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Protective effects of *Aloe vera* gel on cisplatin-induced oxidative stress, apoptosis and neurons structure in rat hippocampus

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Abstract

Cisplatin (CP) as an important chemotherapeutic drug is used for the treatment of various malignancies; but it has some side effects on central nervous system, in particular hippocampus. The present study was aimed to determine the protective effects of *Aloe vera* (AV) gel on CP-induced oxidative stress, apoptosis and neurons structure changes in the hippocampus of rats. Forty-eight rats were divided into six groups including control, CP (5.00 mg kg^{-1} per week; intraperitoneally), CP + AV (400 mg kg^{-1} per day; orally), CP + metformin (200 mg kg^{-1} per day; orally), AV (400 mg kg^{-1} per day; orally) and metformin (200 mg kg^{-1} per day; orally). At the end of treatment, brain samples were obtained for analysis of apoptotic genes expression and anti-oxidant markers as well as histological study. The results showed that CP caused an increase in malondialdehyde level and a decrease in glutathione peroxidase, superoxide dismutase and catalase levels in CP group compared to control. The AV gel could diminish oxidative stress in the hippocampus of CP group and it resulted in down-regulation of Bax, caspase-3 and caspase-8 and up-regulation of Bcl-2 in CP group. It could ameliorate degenerative changes in hippocampus after exposure to CP. Our results showed that AV gel ameliorated oxidative stress, apoptosis and neuronal loss in the hippocampus of rats under CP treatment.

Introduction

Cisplatin (CP) is a very effective anti-cancer drug widely used in the treatment of a variety of malignancies.[1] The anti-cancer activity of CP is due to its direct interaction with DNA suppressing gene transcription and protein synthesis. It also can induce oxidative stress and apoptosis in tumor cells.[2] Cisplatin is very useful for cancer therapy; but, it is very toxic and has dose-dependent side effects such as nephrotoxicity, neurotoxicity, hepatotoxicity and bone marrow injury.[3-5] Neurotoxicity is one of the most common and dose-limiting side effects of CP observed in treated patients.[6] Cisplatin induces neurotoxicity through several mechanisms such as DNA damage, apoptosis, inflammation, mitochondrial dysfunction and oxidative damage.[7]

Oxidative stress and apoptosis have been known as main mechanisms of CP-induced neurotoxicity.[8] Apoptosis process can be triggered by two main signaling pathways including an extrinsic pathway involving death receptors centralized at the surface of the cells and intrinsic pathways involving mitochondria.[9] Most of the apoptotic pathways lead to the activation of caspases. Caspase-8 and caspase-3 are the main upstream initiator and downstream effector caspases in the intrinsic pathway, respectively.[10] The activation of effector caspases leads to apoptosis presenting distinguished biochemical and morphological changes including cell shrinking, chromatin condensation, nuclear fragmentation, alterations of the plasma membrane and the end formation of apoptotic bodies.[11] The administration of CP is associated with increased formation of free radicals and excess cellular levels of reactive oxygen species (ROS) causing damages to the biochemical components such as proteins, enzymes, nucleic acids and lipids; also, membranes and organelles resulting in activation of caspases and cell death.[12,13]

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The brain contains plenty of polyunsaturated fatty acids that are very sensitive to peroxidation.[14] Also, its anti-oxidant activity is lower than other tissues, because it's not rich in anti-oxidant defense.[15] Therefore, neural tissue is more sensitive than other tissues to oxidative damage. The hippocampus, one of the most important regions of the brain being critical for memory and spatial navigation, includes a proliferating stem cell niche being particularly vulnerable to anti-cancer drugs such as CP.[16,17] Previous findings have shown that some drugs can be used as a protective agent against neurotoxicity of CP. It has been reported that metformin (MT) as a most common used anti-diabetic drug can ameliorate CP-induced morphological abnormalities in the brain at the level of white matter organization, neuronal arborization and dendritic spine density. Neuro-protective effect of MT has been attributed to its anti-oxidant properties.[18]

Dietary supplementations of plant-derived anti-oxidants have been recently considered as a hopeful way for reducing or preventing of CP-induced cytotoxicity in experimental models. Recent studies have showed that thymoquinone, an effective and natural anti-oxidant compound, can ameliorate CP neurotoxicity in a dose-dependent manner by promoting the neuronal cell viability in mice.[19] The other study has reported that anti-oxidant properties of curcumin can protect rat against oxidative stress and cognitive impairment induced by CP. Neuro-protective effect of curcumin has been also ascribed to its anti-oxidant properties.

