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Investigation of the Effects of White Tea Against Cerebral Cortex Damage Induced by Cisplatin

Investigación de los Efectos del té Blanco Contra el Daño de la Corteza Cerebral Inducido por Cisplatino

Osman Ersagun Batcik[1]; Tolga Mercantepe[2] & Adnan Yilmaz[2]

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SUMMARY: The aim of this study was to examine the potential preventive properties of white tea extract, a potent antioxidant, against cisplatin-induced damage to the cerebral cortex. Thirty-six male Sprague Dawley rats were distributed into healthy control, sham, (only White tea), cisplatin, and cisplatin+White tea groups. After white tea alone was administered per oral at a rate of 1.5 % for twelve days in experimental group rats only a single dose of 7 mg / kg cisplatin (CIS) was be administered intraperitoneally. We observed that oedematous areas in degenerative neurons in the cisplatin administration group. However, we monitored that degenerative neurons and oedematous were decreased in the white tea treatment group compared to the cisplatin group. In addition, we found increased in MDA and Caspase-3 levels in the Cisplatin application group compared to the control group ($p<0.05$). On the contrary, we found decreased in GSH levels in the Cisplatin application group compared to the control group ($p<0.05$). On the other hand, we found decreased in MDA, Caspase-3 levels and increased GSH levels in the Cisplatin application group compared to the control group ($p<0.05$). White tea has shown therapeutic effects on cisplatin-induced cerebral cortex injury via decreased oxidative stress and Caspase-3 expression.

KEY WORDS: Caspase-3; Cisplatin; GSH; MDA; White tea.

INTRODUCTION

Based on data from the World Health Organization, it is projected that a total of 7 million fatalities will occur within 30 years because of cancer, with an additional 17 million individuals expected to succumb to mortality by the year 2030 (Stewart & Wild, 2014). The treatment modalities for cancer exhibit variability based on the specific form of cancer, encompassing distinct stages including surgical intervention, radiation, and chemotherapy (World Health Organization, 2020). Cisplatin, also known as cis-diamminedichloroplatinum (II), was first identified in 1965. It is employed as a chemotherapeutic drug for the management of solid tumors, including bladder, cervix, head and neck, esophagus, small cell lung cancer, and more recently, triple-negative breast cancer (Abd-Elrazek *et al*., 2020). Nevertheless, there have been reports indicating that cisplatin induces harm in multiple tissues, particularly the kidneys (Mendonça *et al*., 2013; Morsy *et al*., 2013). Recent investigations have placed particular emphasis on the neurotoxic effect of the substance (Podratz *et al*., 2020). Cisplatin is believed to impact many signal transduction pathways that contribute to tissue damage, with a primary emphasis on oxidative stress and apoptosis (Ozkok & Edelstein, 2014; Oz *et al*., 2015). While the precise mechanism underlying the harmful effects of cisplatin on tissues remains incompletely understood, existing literature suggests that it induces oxidative stress by promoting the generation of free oxygen radicals through the process of lipid peroxidation (Arany & Safirstein, 2003). The biomarker of lipid peroxidation, known as MDA, is widely recognized and dependable (Tsikas, 2017). Furthermore, GSH, known for its potent ability to scavenge oxidants, is the predominant antioxidant enzyme (Di Meo & Venditti, 2001). Furthermore, the literature has documented that cisplatin induces apoptosis by elevating the amount of caspase-3 in cells (Kumburovic *et al*., 2019).

The antioxidant properties of white tea from *Camellia sinensis* stand out because it has a lot of flavonoids and catechins, especially epigalactocatechingallate (EGCG) (Dias

[1] Department of Brain and Nerve Surgery, Medicine Faculty, Recep Tayyip Erdogan University, Rize,Turkey.
[2] Histology and Embryology and Biochemistry, Medicine Faculty, Recep Tayyip Erdogan University, Rize,Turkey.

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et al., 2016). Recent investigations have demonstrated that white tea could decrease lipid peroxidation and enhance glutathione (GSH) levels (Yıldırım *et al*., 2020). Furthermore, there have been reports indicating that white tea exhibits a reduction in caspase-3 levels (Saral *et al*., 2019).

