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Caffeic Acid Phenethyl Ester Ameliorated Ototoxicity Induced by Cisplatin in Rats

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Summary

Caffeic acid phenethyl ester (CAPE), an active component of propolis, exhibits antioxidant properties. This experimental study was designed to determine the effect of CAPE on ototoxicity induced with cisplatin. Twenty-four adult Wistar albino rats were divided into four groups: cisplatin (n=6), saline (n=6), CAPE (n=6), and cisplatin plus CAPE (n=6). Rats were tested before and 5 days after cisplatin treatment with or without chemo protection. The Distortion Product Otoacoustic Emissions (DPOAEs) were elicited from the control and experimental animals utilizing the standard commercial Otoacoustic Emission (OAEs) apparatus. The animals in all groups were sacrificed under general anesthesia on the fifth day following last OAE measurements. For biochemical investigations, the blood samples were drawn from inferior vena cava

On day 0, the initial baseline DPOAEs measurement results presented similar values while comparing the groups in drug free phase ($p>0.05$). On day 5, intrasubject measurement parameters of DPgrams and I/O functions of cisplatin group were significantly deteriorated ($p<0.05$). The second measurements of the other groups revealed no significant differences between their DPgrams and I/O functions in all frequencies ($p>0.05$). Among the biochemical parameters, plasma xanthine oxidase (XO) activity was found to be more elevated in the cisplatin group than the saline group ($p<0.05$). CAPE led to more decreased XO activity than cisplatin ($p<0.05$). The results of this study show that prophylactic administration of CAPE for cisplatin ototoxicity ameliorated hearing deterioration in rats.

Key words: **Ototoxicity, cisplatin, antioxidant agents, caffeic acid phenethyl ester, otoacoustic emissions, outer hair cells, rat.**

INTRODUCTION

Cisplatin, which is now known as one of the most potent antitimor drugs, is an effective therapeutic agent for various malignancies such as head and neck squamous cell carcinoma, testicular, ovarian, bladder, uterine cervical, osteogenic and lung carcinomas. However, its therapeutic efficacy is often limited by severe side-effects, such as ototoxicity, nephrotoxicity and peripheral neuropathies. Cisplatin impairs cochlear function in experimental animal studies and in patients in whom hearing loss develops, resulting in loss of outer hair cells [1]. In human beings, following high doses of cisplatin administration, typically cumulative, bilateral, and irreversibly profound sensorineural hearing loss and tinnitus can develop [2]. This is characterized by a usually permanent sensorineural hearing loss starting at high frequencies and extending progressively to lower frequencies [3]. Therefore, a search for a method to prevent cisplatin ototoxicity has been an ongoing endeavor. The mechanism of cisplatin ototoxicity is based on the generation of the reactive oxygen species (ROS), which interfere with the

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antioxidant protection of the organ of Corti [4-6].

One of the most important enzymatic sources of superoxide radicals is xanthine oxidase (XO). This oxidant enzyme is located in the cells and catalyzes the conversion of hypoxanthine and xanthine to uric acid. Current interest in XO originates from its proposed role in oxidative injuries. The major intracellular antioxidant enzyme superoxide dismutase (SOD) specifically converts superoxide radicals to hydrogen peroxide. The production of ROS surpasses the antioxidant capacity resulting in attacks of polyunsaturated fatty acids on the membranes and consequent lipid peroxidation. Malondialdehyde (MDA) has been used as a sensitive indicator of *in vivo* lipid peroxidation [7]. Nitric oxide (NO) is known to be a ROS and is produced by the family of nitric oxide synthase enzymes. Therefore, it should be useful to determine the interference of NO generation in cisplatin administration.

Caffeic acid phenethyl ester (CAPE), an active component of propolis extracted from honey, exhibits antiviral, anti-inflammatory, and immunomodulatory properties and is shown to inhibit the growth of different types of transformed cells [8-10]. In transformed cells, CAPE alters the redox state and induces apoptosis. It has been reported that CAPE suppresses lipid peroxidation, displays antioxidant activity and inhibits lipooxygenase activities [11,12].

The purpose of the present study was to investigate the probable protective effect of CAPE against cisplatin ototoxicity using otoacoustic emission (OAEs) and the indices of plasma oxidant/antioxidant parameters.