Aloe vera (AV) is one of the most common used herbs worldwide. Several pharmacological effects have been reported for AV gel including anti-cancer,[20] neuro-protective,[21] anti-oxidant,[22] immunomodulatory,[23] anti-inflammatory,[24] anti-depressant and memory-enhancing properties.[25] Beneficial properties of AV gel attribute to numerous phytochemicals substances such as α-tocopherol (vitamin E), ascorbic acid, flavonoids, carotenoids, minerals, enzymes, proteins and carbohydrates.[26]

It has been proposed that the anti-oxidant activity of AV may be a major property of this plant medicines used in the treatment of several diseases.[27] There are several investigations providing experimental evidence for the neuro-protective effect of AV in various diseases. The studies have shown that pre-treatment with AV polysaccharides can decrease cerebral ischemia and reperfusion injury following traumatic hemorrhage in rats through preventing inflammatory response and lipid peroxidation in the brain.[28] Other study has reported that AV could ameliorate anti-oxidant activity in the cerebral cortex and hippocampus leading to improvement of motor and memory performances in diabetic mice.[29] The therapeutic effect of AV-supplemented diet has also shown in an experimental model of multiple sclerosis.[30]

It has been found that oxidative stress and apoptosis play a major role in the pathogenesis of CP-induced neurotoxicity. Because of anti-oxidant property of AV gel, it was hypothesized that it may attenuate the neurotoxicity in the hippocampus of CP-treated rats. The present study was aimed to determine whether AV gel may alleviate oxidative stress and apoptosis induced by CP in the hippocampus of rats.

Materials and Methods

Animals. Forty-eight healthy adult male Wistar rats (180 - 200 g) were purchased from the experimental animal holding of Jundishapur University of Medical Sciences, Ahvaz, Iran. Initially, all rats were housed in conventional conditions for 1 week before experiments began. The animals were kept under standard laboratory conditions, the temperature of 22.00 ± 2.00 ˚C and humidity of 40.00 ± 5.00% with 12/12 hr light/dark cycle. They had access to food pellets (Pars Animal Feed Co., Tehran, Iran) and water *ad libitum*. All experimental assays were approved by the Ethics Committee of Shahid Chamran University of Ahvaz, Ahvaz, Iran, for animal and human experiments (EE/98.24.3.26304/scu.ac.ir).

Experimental groups. After one week of acclimatization, the rats were randomly divided into six equal groups (eight in each group) as follows**:**

The control group: Rats were fed by a standard diet. CP group: Rats received CP intra-peritoneally (IP) at a dose of 5.00 mg kg^{-1} once a week for 4 weeks.[31] CPAV group: Rats received cisplatin (IP) at a dose of 5.00 mg kg^{-1} per week and AV (orally) at a dose of 400 mg kg^{-1} per day for 4 weeks.[32] CPMT group: Rats received cisplatin (IP) at a dose of 5.00 mg kg^{-1} per week and metformin (orally) at a dose of 200 mg kg^{-1} per day for 4 weeks.[8] AV group: Rats received *Aloe vera* (orally) at a dose of 400 mg kg^{-1} per day for 4 weeks. MT group: Rats received metformin (orally) at dose of 200 mg kg^{-1} per day for 4 weeks.

Plant materials preparation and assessment of its anti-oxidant properties. *Aloe vera* gel powder extracted from *Aloe barbadensis* was obtained from Barij Essence Pharmaceutical Company (Kashan, Iran). The phenolic content in the gel was estimated using Folin–Ciocalteu (FC) and aluminium chloride colorimetric assay as described previously.[33] Briefly, 10.00 mg of the dried extract was dissolved into 1.00 mL deionized water (DW). A series of gallic acid concentrations (0.00 - 500 μg mL^{-1}) were prepared in ethanol. Aliquots (40.00 μL) of each AV gel solution and the standard were respectively mixed with 20.00 μL of 1.00 N FC reagent (Sigma-Aldrich, St. Louis, USA). The mixtures were incubated for 3 to 5 min at room temperature followed by addition of 20.00% sodium carbonate (Sigma-Aldrich) solution; then, incubated for 30 min at room temperature. The absorbance was measured at 700 nm using multimode plate reader (PowerWave HT; BioTek, Winooski, USA). The total phenolic content was determined from the standard calibration curve. Results

were expressed as the percentage of gallic acid equivalents (GAE) per 100 g dry weight. The flavonoids content was measured using aluminium chloride colorimetric assay as described previously. Catechin solutions (0.00 - 25.00 μg mL^{-1}; Sigma-Aldrich) were prepared for flavonoid assessment. Aliquots (25.00 μL) of each AV gel (10.00 mg in 1.00 mL DW) and the standard were mixed with 125 μL DW followed by addition 8.00 μL of 5.00% sodium nitrate (Sigma-Aldrich). The mixture was allowed to stand for 5 min at room temperature. Then, 10.00% aluminium chloride solution (Sigma-Aldrich) was added into the 15.00 μL of the mixture. The absorbance was measured at 517 nm using the multimode plate reader (BioTek). Total flavonoid content was expressed as the percentage of catechin equivalents (CAE) per 100 g dry weight being determined from the standard calibration curve.