Our objective in this study was to examine the potential preventive properties of white tea extract, a potent antioxidant, against cisplatin-induced damage to the cerebral cortex.

MATERIAL AND METHOD

Animaly study. This study utilized a sample of 36 Sprague Dawley rats, with weights ranging from 240 to 280 grams and an age range of 3 to 4 months. The breeding process for all animals has adhered to the guidelines outlined in the "Guide to the Care and Use of Laboratory Animals," authored by the National Academy of Sciences and published by the National Institutes of Health. The research was conducted in accordance with the protocol authorized by the Animal Experiments Local Ethics Committee (2018/60) of Recep Tayyip Erdog˘an University.

Rats are randomly composed of four groups:

- Control (saline, Group 1): only 1 ml saline intraperitaonal saline was applied.
- Sham (White Tea Only, Group 2): White tea alone was administered per oral at a rate of 1.5 % for twelve days.
- Cisplatin (7 mg/kg, Group 3): At the end of the 12th day, only a single dose of 7 mg / kg cisplatin (CIS) was administered intraperitoneally.
- Cisplatin + White Tea (Group 4): White tea was administered per oral at a rate of 1.5 % for twelve days. At the end of the 12th day, only a single dose of 7 mg / Kg cisplatin (CIS) was administered intraperitoneally.

B**iochemical Analysis.** Malondialdehyde (MDA) values were checked to determine the lipid peroxidation level in brain tissue homogenates. MDA level was measuredusing Draper and Hadley's double heating technique with the color formed after the reaction was measured spectrophotometrically. MDA levels are given in nmol/mg tissue.

Glutatyon (GSH) values were checked to determine the antioxidant level in brain tissue homogenates. MDA level was measured using Ellman's methods with the color formed after the reaction was measured spectrophotometrically. MDA levels are given in nmol/mg tissue.

Histopathological Analysis. Samples of brain tissue to be removed from rats were fixed in a bouin fixer (SigmaAldrich, St. Louis, MO, USA) for 24 hours. After the fixation process, the samples were subjected to dehydration, clearing and paraffin embedding stages, respectively, using the Shendon citadel 200 (Thermo, Germany) tissue tracking device. Then, samples were blocked with hard paraffin (Merck, Darmstadt, Germany) and sections 3-5 µm thick were cut with a microtome (Leica, RM2125RT, Germany). The sections were stained with hematoxylin (Harris hematoxylin, Merck, Germany) and Eosin (H&E) (Eosin G, Merck, Germany) stains. The staining sections were examined in a light microscope (Olympus BX51, OlympusCorparation, Tokyo, Japan) and the photographs were taken with Olympus DP71 camera (OlympusCorparation, Tokyo, Japan).

Immnuohistochemical Analysis. In our study, Caspase-3 (1: 100, ab4051, Abcam, England), and Goat Anti-Rabbit IgG H&L (HRP) (ab205718) second antibodies were used to identify apoptotic cells in tissues. microtome of the paraffin-fixed brain tissue in 10 % neutral formalin block (Leica RM2125RT, Germany) 1-3 µm sections were mounted on positively charged glass slides (Patolab Biomedical, Turkey). After the deparaffinization process, sections were processed for antigen retrieval in accordance with the instructions for use of primary antibodies. Sections were kept in 3 % H_2O_2 for 12 min, then washed with phosphate buffer (Ph: 7.4, Sigma-Aldrich, Germany) for 15 minutes in the secondary block solution. Following this procedure, the sections were incubated in primary antibody and secondary antibody for one hour each. This was counterstained with Harris hematoxylin (Merck, Darmstadt, Germany) following incubated to diaminobenzidine (DAB).

Semi-quantitative Analysis. The histopathological evaluation of the H&E-stained sections of the brain tissue were calculated in terms of experimental groups by the blinded histologist as shown in Table I.

Semi-quantitative Analysis was performed scoring in 20 different areas randomly determined for each preparation.

Table I. Histopathologic Score (HPS) Methods.