MATERIALS AND METHODS

Chemicals

Cisplatin (Cisplatinum Ebewe 0.5 mg/ml) was obtained from Liba Drug Company, Turkey. CAPE was purchased from Sigma, Germany. The other chemicals were of the highest quality commercially available.

Animals

Twenty-four adult female Wistar albino rats, initially weighing between 150 g and 220 g at the age of 12 weeks were used in this study. The animals were fed with commercial standard diet and water *ad libitum* during the experimental period. They were kept at room temperature of 20-22°C, relative humidity of 50±5% and 12:12 h light and dark cycles. They were housed in plastic cages containing wood-chip bedding with three or four rats per cage. The presence of Preyer's reflex was used for initial acceptance of each adult animal subject to the study. The ear canals and tympanic membranes of the animals were examined initially by otomicroscopy. Additionally, normal findings in DPOAEs confirmed the health of the hearing of the adult rats and made them eligible for the investigation. All animals were housed for acclimatization for 1 week before the experiments. This research was performed under the animal usage protocol approved by the local Institutional Animal Care and Use Committee.

Anesthesia

Rats were anesthetized with a cocktail of ketamine hydrochloride (30 mg/kg) and xylazine (6 mg/kg) which were administered intraperitoneally (i.p.) just before the OAE recordings and sacrifice processing.

Experimental design

The animals were randomly divided into four groups, of 6 rats each (group A, control; group B, CAPE; group C, cisplatin; group D, cisplatin plus CAPE). Group A animals received i.p. vehicle with 0.2 ml of 10% ethanol as a solubility agent of CAPE. Group B was administered only CAPE for 6 days (10 µmol/kg/day). Group C was injected with cisplatin i.p. at time 0 (16 mg/kg body weight), once only. Group D received 10 µmol/kg/day CAPE i.p. 24 hours before and at the same time as single dose cisplatin injection (16 mg/kg i.p.) and every 24 hours for 5 consecutive days. Body weights, clinical signs and food and water consumption were recorded regularly. Rats were tested before and on the 5th day after cisplatin intervention with or without chemo protection. The animals in all the groups were sacrificed on day 5 following the last OAE measurements under sedation, after the blood samples were taken from the inferior vena cava. The plasma was separated by centrifugation and stored at -30°C until analysis. Experiments were performed between 4.30 and 12:00 p.m. daily.

OAE measurements

Repeated OAE recordings were made on the right ear of each animal which had normal ear canal and tympanic membrane examined otomicroscopically. Only those rats with normal replicable OAEs before the administration of any substance on day 0 were accepted in this study. OAE recordings were performed in a quiet room. The DPOAEs were elicited from the control and experimental animals utilizing the standard commercial OAE apparatus of ILO-96 cochlear emission analyzer (Otodynamics Ltd., London, UK). Following the anesthetic intervention, the primary tones produced by the two separate speakers were introduced into the animal's outer ear canal through an inserted earphone probe using a plastic adapter that seals the probe in the outer ear canal.

DPOAEs were measured as sound pressure level (SPL) by using stimuli of constant intensity and frequency changes. For DPgram, the intensities of primary stimuli were set as equilevel (L1=L2) at 65 dB.

The frequencies (f_1 and f_2) were adjusted in such a manner that f_2/f_1=1.21. DPOAEs were determined as DPgrams, where the intensity levels of the primary tones held constant and DPOAE data were recorded at different frequency regions, ranging from 1001 to 6299 Hz, and plotted as a function of f_2. The resolution of the DPgram was obtained at four points per octave. Detection thresholds and suprathreshold measures in the form of I/O functions were obtained by decreasing the primary tones from 75 to 36 dB SPL, in 3-dB steps. The level of the noise floor was measured at the frequency which was 50 Hz above the DPOAE frequency, using similar averaging techniques. An emitted response was accepted if the DPOAEs at $2f_1$-f_2 amplitude were ≥3 dB above the magnitude of the noise-floor level at the $2f_1$-f_2 + 50 Hz frequency for DPgram and I/O functions. Both types of testing methods were recorded till the responses attained their highest level, then the test was stopped when further measurements led to no increase in DPOAE amplitude levels.

