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Research Article

Telfairia occidentalis Leaf Extract Protects the Cerebellar Cortex against Cisplatin-Induced Oxidative Damage in Wistar Rat

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Abstract
Cisplatin therapeutic use is limited to its severe nephrotoxicity, hepatotoxicity and neurotoxicity by the generation of free radicals resulting in oxidative stress. This study evaluates the protective effects of *Telfairia occidentalis* (TO) leave extract against cisplatin-induced oxidative stress in the cerebellar cortex of Wistar rat. Forty adult male Wistar rats weighing between 160 g and 180 g were divided into 5 groups of 8 animals each. Group I rats received water and served as the control group. Group II rats received 2.5 mg/kg intraperitoneal (i.p.) injection of cisplatin for 2 weeks (twice weekly), group III rats received a combination of cisplatin and TO for 2 weeks (twice weekly), group IV rats received 400 mg/kg body weight of TO orally for 2 weeks (twice weekly), while group V rats received a combination of cisplatin and 500 mg/kg vitamin E orally for 2 weeks. Twenty-four hours after the last administration, the animals in all the groups were weighed done and killed. Their brains were dissected, weighed and some preserved for biochemical analysis (oxidative stress). The cerebella were fixed in 10% formol saline for histological and immunohistochemical studies. A decreased body weight, non-significant increased lipid peroxidation, decreased levels of catalase, as well as decreased Purkinje cell density and increased astrocyte population was observed in cisplatin-treated rats. Co-administration of TO and vitamin E with cisplatin, improved the changes observed above. In conclusion, 10 mg/kg body weight cisplatin administered intraperitoneally to adult male Wistar rats, induced mild oxidative damage in the cerebellum. Co-administration of 400 mg/kg body weight aqueous extract of TO, decreased the rate of oxidative damage induced by cisplatin.

Key Words: Oxidative stress, cisplatin, neurotoxicity, cerebellum, *Telfairia occidentalis*

INTRODUCTION

Cisplatin, a platinum-based antineoplastic agent, is one of the most effective chemotherapeutic drugs used in the treatment of solid tumors, especially of the testis, colon, head and neck, ovary and bladder (Shah and Dizon, 2009; Zhang *et al*., 2010). However, nephrotoxicity, hepatotoxicity, neurotoxicity and ototoxicity have been reported to be some of the adverse side effects upon the use of cisplatin (Ek Born *et al*., 2003; Iraz *et al*., 2005; Yao *et al*., 2007; Podratz *et al*., 2011). A major clinical issue affecting 10–40% of patients treated with cisplatin or oxaliplatin is severe peripheral neuropathy causing sensory, motor, and autonomic dysfunction, with symptoms including cold sensitivity and neuropathic pain (Podratz *et al*., 2011). The biochemical basis of cisplatin neurotoxicity is uncertain, but the generation of free radicals resulting in oxidative stress has been implicated (Altun *et al*., 2010). Cisplatin appears to affect the axons, myelin sheath, neuronal cell body and the glial structures of the neurons (Stillman and Cata, 2006), interferes with DNA replication and metabolic function of the neurons (Dunlap and Paice, 2006). McKeage *et al*. (2001) reported that cisplatin has poor penetration through the blood-brain barrier (BBB), however, the amount of cisplatin that crosses the BBB is sufficient to induce neurotoxicity. Cisplatin has been reported to produce about three times more platinum-DNA adducts in the dorsal root ganglion than equimolar doses of oxaliplatin, consistent with clinical observations that cisplatin is associated with greater neurotoxicity (Ta *et al*., 2006).

Several agents have been used in an attempt to ameliorate or prevent cisplatin neurotoxicity (Alber *et al*., 2001). The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth. Herbal medicine was practiced by people in Africa, Asia, Europe and the Americas (Wargovich *et al*., 2001). The medicinal plant, Telfairia occidentalis (TO), a fluted pumpkin, darkish-green leafy vegetable popularly called 'Ugwu' in Igbo (Nigeria), is used in soup and herbal preparations for the management of diseases in Nigeria. Studies have shown that TO is very rich in flavonoids, phenolic compounds and iron, as such, possesses antioxidant, antihepatic, anticancer, antidiabetic, anti-inflammatory and neuroprotective properties (Aderibigbe *et al*., 1999; Oboh, 2005; Kayode *et al*., 2010; Adejuwon *et al*., 2014). As a result of the health benefits of TO, this study was designed to evaluate the protective effect of aqueous extract of TO on cisplatin-induced oxidative stress in rat cerebellum.