Sampling and tissue preparation. Rats were decapitated after being euthanized by mixture of 100 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 10.00 mg kg^{-1} xylazine (Alfasan). Their brains were removed and hippocampi were quickly dissected on ice, rinsed with saline and frozen at – 80.00 ˚C for quantitative polymerase chain reaction (qPCR) analysis. To determine the oxidant/anti-oxidant factors in the hippocampus, obtained tissue was homogenized in 500 μL RIPA lysis buffer (NaCl: 150 mM; SDS: 0.10%; Tris: 25.00 mM; pH: 7.40, NaF: 1.00 mM; phenylmethylsulfonyl fluoride: 1.00 mM) with a homogenizer (Heidolph, Schwabach, Germany). Homogenate was centrifuged (5415 R; Eppendorf AG, Hamburg, Germany) at 10,000 RPM for 15 min at 4.00 ˚C. The supernatant was collected and stored at – 70.00 ˚C for subsequent analyses. The protein concentration of supernatant was estimated using the Bradford method.[34]

Lipid peroxidation assay. The level of lipid peroxidation was determined through the content of malondialdehyde (MDA) in tissue. Tissue MDA was determined using the thiobarbituric acid reactive substance assay as described by Buege and Aust with slight modifications.[35] Briefly, 100 μL of tissue lysate was mixed thoroughly with 200 uL of a stock solution of 15.00% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 M hydrochloric acid. The solution was stood for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 5,000 RPM for 10 min. Quantification of the thiobarbituric acid reactive substances was determined by comparing the absorption with the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The MDA results were expressed as nmol mg^{-1} protein.

Determination of catalase (CAT) activity. Catalase activity was determined spectrophotometrically by the method of Koroliuk *et al*.[36] Briefly, 10.00 μL of the sample was incubated with 100 μmol mL^{-1} of H_2O_2 (Merck, Kenilworth, USA) in 0.05 mmol L^{-1} Tris-HCl (Sigma-Aldrich) buffer at pH of 7.00 for 10 min. The reaction was terminated by rapidly adding 50.00 μL of 4.00% ammonium molybdate (Sigma-Aldrich,). Yellow complex of ammonium molybdate and H_2O_2 was measured at 410 nm using the multimode plate reader (BioTek). One unit of CAT activity was defined as the amount of enzyme required to decompose 1.00 μmol H_2O_2 per min. Catalase activity was expressed as U mg^{-1} protein.

Determination of superoxide dismutase (SOD) level. Superoxide dismutase activity was determined using the nitro blue tetrazolium (NBT) method.[37] The assay involves the production of superoxide from O_2 using reduced β-nicotinamide adenine dinucleotide (NADH) as a reductant and phenazine methosulphate (PMS) as a catalyst in the presence of an indicator, NBT, which turns blue when reduced by superoxide. The color change during the reaction was monitored spectrophotometrically in the visible range at 560 nm. When SOD enzyme is added to the reaction, competes with NBT to react with superoxide. The NBT reduction inhibition percentage was used to quantify superoxide-scavenging level. The tissue homogenate (10 μL) was mixed with sodium pyrophosphate buffer (Merck) (100 mM; pH: 8.30), 1.28 mM PMS (Sigma-Aldrich) and 0.18 mM NBT (Sigma-Aldrich). The reaction was started by the addition of 12.72 mM NADH (BioBasic, Toronto, Canada). The reaction mixture was then incubated at 30.00 ˚C for 90 sec and stopped by the addition of 1.00 mL glacial acetic acid (Merck). The absorbance was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required inhibiting chromogen production by 50.00% in 1 min under the assay conditions. The SOD activity was expressed as U mg^{-1} protein.

Determination of glutathione peroxidase (GPx) activity. The activity of GPx was evaluated with GPx detection kit according to the manufacturer's instructions (Randox Laboratories, Crumlin, UK). The GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The changes in absorbance were quantified at 340 nm and the enzyme activity was represented as U mg^{-1} protein.

Quantitative analysis of apoptotic/anti-apoptotic genes expressions in the hippocampus. To determine whether AV gel could change the expression of apoptosis-related genes (Bax, Bcl2, caspase-3 and caspase-8) in hippocampus of rats under CP treatment, the qPCR analysis was done.

Total RNA isolation. Total RNA was isolated from frozen hippocampus tissues using RNX™ RNA isolation kit (SinaClon Inc., Tehran, Iran) as recommended by the manufacturer. The samples were treated with DNase I enzyme to avoid DNA contamination. The purity of RNA at

260/280 optical density (OD) ratio was evaluated using an Eppendorf μCuvette measuring cell (BioPhotometer D30; Eppendorf). Only high purity samples (OD: 260/280 > 1.8) were subjected to further manipulations.

cDNA synthesis. The cDNA was synthesized from RNA samples using the YTA cDNA synthesis kit (Yekta Tajhiz, Tehran, Iran) and Thermal Cycler (MasterCycler; Eppendorf).