The study was started with DPgram and I/O function measurements of all animals to determine the baseline hearing status. Two measurements were made in all animals during experiments on days 0 and 5. Otomicroscopic examination of the rats was performed before every DPOAE testing to exclude any middle ear pathology that may impair DPOAE measurements. For each animal, I/O functions at 3, 4, 5 and 6 kHz were recorded and the detection threshold was noted. The f_2 frequencies examined for DPgram ranged from 1001 to 6299 Hz (1001, 1184, 1416, 1685, 2002, 2380, 2832, 3369, 4004, 4761, 5652, 6299 Hz). Separate threshold and I/O functions were calculated for each group of subjects. After the animals were sacrificed, disappearance of the DPOAE of the rats was observed to demonstrate the validity of measurements.

Biochemical determination

Blood samples were drawn into EDTA-containing commercial tubes. After immediate centrifugation, plasma samples were stored as frozen at -30°C. The plasma MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90°-100°C (A). In the TBA test reaction, MDA or MDA-like substances and TBA react together for the production of a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 90°C for 15 min. The sample was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm (Ultra spec Plus, Pharmacia LKB Biochrom Ltd, UK). The results were expressed as micromole per liter plasma (µmol/L) according to a standard graphic, which was prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane.

Since NO is a very labile molecule, its direct measurement in the biological samples was very difficult. In aqueous solution, NO reacts with molecular oxygen and accumulates in the plasma as nitrite (NO_2^-) and nitrate (NO_3^-) ions. Therefore, the stable oxidation end products of NO, namely NO_2^- and NO_3^- can be readily measured in biological fluids and have been used *in vitro* and *in vivo* as indicators of NO production [13]. Therefore, plasma nitrite concentration was accepted as an index of $NO^\cdot$. For total nitrite detection, deproteinized plasma was treated with copperized cadmium (Cd) granules to reduce NO_3^- to NO_2^-. Nitrite concentrations were quantified by a colorimetric assay based on the Griess reaction [14]. Briefly, a chromophore with a strong absorbance at 545 nm is formed by reaction of nitrite with a mixture of N-naphthylethylene diamine and sulphanilamide. A standard curve was established with a set of serial dilutions (10^{-8} to 10^{-3} mol/L) of sodium nitrite. Results were expressed as µmol/L plasma.

The principle of the total SOD (EC 1.15.1.1) activity method is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by xanthine/xanthine oxidase system [15]. Activity was assessed in the ethanol phase of the plasma after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of the plasma and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as Units per milliliter plasma (U/mL).

Plasma XO (EC 1.2.3.2) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm [16]. A calibration curve was constructed by using 10-50 milliUnits/mL concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1 µmol uric acid formed per minute at 37°C, pH 7.5. Results were expressed in Units per liter plasma.

Statistical analysis

Each animal served as its own control for the OAE recordings. Results were analyzed statistically by Kruskal Wallis (SPSS® 10.0, SPSS) to determine differences in amplitudes of DPOAEs and corresponding noise floor differences and thresholds for each frequency. The effects of CAPE and cisplatin were evaluated among intrasubject and intersubject variation, by considering the baseline measurements and median values. Biochemical enzymes were analyzed utilizing the Mann Whitney U test (SPSS® 10.0, SPSS). Weight loss was analyzed statistically by ANOVA (SPSS® 10.0, SPSS). A significance level of $p<0.05$ was used in statistical test analysis.

RESULTS

The animals tolerated the anesthesia well. There were no general health differences among rats that received vehicle or CAPE. However, the animals being injected with cisplatin alone and cisplatin plus CAPE showed significant weight loss on day 5 compared with other groups (*Figure 1*). In addition, in this group, food and water consumption decreased day by day.

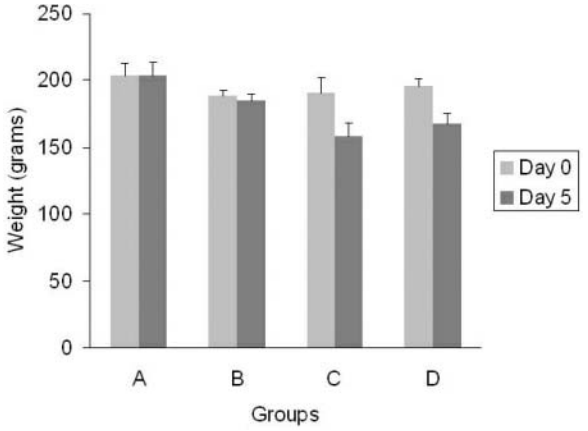

FIGURE 1 - Mean weights of the rats on days 0 and 5. Control group with ethanol (A); control group with CAPE only (B); rats treated with cisplatin (C); rats treated with cisplatin plus CAPE (D). Error bars are S.E.M.