MATERIALS AND METHODS

Plant materials

The leaves of *Telfairia occidentalis* used in this study were harvested from a farm around Awolowo Hall of the University of Ibadan, Oyo State in January, 2017. The plant taxonomical

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identification and authentication of these leaves was done in the Department of Botany, University of Ibadan, with a voucher number UIH- 22661.

Aqueous extraction of *Telfairia occidentalis*

The leaves were oven-dried and then blended into powder with a milling machine in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. One thousand five hundred grammes (1500 g) of dried leaves of *Telfairia occidentalis* was weighed, granulated into powder, soaked in 7.5 litres of distilled water for 48 hours and mixed thoroughly. The mixture was filtered using Teflon® filter paper. The solvent in the filtrate was removed using a Rotatory evaporator (Bibby Sterling®, Germany) leaving only the soluble organic matter. The percentage yield of the aqueous extract of *Telfairia occidentalis* was 16.67% (250 g).

Phytochemical screening of the leaf extracts of *Telfairia occidentalis*

Phytochemical screening of the leaves of *Telfairia occidentalis* was done in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. The following compounds as described by Trease and Evans (2002) were screened for; alkaloids, flavonoids, anthraquinone, saponins, tannins and cardinolides.

Animals

Forty healthy male adult rats of Wistar strain, weighing between 180 and 200 g and obtained from the Central Animal House of the Faculty of Basic Medical Sciences, University of Ibadan were used for the study. The rats were weighed and divided into five groups of eight animals per group. The animals were maintained in wire mesh cages, under hygienic, freely ventilated and naturally illuminated animal house of the Department of Veterinary Physiology, University of Ibadan. All animals received human care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals (prepared by the National Academy of Science and published by the National Institutes of Health).

Grouping of animals

Group I: Rats received distilled water orally for 14 days and served as the control

Group II: Rats received 2.5 mg/kg body weight cisplatin intraperitoneally (i.p.) twice weekly for 14 days.

Group III: Rats received 400 mg/kg extract of TO orally for 14 days + 2.5 mg/kg cisplatin i.p. twice weekly for 14 days.

Group 1V: Rats received 400 mg/kg extract of TO orally for 14 days.

Group V: Rats received 2.5 mg/kg cisplatin i.p. twice weekly for 14 days + 500 mg/kg vitamin E orally for 14 days.

At the end of the administration, the rats were weighed done and killed 24 hours after the last administration. The brain of the rats was dissected out. While, some cerebella preserved in phosphate buffered saline (PBS) at a pH 7.4 and temperature of 4^{o}C for biochemical (oxidative stress) analysis, others were fixed in 10% formol-saline for histological, histomorphometric and immunohistochemical studies.

Body weight: Body weight of the animals using a Swiss microwa balance (type 7720).

Biochemical analysis: The cerebella of the control and experimental rats were homogenized in eight volumes of 50 mM of Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000 ×*g* for 15 minutes at 4 ^{o}C. The supernatant was collected for biochemical assays of the following markers;

Lipid peroxidation (LPO), quantified as malondialdehyde (MDA) employing the method described by Farombi *et al*., (2000) and expressed as micromoles MDA/mg tissue (µmol/mg).

Reduced glutathione (GSH) was determined according to Jollow *et al*. (1974) and expressed as µg/ml/mg tissue.

Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) and expressed as unit/mg tissue.

Catalase activity was determined by the method of Beutler *et al* (1963) and expressed as unit/mg tissue.

Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al*. (1973) and expressed as µg/mg tissue.

Histological and Histomorphometric studies: Cerebellar tissues were processed employing routine paraffin embedding techniques and stained with Haematoxylin and Eosin (H and E) for histological and histomrophometric evaluations.