Real-time (RT) PCR analysis. The RT-PCR was performed using the Roche Light-Cycler Detection System (Basel, Switzerland) by the qPCR™ Green Master Kit for SYBR Green I® (Yekta Tajhiz). The 12.50 μL reaction for each examined gene was prepared from 6.25 μL of 2X master mix, 0.25 μL of each forward primer (10.00 μM), 3.00 μL cDNA of the sample and 2.25 μL of nuclease-free water. The cycling parameters were 95.00 ˚C for 4 min and 40 cycles of 95.00 ˚C for 15 sec followed by 60.00 ˚C for 30 sec. The GAPDH mRNA fragment was used as a housekeeping gene to normalize the expression data. The primer sequences are described in Table 1.

Histological study. To determine the neuronal survival in the cornu ammonis 1 (CA1), cornu ammonis 3 (CA3) and dentate gyrus (DG) regions of the hippocampus, brains were removed and fixed in 10.00% buffered formalin. Then, the brains were processed by standard method for light microscopy study. Tissue sections of 5.00 μm thickness were prepared from hippocampus by a microtome (RM 2125; Leica Biosystems Nussloch GmbH, Nußloch, Germany) and stained with Hematoxylin and Eosin (H & E).

Statistical analysis. Data analyses were done using the SPSS Software (version 18.0; IBM Corp., Armonk, USA). The data are reported as mean ± standard deviation. One way analysis of variance followed by Tukey test for multiple comparisons was used to assess the variation of the means among the groups. A *p*-value less than 0.05 was statistically significant.

Results

Phytochemical content. The total phenol and flavonoid contents of AV gel were 49.81 μg GAE mg^{-1} and 56.42 μg QE mg^{-1} of extract, respectively.

The lipid peroxidation levels. Cisplatin significantly increased MDA level in hippocampus tissue by 2.19 ± 0.20 nmol mg^{-1} compared to 0.67 ± 0.14 nmol mg^{-1} in Control group ($p < 0.05$; Table 2). Administration of AV gel or MT alone had no significant effect on MDA levels (0.93 ± 0.28 nmol mg^{-1} and 0.39 ± 0.09 nmol mg^{-1}, respectively) compare to control group. Administration of AV gel to the CP-treated rats resulted in a significant reduction of MDA level (1.06 ± 0.18 nmol mg^{-1}) compared to the CP group (Table 2).

Anti-oxidant enzymes activities. Table 2 shows the changes in the activities of anti-oxidant enzymes (SOD, CAT and GPx) in hippocampus tissues of control and experimental rats. There was a decrease in the activities of SOD, CAT and GPx in the hippocampus of CP group compared to the control group ($p < 0.05$; Table 2). Treatment of CP group with AV gel significantly attenuated the reduction of anti-oxidant enzymes activities ($p < 0.05$; Table 2).

The expression of apoptotic/anti-apoptotic genes. The results of the current study showed that the expression of pro-apoptotic gene, Bax, was significantly higher in the hippocampus of CP group compared to control group ($p < 0.05$; Fig. 1). The Bax transcription was down-regulated in CP rats together with administration of AV gel ($p < 0.05$). *Aloe vera* had a more inhibitory effect on hippocampus expression of Bax in CP-treated rats in comparison with MT-treated animals; but significant changes were not observed ($p > 0.05$; Fig. 1). The mRNA level of anti-apoptotic gene, Bcl-2, was significantly lower in hippocampus of CP group compared to the control group ($p < 0.05$; Fig. 2). Administration of AV and MT along with CP led to up-regulation of Bcl-2 compared to CP group ($p < 0.05$; Fig. 2). The AV or MT groups presented no significant difference regarding the expression of Bcl-2 gene in relation to control group ($p > 0.05$; Fig. 2).

The expression of caspase-3 and caspase-8 genes was significantly up-regulated in the hippocampus of the CP group rats compared to control animals ($p < 0.05$; (Figs. 3 and 4). The treatment of CP rats by AV gel or MT could significantly inhibit the up-regulation of caspase-3 and caspase-8 in the hippocampus ($p < 0.05$; Figs. 3 and 4).

Table 1. Characteristics of primers which were used for real-time PCR analysis.

Gene	Sequences	Size (bp)	Accession number
Bax	F:TGCTACAGGGTTTCATCCAG R:TGTTGTTGTCCAGTTCATCG	145	NM_017059.2
Bcl-2	F:ATCGCTCTGTGGATGACTGAGTAC R:AGAGACAGCCAGGAGAAATCAAAC	135	NM_016993.1
Caspase-3	F:AATTCAAGGGACGGGTCATG R: CAGATCCCGTGTATTGTGTCA	181	XM_006253130.3
Caspase-8	F: AAAGCCCAGGTTTCTGCCTA R: ATCAAGCAGGCTCGAGTTGT	141	NM_022277.1
GAPDH	F: AGTTCAACGGCACAGTCAAG R: TACTCAGCACCAGCATCACC	119	XM_017593963.1

Histological evaluation. The light microscopic study showed normal morphology in CA1 pyramidal neurons and granular cells in DG of the hippocampus in control group. Normal pyramidal neurons and granular cells are rounded cells with distinctic euchromatic nuclei (Figs. 5A and 6A). In the CP group, plenty of pyramidal neurons in the CA1 region showed degenerative changes including being shrunken with heterochromatic nuclei (Fig. 5B).