Hearing studies with OAE

DPgram and I/O functions of study groups corresponding to days 0 and 5 are presented in *Figures 2-5*. All rats had otomicroscopic examination and DPOAE testing in advance, DPgrams and I/O functions were recorded. On day 0, the initial baseline DPOAE measurements presented similar values to those of the groups prior to drug administration ($p>0.05$). On the fifth day, intrasubject measurement parameters of DPgrams and I/O functions of the cisplatin group were found significantly deteriorated ($p<0.05$). The second measurement of the CAPE, CAPE and cisplatin and control groups revealed no significant differences between their DPgrams and I/O functions in all frequencies ($p>0.05$).

In the DPgrams, for all sessions, the emission amplitude levels were greater than the noise floor throughout the testing frequencies. The analysis of the results in the median amplitudes of DPgrams and I/O functions revealed statistically significant differences between the groups of C and D ($p<0.05$), suggesting that CAPE has a preventive effect against cisplatin ototoxicity.

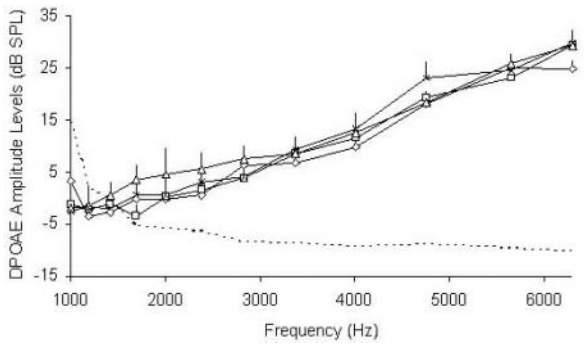

FIGURE 2 - DPgrams from rats treated with cisplatin and/or CAPE. Determination of DPOAEs on day 0. Control group with saline (◇); control group with CAPE only (□); rats treated with cisplatin (△); rats treated with cisplatin plus CAPE (×). The dotted line is noise level. The values are expressed as the ± SEM.

Biochemical analysis of plasma

Plasma NO, SOD, XO activities and MDA levels of the animals are shown in *Figure 6*. XO activity was found to be significantly elevated in the cisplatin group ($p<0.05$). The administration of CAPE had a decreasing effect on XO activity ($p<0.05$).

DISCUSSION

Cisplatin is an effective therapeutic agent for several human cancers including head-neck, testicular, ovarian, bladder, uterine cervical, osteogenic and lung carcinomas. Cisplatin, which contains a heavy metal platinum, is widely used and has serious side effects, the main dose-limiting ones being ototoxicity and nephrotoxicity. Although the use of intravenous hydration and diuresis effectively decreases the severity of nephrotoxicity, ototoxicity still poses the major limitation of achieving effective doses of cisplatin chemotherapy [17,18]. This may result from generation of ROS, which damage various tissues including the structures of the cochlea [5,19]. ROS that react with membrane lipids to produce toxic aldehydes such as 4-hydroxynonenal which were found to cause apoptosis and cell death in the organ of Corti explants and in cell cultures of the spiral ganglion [5]. There have been numerous clinical and experimental studies performed to protect cochlea from cisplatin otoxicity.

According to our study protocol, ototoxicity was assessed on the 5th day after cisplatin administration to investigate the short-term ototoxic effects of cisplatin. Cisplatin was injected in a single high dose, because in most oncology clinics, high doses of cisplatin are usually administered once per 3 or 4 weeks. We tested the protective effect of CAPE for cisplatin ototoxicity. We hypothesized that CAPE would effectively protect cochlea by its antioxidant and anti-inflammatory effects on cisplatin-induced