Score	Odematous areas	Score	Necrotic Neurons
0	≤%25	1	≤%25
1	≤%50	2	≤%50
2	>%50	3	>%50

Statistical analysis. All data obtained as a result of the analysis in our study were calculated using the SPSS 18.0 (IBM, Armonk, NJ, USA) statistical software. The data obtained as a result of semi-quantitative analysis were

calculated as median ± 25 % -75 % interquartile range, considering the maximum and minimum values. After analyzing the non-parametric Kruskal Wallis test using the differences between the groups followed by a Tamhane T2 test, the numerical data of the groups were analyzed (P-value <0.05 was chosen as significant).

RESULTS

Biochemical Results

MDA Levels. Only white tea treatments (sham) group MDA levels similar to control group (Table II). On the other hand, MDA tissue values were higher in cisplatin group than in the control group ($p<0.05$). In contrary, in the Cisplatin+White tea treatment group MDA tissues levels decreased than cisplatin application group ($p<0.05$).

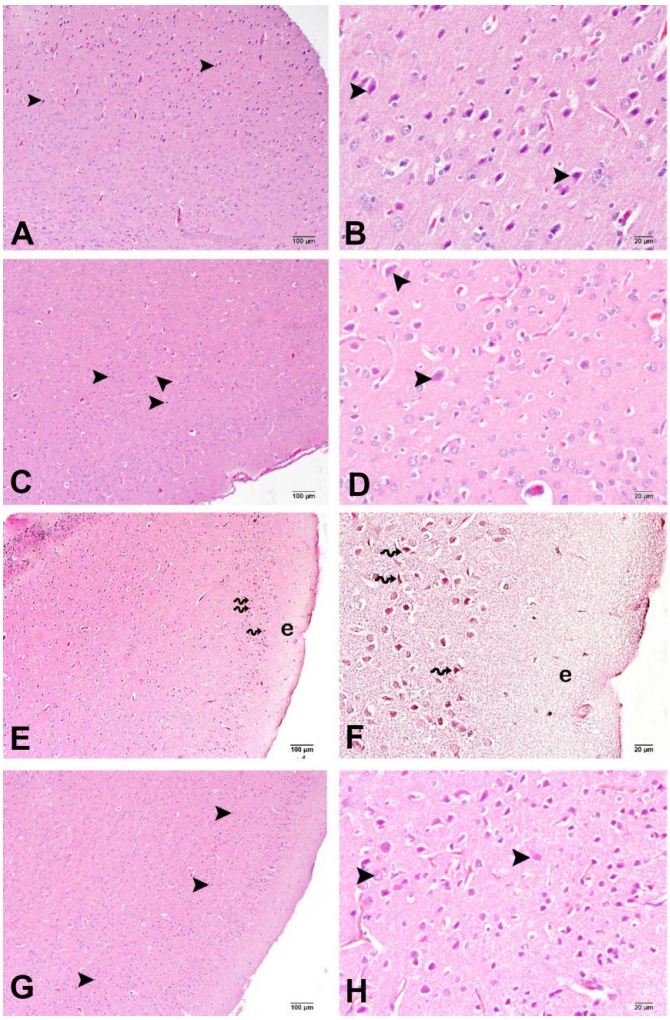

Fig. 1. Representative light microcopy image of cerebral cortex tissue. H&E. A-B Control Group: Healthy morphological structures of the cerebral cortex tissue. C-D Sham Group; Typically morphological structures of the cerebral cortex tissue. E-F Cisplatin Group: Cerebral cortex area demonstrates a great number of degenerative neurons with perineural vacuolizations (spirally arrow). Intense brain edema, prominent in subpia mater region (e). Vascular congestion (tailed arrow). (C, x100 magnification) Trauma+Dex Group; normal findings. G-H Cisplatin+WT Group: We show decreased degenerative neurons. Also, typicaly neuron we see (arrow head).

GSH levels. Only white tea treatments (sham) group GSH levels similar to control group (Table II). But, in the cisplatin application group GSH tissue values were decreased than in the control group ($p<0.05$). However, in the Cisplatin+White tea treatment group GSH tissues levels increased than cisplatin application group ($p<0.05$).

Table II. Biochemical Results (mean±standard deviation).