Immunohistochemistry: Cerebellar tissues were immunostained with Glial fibrillary acidic protein (GFAP) for astrocyte population (neuroglia) using the Avidin biotin immunoperoxidase method. Briefly, cut formalin-fixed paraffin sections were treated with 3% hydrogen peroxide (H_2O_2) for 15 min, to block endogenous peroxidase. Then, washed in phosphate buffered saline (PBS) and treated with GFAP primary antibody (GFAP, mouse monoclonal antibody 1:100 dilution, Leica Biosystems Inc. Ilinois, USA) at room temperature for 60 min. The sections were washed in 3 changes of PBS for 5 min each, incubated with horseradish peroxidase (HRP) secondary biotinlated anti-mouse antibodies and washed in 3 changes of PBS for 5 mins. The sections were then incubated with diaminobenzidine (DAB) for 3 to 5 min and counterstained with Haematoxylin solution for 2 min and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in DPX. Images were captured from the cerebellar cortex with a 500-pixel Leica binocular microscope.

Measurement of gross and microscopic parameters: The gross parameters measured and instruments used include: Body weight and percentage weight gain/loss of animals in all groups. Mettler analytical balance

The microscopic parameters measured and the methods used include:

i. Morphological changes in cerebellar cortex stained with H & E, using with a 500 pixel Leica binocular microscope.
ii. Histomorphometry: Thickness of the molecular layer (ML) and the density of the Purkinje cells (Pc) of cerebellar cortex using the software Motic software 2.0.
iii. Immunohistochemistry of the cerebellar cortex using, Glial fibrillary acid Protein (GFAP) for astrocyte population.

Statistical Analysis

The data obtained were further analyses employing a one-way ANOVA, followed by Tukey Post-hoc for multiple comparisons using GraphPad prism 6.0 version and expressed as mean ± SEM, with the level of significance at $p< 0.05$

RESULTS

Phytochemical screening: Phytochemical studies carried out on the leaves of *Telfairia occidentalis* showed that it contained large amount of flavonoids, small amount of saponins, tannins and cardinolides. While, alkaloids and anthraquinones was absent.

Body weight: A decreased weight gain was observed in cisplatin-treated rats compared with the control at $p<0.05$. Administration of TO and VE to cisplatin-treated rats, improved the % weight gain of the rats (Table 2).

Biochemical evaluation: A non-significant increase in LPO and decrease in catalase, SOD and GPx was seen in the cisplatin-treated rats compared with the control, TO and VE group at $p>0.05$ (Table 3).

Histology and Histomorphometry: The cerebellar cortex of all the groups studied showed a three-layered cytoarchitecture, namely molecular, Purkinje and granule layers (Plate 1). The ML thickness of the control and treated groups was not significantly different at $p>0.05$. A decreased Pc density and increased astrocyte population was seen in the cisplatin-treated rats compared with the control and TO groups at $p<0.05$ (Table 4, Plates 1 and 2).

Table 1:
Phytochemical consttituent of *Telfairia occidentalis* leaf extracts

Phytochemicals	Composition
Alkaloids	-
Flavonoids	+++
Anthraquinone	-
Saponins	+
Tannins	+
Cardinolides	+

+++ = present in large quantity
+ = present in trace or small amount ; – = absent

DISCUSSION

Cisplatin is a potent antitumor drug against various types of malignant tumors. In spite of the chemotherapeutic effectiveness of cisplatin, its use has been limited in the clinic as a result of its adverse side effects, including vomiting, nausea, digestive disorders, anorexia, cachexia, nephrotoxicity, neurotoxicity, ototoxicity and hepatotoxicity (Arany and Safirstein, 2003; Agustsson *et al*. 2007; Rydén *et al*. 2008; Podratz *et al*. 2011). In this study, cisplatin-treated rats showed significantly decreased body weight compared with the control and other treated rats. The mechanism for the decreased body weight is not clear but cisplatin has been shown to increase lipolysis and decrease lipogenesis as a result of reduction of food intake (Jeevanandam *et al*., 1986; Rydén *et al*., 2008). Such reduction of the body weight in cisplatin-treated rats may be due to gastrointestinal toxicity and concomitant loss of the animal appetite with subsequent reduction of food ingestion (Atessahin *et al*., 2005).

This finding is consistent with the reports of Garcia *et al*. (2013), that cisplatin induced lipid catabolism and decreased body weight in male mice. Co-administration of TO with cisplatin improved the body weight of the rats compared with the control. TO leaves are important sources of nutrients especially, vitamins and minerals, and also contain adequate amounts of proteins but low in fiber (Ladeji *et al*., 1995). These nutrients may increase apatite, which increases food intake and improves body weight. This corroborates previous studies which showed that TO is associated with weight gain because of its high protein and vitamin content (Fasuyi, 2006).