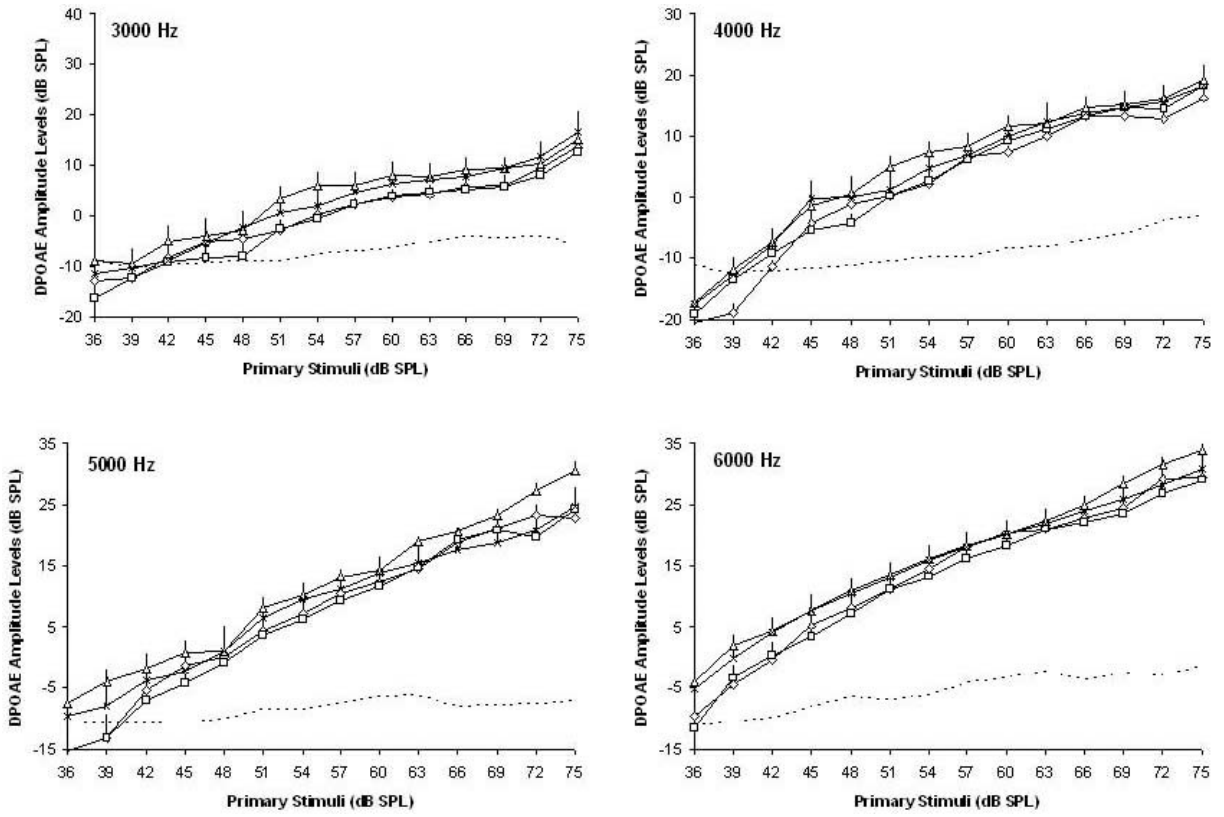

FIGURE 3 - I/O functions of the DPOAEs at 3000, 4000, 5000, and 6000 frequencies of rats treated with cisplatin and/or CAPE on day 0. Control group with saline (◇); control group with CAPE only (□); rats treated with cisplatin (△); rats treated with cisplatin plus CAPE (×). The dotted line is noise level. The values are expressed as the ± SEM.

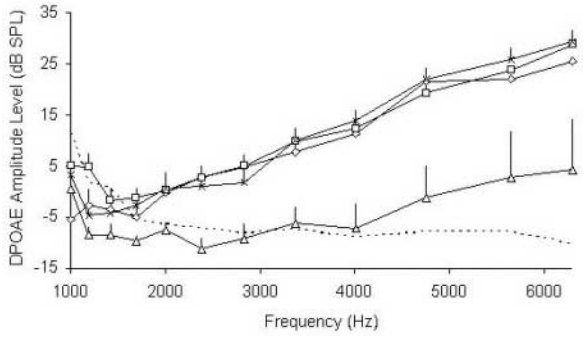

FIGURE 4 - DPgrams from rats treated with cisplatin and/or CAPE. Determination of DPOAEs on the 5th day. Saline (◇); CAPE (□); rats treated with cisplatin (△); rats treated with cisplatin plus CAPE (×). The dotted line is noise level. The values are expressed as the ± SEM.

ototoxicity. To our knowledge, this is the first study to adapt these effects of CAPE in an attempt to prevent cisplatin-induced injury. Rats were tested before and on the 5th day after cisplatin intervention with or without chemo protection. We have monitored hearing loss using DPOAEs which is highly selective for cochlea. The most important benefits of OAEs are their non-invasive capacity and objectivity to determine the primary stages of the sound process, to identify and evaluate the biomechanical activity of the outer hair cells [19]. DPOAEs are well described methods for detecting the effects of cisplatin on the cochlea even before it was revealed by pure-tone audiometry [20].