Histologic sections of CPAV and CPMT groups showed morphologic changes reduction in CA1 compared to CP group (Figs. 5C and 5D). The granular cells in DG of hippocampus in CP groups showed severe degenerative changes being shrunken with dense and heterochromatic nuclei (Fig. 6B). Meanwhile, the degenerative changes in CPAV and CPMT groups were attenuated in DG of hippocampus compared to CP group (Figs. 6C and 6D).

Table 2. Effects of aloe vera gel on lipid peroxidation (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities in hippocampus of rat. Values are presented as mean ± SD.

Groups	MDA (µmol mg^{-1})	SOD (U mg^{-1})	GPx (U mg^{-1})	Catalase (U mg^{-1})
Control	0.67 ± 0.14^{a}	3.24 ± 0.23 ^{a}	7.46 ± 0.03 ^{a}	24.44 ± 0.89^{a}
CP	2.19 ± 0.20 ^{b}	1.27 ± 0.18 ^{b}	3.15 ± 0.39 ^{c}	14.58 ± 1.12 ^{b}
CPAV	1.07 ± 0.18 ^{a}	2.23 ± 0.10^{a}	5.37 ± 0.26 ^{b}	21.88 ± 0.97 ^{a}
CPMT	1.45 ± 0.17 ^{b}	1.85 ± 0.08 ^{b}	4.57 ± 0.45 ^{b}	24.17 ± 2.70 ^{a}
AV	0.93 ± 0.28 ^{a}	2.14 ± 0.03 ^{a}	9.04 ± 0.48 ^{a}	25.88 ± 1.77 ^{a}
MT	0.39 ± 0.09 ^{a}	2.37 ± 0.98 ^{a}	8.71 ± 0.28 ^{a}	21.48 ± 1.88 ^{a}

CP: Cisplatin, CPAV: Cisplatin+aloe vera, CPMT: Cisplatin+metformin, AV: Aloe vera, and MT: Metformin.
^{abc} Different letters in each column denote significant differences ($p < 0.05$).

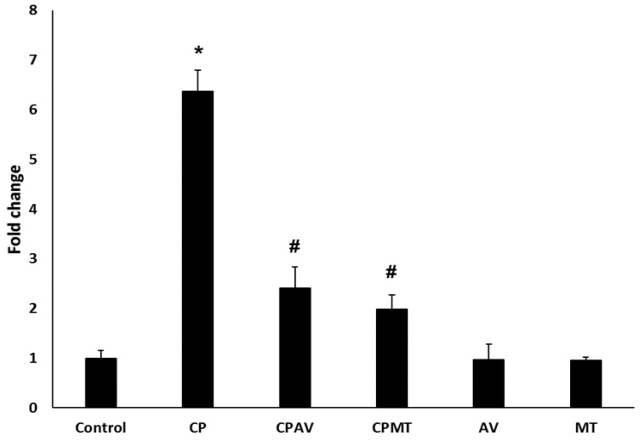

Fig. 1. The mRNA level of Bax gene in hippocampus of rats in all experimental groups. The expression of each gene was determined relative to GAPDH expression as a calibrator gene. * $p < 0.05$ versus control group and # $p < 0.05$ versus cisplatin (CP) group. AV: *Aloe vera*; MT: Metformin.

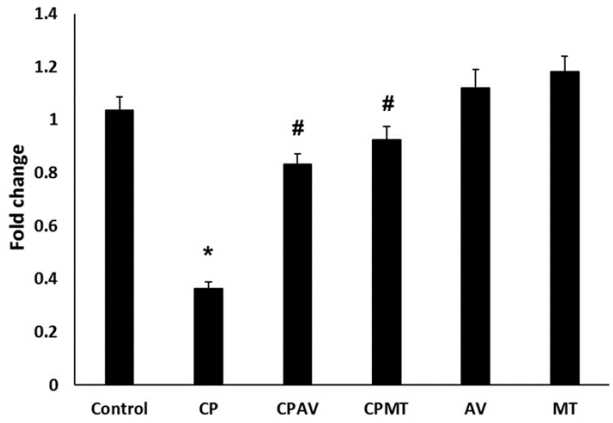

Fig. 2. The mRNA level of Bcl-2 gene in hippocampus of rats in all experimental groups. * $p < 0.05$ versus control group and # $p < 0.05$ versus cisplatin (CP) group. AV: *Aloe vera*; MT: Metformin.