Cisplatin has been reported to induce neurotoxicity by generation of reacting oxygen species (ROS), resulting in oxidative stress. The oxidative stress can cause cell damage when losing the imbalance between ROS production and antioxidant defense (Blokhina *et al*., 2003) and has been implicated in a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), cancer, aging, memory loss and depression (Chatterjee *et al*., 2007).

Table 2:
Mean body weight (g) and percentage weight gain/loss (%) of the control and treated rats

Group	Initial body Weight (g)	Final body Weight (g)	Weight gain/ loss (g)	% weight gain/ loss
Control	167.63±6.23	178.75±5.90	11.12±1.46	6.65±0.97
Cisplatin	179.63±8.40	186.63±8.67	7.00±1.93a	3.90±1.12a
Cisplatin+TO	174.75±7.00	183.50±6.12	9.75±1.91	5.04±1.24
TO	177.13±8.08	186.63±8.30	9.50±2.98	5.38±1.72
Cisplatin+VE	172.63±8.08	182.25±8.30	9.63±1.51	5.57±0.81

Table 3:
Biochemical evaluation of the control and treated rats' cerebellum

Group	LPO µmol/mg	GSH µg/ml/mg	Unit/mg Catalase	SOD Unit/mg	GPx µg /mg
Control	0.01±0.00	0.11±0.02	0.70±0.38	1.45±0.39	4.15±0.11
Cisplatin	0.02±0.00	0.11±0.02	0.57±0.34	1.11±0.05	3.90±0.26
Cisplatin+TO	0.01±0.00	0.10±0.03	0.71±0.19	1.11±0.12	3.95±0.30
TO	0.01±0.00	0.13±0.03	0.60±0.19	1.20±0.20	4.23±0.23
Cisplatin+VE	0.01±0.00	0.10±0.05	0.60±0.27	1.41±0.30	3.95±0.13