According to the biochemical and histopathological findings, CAPE was found to prevent ischemia-reperfusion injuries by several ways in different organ models [21-23]. The results of this study indicate that prophylactic administration of CAPE protects the cochlea against cisplatin ototoxicity. None of the biochemical parameters in plasma were changed upon cisplatin administration and CAPE application except XO. It can be speculated that cisplatin as well as CAPE cannot affect plasma oxidant/antioxidant systems. CAPE is a natural product extracted from propolis of honey and used for isolation and disinfection of hives with no known side effects in bees [13]. The main reason for CAPE preference in this study was its well known strong antioxidant, antimitotic, anti-inflammatory and wound healing properties [23,24]. It has also been shown that CAPE is a

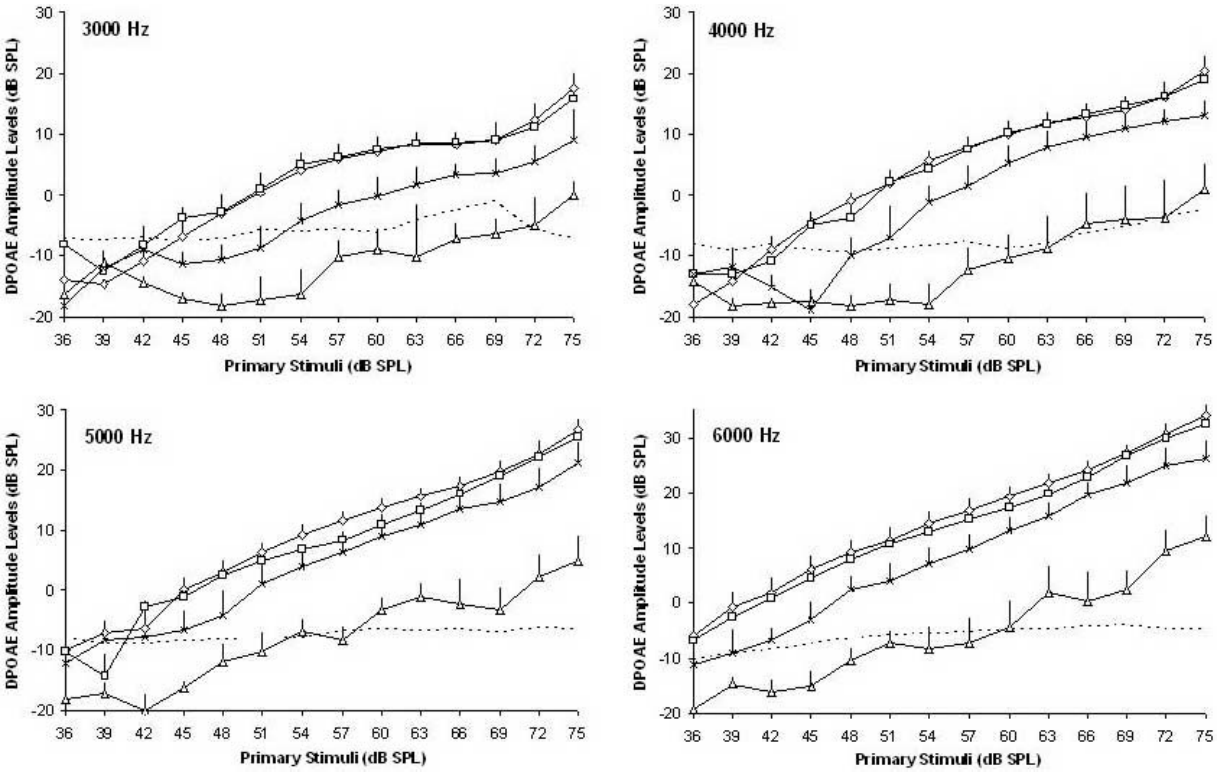

FIGURE 5 - I/O functions of the DPOAEs at 3000, 4000, 5000, and 6000 frequencies of rats treated with cisplatin and/or CAPE on the 5th day. Control group with saline (◇); control group with CAPE only (□); rats treated with cisplatin (△); rats treated with cisplatin plus CAPE (×). The dotted line is noise level. The values are expressed as the ± SEM.