Group	MDA (nmol/mg tissue)	GSH (nmol/mg tissue)
Control	0.47±0.52	39.48±1.06
Sham (WT)	0.46±0.43	38.87±1.04
Cisplatin	0.98±0.62[a]	19.58±1.91[a]
Cis+WT	0.68±0.57[a,b]	28.65±1.12[a,b]

aP≤0.05 Compared to Control Group, b P≤0.05 Compared to Cisplatin Group, One-Way ANOVA-Tukey HSD

Histopathological Results. We observed that the cerebral cortex was normal in the control group brain tissue sections (Fig. 1a-b). We did not observe any difference between the control group and the white tea administration groups alone (Fig. 1a-d, Table III). On the other hand, we observed oedematous areas in degenerative neurons in the cisplatin administration group (Fig. 1e-f, Table III). However, we monitored that degenerative neurons and oedematous were decreased in the white tea treatment group compared to the cisplatin group (Fig. 1g-h, Table III).

Table III. Histopathological Scoring (HPS) (median-25%-75% IQR).

Groups	Edema	Degenerative Neurons	HPS
Control	0.00(0-0)	0.00(0-1)	0.00(0-1)
Sham (WT)	0.00(0-0)	0.00(0-1)	0.00(0-1)
Cis	2.00(2-3)[a]	2.00(2-3)[a]	4.00(2-4)[a]
Cis+WT	0.50 (0-1)[b]	1.00(0-1)[c]	1.50(0-2)[d]

Immunohistochemical Analysis. When sections of brain tissue incubated with Cleaved Caspase-3 primary antibody were examined under a light microscope; we found that the number of neurons that showed cerebral cortex cleaved caspase-3 positivity in the cisplatin group increased significantly compared to the control group (Fig. 2e-f, Tables IV and V). On the other hand, we demonstrated that cleaved caspase-3 positive neurons decreased in the white tea treatment group compared to the cisplatin application group (Fig. 2e-f, Tables IV and V).

Table IV. Immunohistochemical stainings and Claeved Caspase-3 positivity score.

Score	Positivity
0	None (< %5)
1	Mild (< %25'ten az)
2	Moderate (%25-50)
3	Severe (%51-75)
4	Very Severe (> %75)

Table V. Scoring of immunohistochemical stainings with incubed Cleaved Caspase-3 primary antibody (median-25%-75% IQR).

Group	Cleaved Caspase-3 posivitiy score
Control	0.00(0-0)
Sham (WT)	0.00(0-0)
Cis	3.00(2-3)[a]
Cis+WT	1.50(0-2)[b,c]