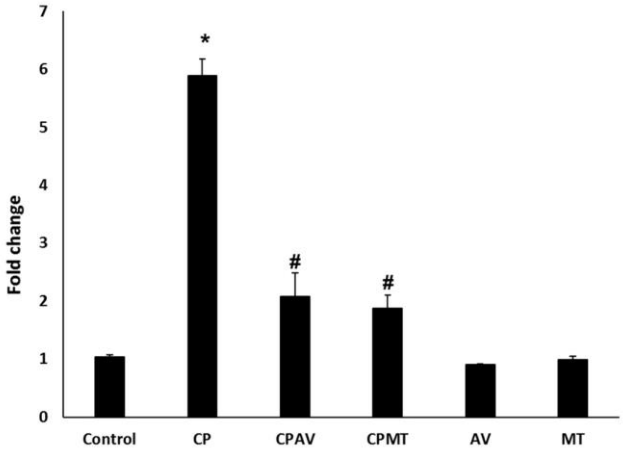

Fig. 3. The mRNA level of caspase-3 gene in hippocampus of rats in all experimental groups. * $p < 0.05$ versus control group and # $p < 0.05$ versus cisplatin (CP) group. AV: *Aloe vera*; MT: Metformin.

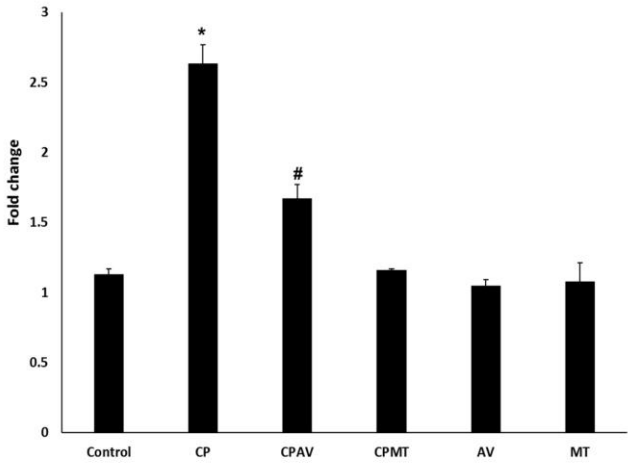

Fig. 4. The mRNA level of caspase-8 gene in hippocampus of rats in all experimental groups. * $p < 0.05$ versus control group and # $p < 0.05$ versus cisplatin (CP) group. AV: *Aloe vera*; MT: Metformin.

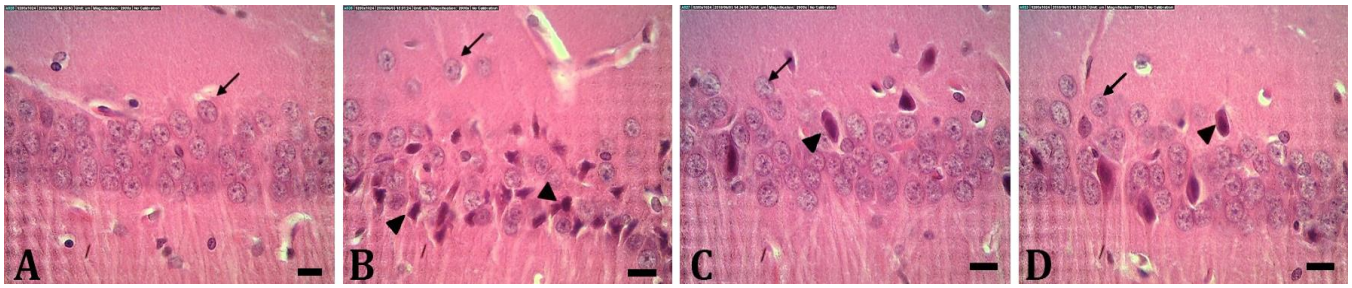

Fig. 5. Photomicrographs of *Aloe vera* (AV) gel and metformin (MT) effects on histological changes of hippocampus induced by cisplatin (CP) in cornu ammonis 1. **A)** Control group: Normal morphology is seen in pyramidal neurons of the hippocampus as clear rounded cells with distinct euchromatic nuclei (arrow). **B)** CP group: CP-induced degenerative changes in pyramidal cells as shrunken cell with heterochromatic nuclei (arrowheads) compared to normal cells (arrow). **C** and **D)** CPAV and CPMT groups, respectively, in which AV gel and MT administrations improved degenerative cell changes induced by CP. Increase in normal cells with distinct euchromatic nuclei (arrow) and decrease in shrunken cell with heterochromatic nuclei (arrowhead), (H & E, Scale bars = 20 µm).