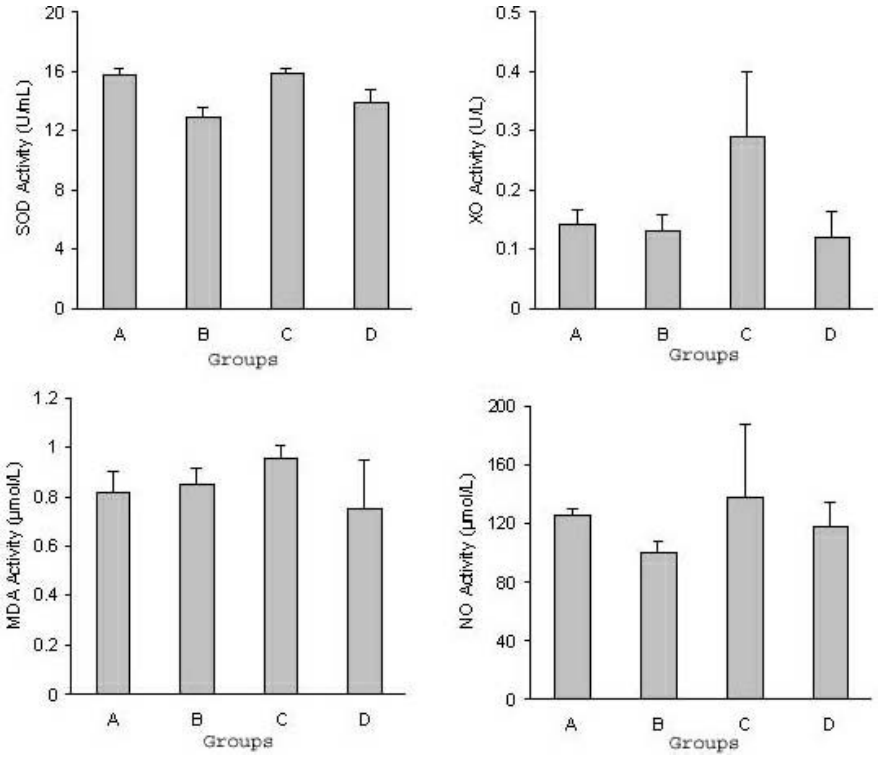

FIGURE 6 - Superoxide dismutase (SOD), xanthine oxidase (XO), malondialdehyde (MDA), and nitric oxide (NO) levels in plasma of the rats treated with cisplatin and/or CAPE. Control group with saline (A); control group with CAPE only (B); rats treated with cisplatin (C); rats treated with cisplatin plus CAPE (D). Bars represent the means of the parameters and the error bars represent standard error of means (SEM).

potent inhibitor of lipoxygenase activity, preventing tissue infiltration of human leukocytes, thus, suppressing inflammation and modulating the immune reaction [12].

High concentration of superoxide and hydrogen peroxide produced by elevated XO activity in the cisplatin group may be responsible for the toxic effects of cisplatin. By measuring the activities of certain enzymes in serum, it is possible to infer the nature of pathological changes in the tissues of the body [7]. All the alterations of enzyme levels or cell activities contribute to the levels or activities of the same enzymes in the extracellular and intravascular regions. Therefore, our findings may consequently show the increased tissue XO activity in the cisplatin group and decreased tissue XO activity in the cisplatin plus CAPE group. However, the blood levels of these enzymes may reflect damage to other organs such as kidney or may indicate damage to red and white blood cells or bone marrow.

CAPE seems to be useful in preventing experimental cisplatin ototoxicity in rats. The authors suggest that CAPE may be beneficial in humans as it has no known harmful side effect on normal cells. We recommend that further studies to be done with different doses of cisplatin and also in longer duration to monitor interferences of CAPE over antitumoral effect of cisplatin. Indeed, these studies will define biochemical aspects of these processes and determine the correct dose of CAPE which is necessary for maximal benefit of CAPE.

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REFERENCES

[1] Campbell KC, Rybak LP, Meech RP, Hughes L. D-methionine provides excellent protection from cisplatin ototoxicity in the rat. Hear Res 1996; 102 (1-2): 90-98.