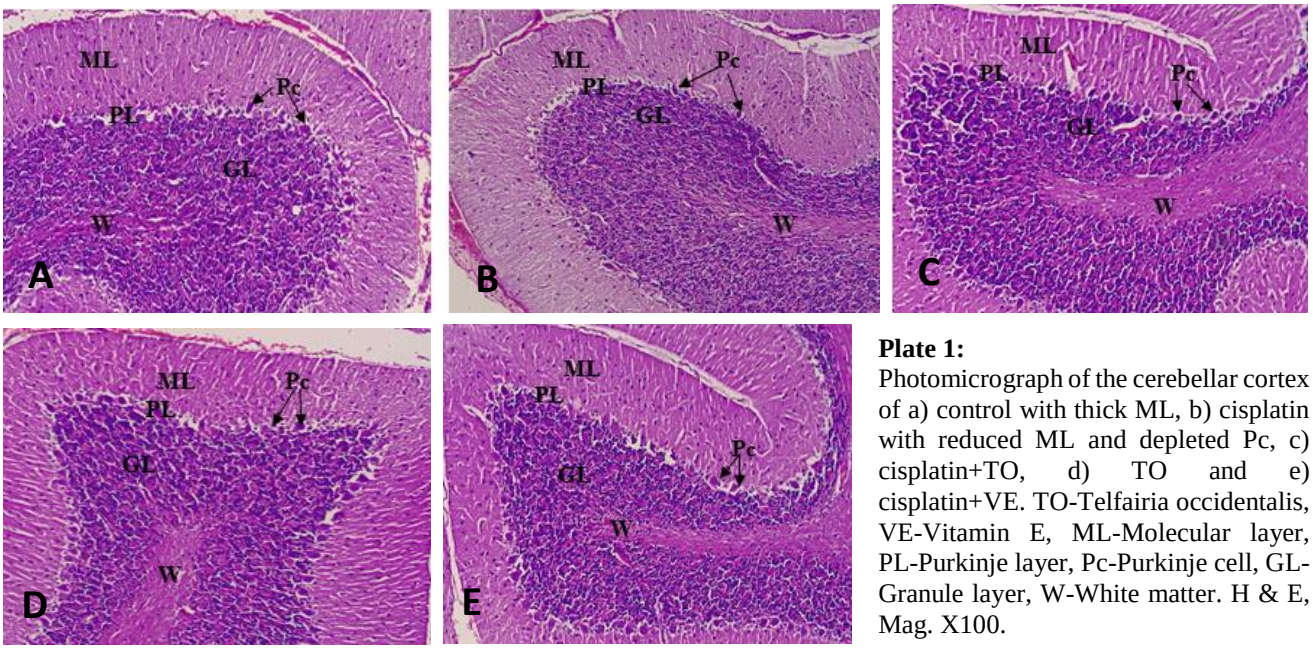

Plate 1:
Photomicrograph of the cerebellar cortex of a) control with thick ML, b) cisplatin with reduced ML and depleted Pc, c) cisplatin+TO, d) TO and e) cisplatin+VE. TO-Telfairia occidentalis, VE-Vitamin E, ML-Molecular layer, PL-Purkinje layer, Pc-Purkinje cell, GL-Granule layer, W-White matter. H & E, Mag. X100.

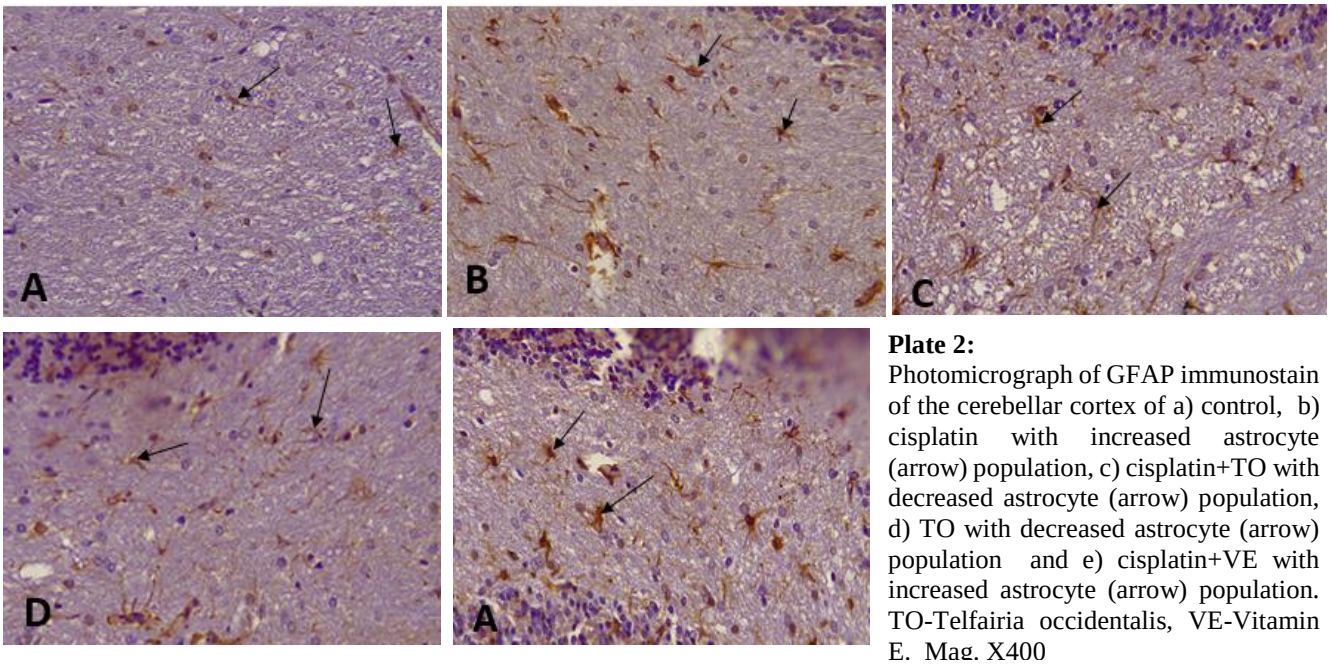

Plate 2:
Photomicrograph of GFAP immunostain of the cerebellar cortex of a) control, b) cisplatin with increased astrocyte (arrow) population, c) cisplatin+TO with decreased astrocyte (arrow) population, d) TO with decreased astrocyte (arrow) population and e) cisplatin+VE with increased astrocyte (arrow) population. TO-Telfairia occidentalis, VE-Vitamin E. Mag. X400

Table 4:
Mean thickness of the molecular layer (mm), Purkinje cell density and astrocyte population of the control and treated rat cerebellar cortex