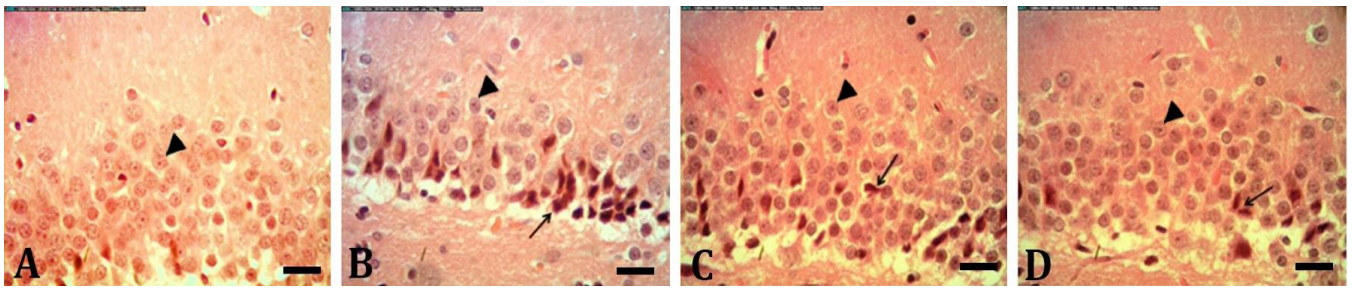

Fig. 6. Photomicrographs of *Aloe vera* (AV) gel and metformin (MT) effects on histological changes of hippocampus induced by cisplatin (CP) in dentate gyrus (DG) regions. **A)** Control group: Normal morphology is seen in granular cells of the hippocampus as rounded cells with distinct nuclei (arrowhead). **B)** CP group: CP-induced degenerative changes in granular cells as shrunken cell with heterochromatic nuclei (arrow) compared to normal cells (arrowhead). **C** and **D)** CPAV and CPMT groups, respectively, in which AV gel and MT administrations ameliorated CP-induced histological changes. Increase in normal cells with distinct euchromatic nuclei (arrowhead) and decrease in shrunken cell with heterochromatic nuclei (arrow), (H & E, Scale bars = 20 µm).

As shown in Table 3, the number of normal pyramidal and granular neurons decreased significantly in CA1, CA3 and dentate gyrus (DG) regions in CP group compared to the other groups ($p < 0.05$). But, the number of the normal neurons increased significantly in the CPAV and CPMT groups compared to CP group. Treatment with AV gel and MT improved hippocampal neurons viability in different regions of the hippocampus ($p < 0.05$).

Table 3. Effects of cisplatin, *Aloe vera* gel and metformin on the number of surviving neurons in CA1, CA3 and dentate gyrus (DG) regions of rat hippocampus. Values are presented as mean ± SD.

Groups	CA1	CA3	DG
Control	16.75 ± 0.17[a]	11.23 ± 0.17[a]	32.14 ± 0.29[a]
CP	10.20 ± 0.62[b]	5.42 ± 0.5[b]	14.71 ± 0.79[b]
CPAV	15.94 ± 0.33[a]	10.04 ± 0.12[a]	30.04 ± 0.05[c]
CPMT	15.80 ± 0.25[a]	10.13 ± 0.16[a]	29.66 ± 0.17[c]
AV	16.25 ± 0.21[a]	10.71 ± 0.14[a]	31.85 ± 0.28[a]
MT	16.76 ± 1.04[a]	11.13 ± 0.28[a]	31.70 ± 0.29[a]

CP: Cisplatin, CPAV: Cisplatin+*Aloe vera*, CPMT: Cisplatin+ metformin, AV: *Aloe vera*, and MT: Metformin.
[abc] Different letters in each column denote significant differences ($p < 0.05$).

Discussion

In this study, the neuro-protective effects of AV gel on CP-induced oxidative stress, apoptosis and neurons structure changes in hippocampus of rats were evaluated. There are various mechanisms for CP-induced neurotoxicity including DNA damage, oxidative stress, apoptotic pathways activation and inflammation. There are many studies describing that oxidative stress and apoptosis may be the main mechanisms for toxicity induced by CP. The administration of CP leads to free radicals increases and excess cellular levels of ROS causing damage to the biochemical components such as nucleic acids, proteins and lipids as well as membranes and organelles which can result in activation of cell death processes like apoptosis.[8,13,38]

The result of this study is that CP significantly increased MDA level and decreased anti-oxidant enzymes (SOD, CAT and GPX) activity in rat hippocampus. These results indicate that CP could lead to oxidative stress in the hippocampus. These findings are consistent with Gulec *et al.,* and Tuncer *et al.,* findings indicating that CP can lead to oxidative stress in the hippocampus. These findings are consistent with Gulec *et al.,* and Tuncer *et al.,* findings

indicating that CP can lead to significant tissue damage characterized by a significant decrease in anti-oxidant enzymes activity and increase in MDA level.[38,39] Moreover, numerous anti-oxidant treatments have been used successfully in order to inhibit neurotoxicity induced by CP. *Aloe vera* is one of the most important herbs having several pharmacological effects including neuro-protective, anti-oxidant and memory-enhancing properties. It has been proposed that the anti-oxidant activity of AV may be a major property of this plant being used in the treatment of several diseases.[27,40]