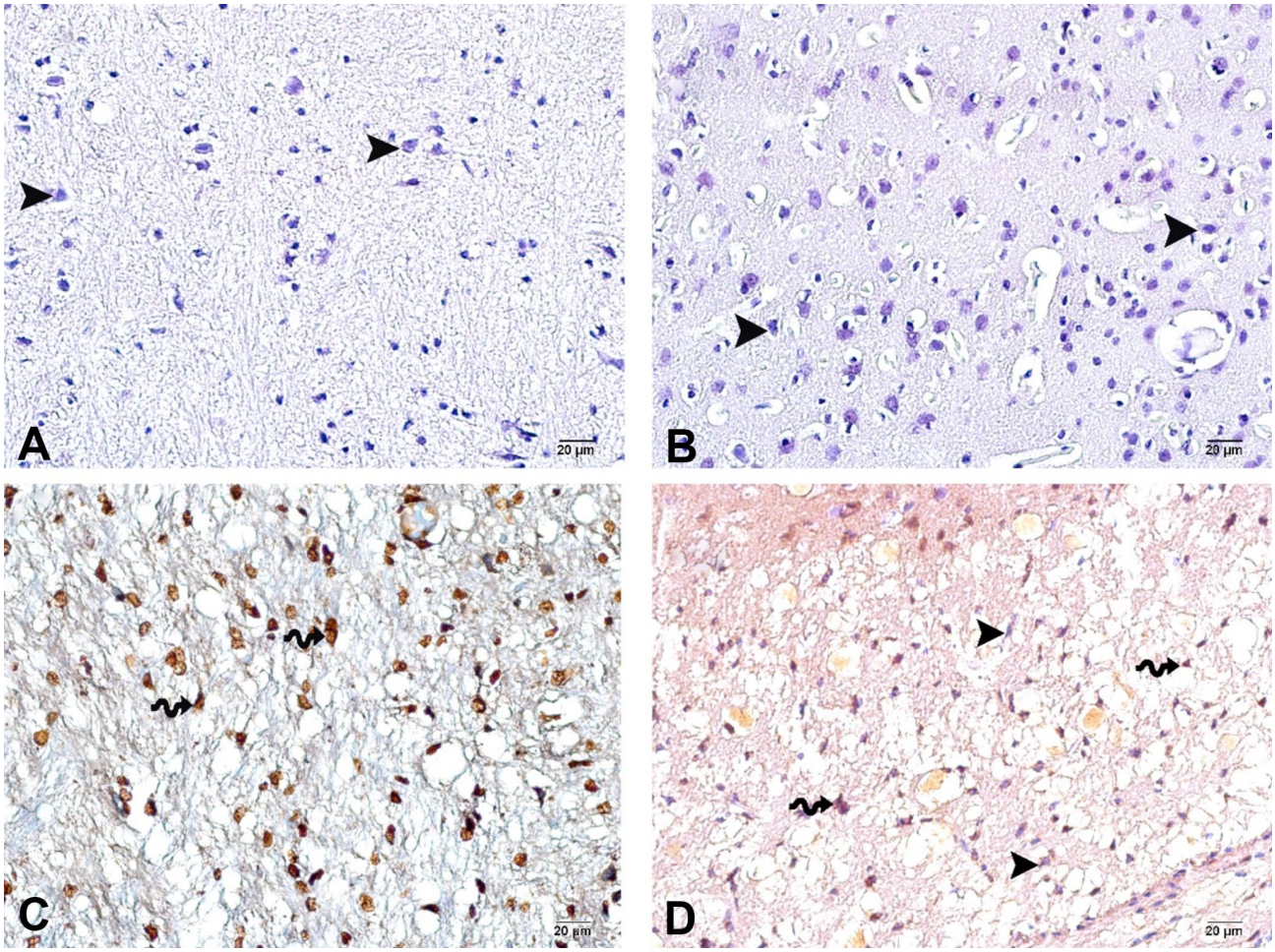

Fig. 2. Representative photomicrographs of the Cleaved Caspase-3 stained sections from the brain tissue by light microscopy. A Control Group-B Sham Group; Caspase-3-negative neurons (arrow head) and oligodendrocytes (blue arrowhead). (B, x400 magnification). C-Trauma Group; Cerebral cortex area demonstrating a great number of apoptotic neurons (spirally arrow) D-Cisplatin+WT Group; Typical neurons (arrow head) in addition to a smaller number of apoptotic nuerons (spirally arrow) were observed.

DISCUSSION

Several studies have documented that the injection of cisplatin leads to degeneration in the tissue of the cerebral cortex and the development of edema in the brain tissue (Cheng *et al*., 2017; Chiang *et al*., 2019; Podratz *et al*., 2020). In our work, we observed the presence of edematous regions along with degeneration in diffuse neurons, particularly in the cerebral cortex's molecular and outer pyramidal layers, after the administration of cisplatin. Furthermore, Chen *et al*. (2019) observed that cisplatin exhibited a reduction in neuronal density within the hippocampus and induced inflammatory responses. Conversely, our investigation did not detect any signs of inflammation in comparison to the experiments

conducted by Chen *et al*. (2019). It is hypothesized that the termination of our investigation occurred during the acute phase prior to the onset of inflammation after the injection of cisplatin. While the precise mechanism underlying the harm of cisplatin in brain tissue remains unclear, previous studies have indicated that cisplatin induces an elevation in free oxygen radicals and subsequently leads to an increase in lipid peroxidation levels (Kumburovic *et al*., 2019). Additionally, it has been reported in studies that oxidative stress has the ability to trigger apoptosis in neurons (Kumburovic *et al*., 2019). According to the findings of Kumburovic *et al*. (2019), cisplatin induces apoptosis in neurons through the upregulation of caspase-3 expression. Similarly, our investigation revealed that cisplatin enhanced the expression of active caspase-3 in neurons located in the cerebral cortex.