Group	Thickness of Molecular layer (mm) n = 10	Purkinje cell density/ 1.3mm^2 n=10	Astrocyte population /1.3mm^2 n=10
Control	0.28±0.05	39.5±4.90	12.2±2.49
Cisplatin	0.25±0.05	32.2±4.51^a	19.2±3.27^a
Cisplatin+TO	0.26±0.04	34.7±3.89	16.4±3.58
TO	0.27±0.04	34.4±2.91	13.4±3.13^b
Cisplatin+VE	0.26±0.04	35.7±4.76	14.2±1.79

The results of the present study showed elevated levels of malondialdehyde (MDA), an indication of lipid peroxidation and decreased level of catalase. El-Beshbishy *et al.* (2011) reported that cisplatin produces oxidative stress through reduction of plasma antioxidant enzymes levels such as catalase, glutathione peroxidase and superoxide dismutase leading to a failure of the antioxidant defense against free radical damage generated by antitumor drugs. Co-administration of TO with cisplatin decreased the rate of lipid peroxidation and other oxidative stress markers studied. The mechanism for TO-decreased rate of lipid peroxidation may be due to its strong antioxidant property as it contains flavonoids and phenolic compounds which help in scavenge oxygen free radicals, thereby preventing oxidative stress. The antioxidative, anticancer and antidiabetic activities of TO are well documented (Eseyin *et al*., 2014; Adejuwon *et al*., 2014).

The nervous system is frequently the site of symptomatic toxicity of antineoplastic agents, with the cerebellar cortex and spinal cord motor neurons being considered as target areas of

cisplatin neurotoxicity (Abou-Elghait *et al*., 2010). Histologically, the present study showed a three-layered (molecular, Purkinje and Granule layers) cytoarchitecture of the cerebellar cortex in all the groups and no change in the thickness of the molecular layer. However, there was significant loss of Purkinje cells in the cisplatin-treated rats compared with the control group. The mechanism involved in the loss of Purkinje cells is not completely clear but cisplatin has been reported to act on the DNA causing its damage (Driessens *et al*., 2003), able to generate ROS and it inhibits the activity of antioxidant enzymes in some tissues (Dillioglugil *et al*., 2005). Co-administration of TO and vitamin E with cisplatin increased the density of the Pc count, probably by its free radical scavenging activity and preventing further oxidative damage of the Pc.

In the central nervous system (CNS), astrocytes express GFAP which is important in many CNS processes, including cell communication and the functioning of the blood brain barrier, formation of glial scars and repair after CNS injury (Venkatesh *et al*., 2013; Paetau *et al*., 1985). Glial fibrillary acidic protein expression can be regarded as a sensitive and reliable immunohistochemical marker that labels most, if not all, reactive astrocytes that are responding to CNS injuries (Sofroniew and Vinters, 2010). In this study, increased expression of GFAP was seen in the cisplatin-treated rats compared with the control and other treated rats, which was indicative of astrocytic response to cisplatin injury (increased astrocyte activation), resulting in astrogliosis. The mechanism involved in this over expression of GFAP is unclear, however, cisplatin has been reported to affect neuronal cell body, glial structures of the neurons (Stillman and Cata, 2006), interferes with DNA replication and metabolic function of the neurons (Dunlap and Paice, 2006), thus increasing GFAP expression. Overexpressed glial cells have been shown to secrete a variety of factors with pro-inflammatory and neurotoxic properties including cytokines such as TNFα, fatty acid metabolites, and free radicals such as nitric oxide (NO) (Raivich *et al*. 1998). Co-administration of TO with cisplatin decreased the overexpression of GFAP probably by mopping up free radicals and repair of DNA damage.

In conclusion, Cisplatin caused decreased body weight, mild increase in lipid peroxidation, depletion of Pc and increased astrocyte population in the cerebellum of rats. Administration of TO and vitamin E, ameliorated the neurotoxicity of cisplatin, as such may be neuroprotective. The results of this study may be beneficial considering the increased side effects of cisplatin in the brain and the consumption of TO leaves which is safe, well-tolerated, available, affordable and containing large amount of flavonoid capable of protecting the brain against cisplatin neurotoxicity. However, more studies should be done to ascertain the exact mechanism and site of action, and the interaction of TO leave extract with cisplatin.

Conflict of interest statement

The authors declared that they have no conflicts of interest

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