In this study, we found that AV gel could significantly decrease the CP-induced oxidative stress together with ameliorating neuronal injury in hippocampus tissue. In the other hand, treatment with AV gel could lead to increase in anti-oxidative defense in hippocampus tissue through increasing GPx, SOD and CAT activities and reducing the MDA level. Guven *et al.,* have reported that AV gel administration has neuro-protective effects regarding anti-oxidant properties against experimental sciatic nerve ischemia/reperfusion injury in rats. They have found that AV gel decreases the MDA levels and increases the SOD activity leading to oxidative stress reduction following ischemia.[24,40]

The AV gel used in the current study contains a large amount of flavonoid and phenols. It has been reported that flavonoids augment memory performance stimulating neurogenesis and protect neurons *versus* oxidative stress. Other studies have reported that AV gel contains a large number of anti-oxidants such as flavonoids, carotenoids, tannins and vitamins (vitamin E, A and C) being very efficient in modulating or inactivating of the ROS. It has been demonstrated that administration of these compounds has a neuro-protective effect against neurotoxicity induced by CP without altering its anti-cancer activity.[29,40,41]

Apoptosis process can be triggered by three major pathways including an extrinsic pathway involving death receptors, intrinsic pathways involving mitochondria and endoplasmic reticulum stress pathway. It has been demonstrated that CP can induct these three pathways; whereas, the mitochondria-mediated pathway is the most important one among these three pathways. During mitochondria-mediated pathway, the cytochrome C is released from mitochondria leading to the activation of caspase-8 and caspase-9 and then, activation of caspase-3 which plays an important role in apoptosis.[9, 42] We evaluated the expression of caspase-3 and caspase-8 using RT-PCR analysis. In the current study, our results indicated the over-expression of Bax, caspase-3 and caspase-8 and down-regulation of Bcl-2 in hippocampus of CP group. In accordance, recent studies have shown that CP administration significantly enhances Bax expression level and decreases Bcl-2 expression level in mice renal tissues leading to apoptosis. In fact, CP causes translocation of Bax in mitochondria and subsequently, over-expressions of caspase-3 and caspase-8 occur promptly after CP administration. This alteration in apoptotic genes expression markedly leads to mitochondrial injury and apoptosis. [43] In another study, Lee *et al.,* have indicated that administration of CP activates several caspases leading to apoptotic cell death.[44] In our study, administration of AV gel markedly protected rats against CP-induced enhancements of Bax, caspase-3 and caspase-8 and decrease of Bcl-2 in hippocampus tissue, suggesting significant protection against CP-induced apoptosis.

Histological findings support the biochemical and molecular findings. In the present study, histological findings indicated the degenerative changes in the plenty of pyramidal and granular cells including being shrunken with heterochromatic nuclei demonstrating the apoptotic effects of CP in hippocampus tissue. In the other hand, oxidative stress elevation induced by CP was coupled with neuronal damage in the hippocampus. Our results demonstrated that administration of AV could improve the hippocampus neurons injury. Recently, Guven *et al.,* have demonstrated the neuro-protective effects of AV on experimental sciatic nerve ischemia/reperfusion injury in rats due to anti-oxidant and anti-inflammatory properties. Their histopathological finding showed that AV gel has a neuro-protective effect on the sciatic nerves, because axonal disorders and myelin breakdown reduced markedly after treatment with AV gel. Also, Wang *et al.,* have reported that AV protects the structure and function of mitochondria in neurons of the brain.[24,45]

Podratz *et al*., and Zheng *et al.,* have reported that animals being treated with the chemotherapeutic drug show vacuolated and swollen mitochondria in the peripheral nerves indicating neuronal apoptosis through pathways linked to caspase activation. Their findings indicated that oxidative stress causes mitochondrial dysfunction and as a central mediator of redox imbalance and apoptosis in peripheral neurons, it is responsible for neuro-degeneration. In the other hand, these findings propose that oxidative stress plays an important role in CP-induced neurotoxicity resulting in neuronal injury and cell death in hippocampus tissue after administration of CP.[2,46] Consistent with our findings, several studies have reported that increased oxidative stress in the hippocampus tissue of rats is associated with cognitive impairment.[47] Also, Negi *et al.,* have reported that oxidative stress in several models of neuropathies is responsible for the neuronal damage and oxidative damage to peripheral neurons causing injury to the myelin sheath, mitochondrial proteins and other anti-oxidant enzymes.[48]

In conclusion, this study demonstrated that oxidative stress and apoptosis can play an important role in CP-induced neurotoxicity. Biochemical, molecular and histological findings suggest that AV gel prevents from CP-

induced neurotoxicity in rats. This study concluded that AV gel with its potential anti-oxidant and anti-apoptotic effects can protect the hippocampus tissue from CP-induced damage.

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Conflict of interest

The authors declare that there is no conflict of interests.

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