Despite the current literature showing the occurrence of cisplatin-induced brain toxicity, the molecular mechanism of cisplatin-related brain damage has not been completely clarified (John *et al*., 2017; Salem *et al*., 2019; Samaha *et al*., 2019). On the other hand, the most widely studied and strongly emphasized mechanism is associated with oxidative stress and increased apoptosis (Salem *et al*., 2012). Indeed, cisplatin increases ROS via the breaks it causes in the DNA (Salem *et al*., 2019). Similarly, in our study, we observed that cisplatin caused damage to the brain tissue by increasing the MDA level and decreasing the GSH level.

Recent studies have shown that white tea, which contains high levels of flavonoids and catechins, reduces the presence of free oxygen radicals and the expression of caspase-3 (Bernatoniene & Kopustinskiene, 2018). Even though there has not been any research specifically looking into how white tea might protect brain tissue, what has been written suggests that white tea may be able to reduce oxidative stress by effectively getting rid of free oxygen radicals caused by cisplatin in many organs (Bernatoniene & Kopustinskiene, 2018). Saral *et al*. (2019) conducted experiments that provided evidence for the inhibitory effect of white tea on apoptosis by reducing caspase-3 expression produced by cisplatin. Similarly, the current investigation revealed that cisplatin reduced the presence of activated caspase-3 in neurons located in the cerebral cortex. Similarly, in our study, we observed that white tea reduced histopathological changes in brain tissue by decreasing the MDA level and increasing the GSH level.

This research employs a plot-based approach and is subject to several limitations. This study exclusively employed cleaved caspase-3 as a biomarker for assessing apoptosis. Our research necessitates the inclusion of studies that investigate mitochondrial calcium levels and other apoptotic chemicals in order to provide support.

According to the aforementioned findings, white tea extract has a neuroprotective effect on cisplatin-induced cerebral cortex injury. This effect is achieved through the mitigation of oxidative stress and apoptosis in neurons. Additional research is necessary to elucidate the mechanisms underlying the neuroprotective function of white tea.

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RESUMEN: El objetivo de esta investigación fue estudiar las posibles propiedades preventivas del extracto de té blanco, un potente antioxidante, contra el daño a la corteza cerebral inducido por el cisplatino. Treinta y seis ratas macho Sprague Dawley se distribuyeron en grupos control sano, simulado (solo té blanco), cisplatino y cisplatino + té blanco. Después de que se administró el té blanco solo por vía oral a una tasa del 1,5 % durante doce días, en ratas del grupo experimental solo se administraró una dosis única de 7 mg/kg de cisplatino (CIS) por vía intraperitoneal. Observamos áreas edematosas en neuronas degenerativas en el grupo de administración de cisplatino. Sin embargo, observamos que las neuronas degenerativas y edematosas disminuyeran en el grupo con tratamiento con té blanco en comparación con el grupo de cisplatino. Además, se observó un aumento en los niveles de MDA y Caspasa-3 en el grupo de aplicación de cisplatino en comparación con el grupo control ($p<0,05$). Por el contrario, encontramos una disminución en los niveles de GSH en el grupo con aplicación de cisplatino en comparación con el grupo control ($p<0,05$). Por otro lado, encontramos una disminución en los niveles de MDA, Caspasa-3 y un aumento de los niveles de GSH en el grupo con aplicación de cisplatino en comparación con el grupo control ($p<0,05$). El té blanco ha demostrado efectos terapéuticos sobre la lesión de la corteza cerebral inducida por cisplatino mediante la disminución del estrés oxidativo y la expresión de caspasa-3.

PALABRAS CLAVE: Caspasa-3; cisplatino; GSH; MDA; Té blanco.

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Corresponding Author:
Osman Ersegun, Assoc. Prof.
Recep Tayyip Erdogan University
Faculty of Medicine
Department of Neurosurgery
53100, Rize
TURKEY

E-mail: osmanersegun@gmail.com