[2] Fausti S, Henry JA, Schaffer HI, Olson DJ, Frey RH, Bagby GC Jr. High frequency monitoring for early detection of cisplatin ototoxicity. Arch Otolaryngol Head Neck Surg 1993; 119 (6): 661-666.

[3] Schweitzer VG. Cisplatin-induced ototoxicity: the effect of pigmentation and inhibitory agents. Laryngoscope 1993; 103 (4 Pt 2): 1-52.

[4] Ravi R, Somani SM, Rybak LP. Mechanism of cisplatin ototoxicity: antioxidant system. Pharmacol Toxicol 1995; 76 (6): 386-394.

[5] Rybak LP, Whitworth C, Somani S. Application of antioxidants and other agents to prevent cisplatin ototoxicity. Laryngoscope 1999; 109 (11): 1740-1744.

[6] Kopke RD, Liu W, Gabaizadeh R, *et al*. Use of organotypic cultures of Corti's organ to study the protective effects of antioxidants molecules on cisplatin-induced damage of auditory hair cells. Am J Otol 1997; 18 (5): 559-571.

[7] Akyol O, Herken H, Uz E, *et al*. The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance. Prog Neuropsychopharmacol Biol Psychiatry 2002; 26 (5): 995-1005.

[8] Hepsen IF, Bayramlar H, Gultek A, Ozen S, Tilgen F, Evereklioglu C. Caffeic acid phenethyl ester to inhibit posterior capsule opacification in rabbits. J Cataract Refract Surg 1997; 23 (10): 1572-1576.

[9] Grunberger D, Banerjee R, Eisinger K, *et al*. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia 1988; 44 (3): 230-232.

[10] Khayyal MT, el-Ghazaly MA, el-Khatib AS. Mechanisms involved in the antiinflammatory effect of propolis extract. Drugs Exp Clin Res 1993; 19 (5): 197-203.

[11] Hepsen IF, Er H, Cekic O. Topically applied water extract of propolis to suppress corneal neovascularization in rabbits. Ophthalmic Res 1999; 31 (6): 426-431.

[12] Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. FEBS Lett 1993; 329 (1-2): 21-24.

[13] Koltuksuz U, Irmak MK, Karaman A, *et al*. Testicular nitric oxide levels after unilateral testicular torsion/detorsion in rats pretreated with caffeic acid phenethyl ester. Urol. Res 2000; 28 (6): 360-363.

[14] Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990; 36 (8 Pt 1): 1440-1443.

[15] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34 (3): 497-500.

[16] Prajda N, Weber G. Malign transformation-linked imbalance: decreased xanthine oxidase activity in hepatomas. FEBS Lett 1975; 59 (2): 245-249.

[17] Sockalingam R, Freeman S, Cherny TL, Sohmer H. Effect of high-dose cisplatin on auditory brainstem responses and otoacoustic emission in laboratory animals. Am J Otol 2000; 21 (4): 521-527.

[18] Tampakopoulou DA, Sie KC. Cisplatin ototoxicity in developing gerbils. Hear Res 1999; 132 (1-2): 51-59.

[19] Lopez-Gonzalez MA, Guerrero JM, Rojas F, Delgado F. Ototoxicity caused by cisplatin is ameliorated by melatonin and other antioxidants. J Pineal Res 2000; 28 (2): 73-80.

[20] Ozturan O, Jerger J, Lew H, Lynch GR. Monitoring of cisplatin ototoxicity by distortion-product otoacoustic emissions. Auris Nasus Larynx 1996; 23: 147-151.

[21] Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. Eur J Cardiothorac Surg 1999; 16 (4): 458-463.

[22] Irmak MK, Koltuksuz U, Kutlu NO, *et al*. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with alpha-tocopherol in rat kidneys. Urol Res 2001; 29 (3): 190-193.

[23] Uz E, Sogut S, Sahin S, *et al*. The protective role of caffeic acid phenethyl ester (CAPE) on testicular tissue after testicular torsion and detorsion. World J Urol 2002; 20 (4): 264-270.

[24] Sahin S, Sogut S, Ozyurt H, Uz E, Ilhan A, Akyol O. Tissue xanthine oxidase activity and nitric oxide levels after spinal cord ischemia/reperfusion injury in rabbits: comparison of caffeic acid phenethyl ester (CAPE) and methylprednisolone. Neurosci Res Commun 2002; 31: 111-121.