Journal of Dietary Supplements

ISSN: 1939-0211 (Print) 1939-022X (Online) Journal homepage: http://www.tandfonline.com/loi/ijds20

Gallic Acid Ameliorates Cyclophosphamide-Induced Neurotoxicity in Wistar Rats Through Free Radical Scavenging Activity and Improvement in Antioxidant Defense System

Ademola Adetokunbo Oyagbemi, Temidayo Olutayo Omobowale, Adebowale Bernard Saba, Ebunoluwa Racheal Olowu, Racheal Omolola Dada & Akinleye Stephen Akinrinde

Journal of Dietary Supplements, Early Online:1–18, 2016

DOI: 10.3109/19390211.2015.1103827

Taylor & Francis
Taylor & Francis Group

ARTICLE

Gallic Acid Ameliorates Cyclophosphamide-Induced Neurotoxicity in Wistar Rats Through Free Radical Scavenging Activity and Improvement in Antioxidant Defense System

Ademola Adetokunbo Oyagbemi[1], Temidayo Olutayo Omobowale[2], Adebowale Bernard Saba[1], Ebunoluwa Racheal Olowu[1], Racheal Omolola Dada[1], & Akinleye Stephen Akinrinde[1]

[1]Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria, [2]Faculty of Veterinary Medicine, Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT. Cyclophosphamide (CPA) is a widely used anticancer chemotherapeutic agent and its toxicity has been associated with its toxic metabolites phosphormide mustard. Therefore, the ameliorative effect of Gallic acid against neurotoxicity was examined in this study. Sixty rats were grouped into 10 rats per group. Group 1 received saline orally. Group 2 received CPA at 100 mg/kg single dose intraperitoneally on day 1. Groups 3 and 4 were treated with Gallic acid (GA) at 60 and 120 mg/kg body weight only for 10 days and also received a single dose of CPA (100 mg/kg) intraperitoneally on day 1, respectively. Rats in groups 5 and 6 received GA at 60 and 120 mg/kg body weight only for 10 days. Groups 3, 4, 5, and 6 received GA orally. The cerebellar and cerebral malondialdehyde (MDA) contents and hydrogen peroxide generation were significantly ($p < .05$) elevated. The cerebellar and cerebral catalase (CAT), superoxide dismutase and glutathione-S-transferase (GST) activities were significantly ($p < .05$) reduced in CPA treated group. The activity of glutathione peroxidase (GPx) was significantly increased in rats that were treatment with CPA. Also, nitrite content was significantly elevated in the brain of rats that received the toxic dose of CPA. All these findings suggest that treatment with GA (60 and 120 mg/kg) ameliorated the neurotoxicity induced by CPA via reduction of oxidative stress and increase in antioxidant defense system. Combining all, chemotherapeutic agents with structure/function similar to GA could be of potential benefit to the pharmaceutical industries as an adjuvant in chemotherapy with little or no side effects.

KEYWORDS. antioxidant, cyclophosphamide, gallic acid, neurotoxicity, oxidative stress

Address correspondence to: Dr. A.A. Oyagbemi, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. (Email: aa.oyagbemi@ui.edu.ng)
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ijds

INTRODUCTION

Cyclophosphamide (CPA) is a widely used anticancer chemotherapeutic agent and its toxicity has been associated with its toxic metabolites phosphormide mustard which are responsible for the avalanche of undesirable side effects (Asiri, 2010). The cardiotoxic effects of CPA have also been reported (Viswanatha Swamy et al., 2013; Nagi, Al-Shabanah, Hafez, Sayed-Ahmed, 2011). CPA-induced testicular lipid peroxidation and apoptosis have been documented (Türk, Ceribaşi, Sakin, Sönmez, & Ateşşahin, 2010).

In addition, the use of CPA as an effective antineoplastic agent is often restricted because of its wide-ranging adverse side-effects including nausea, vomiting, alopecia, hemopoetic suppression, nephrotoxicity, hepatotoxicity, and urotoxicity (bladder weight, edema, and hemorrhage as well as increased urinary bladder epithelial cell apoptosis).

Other side effects of CPA have also been documented including cardiotoxicity, immunotoxicity, mutagenicity, genotoxicity, carcinogenicity, and teratogenicity (Roy, Chakraborty, & Bhattacharya, 2014; Kim et al., 2014; Song et al., 2014; Chabra, Shokrzadeh, Naghshvar, Salehi, & Ahmadi, 2014; Yuan et al., 2014; Tasdemir et al., 2013; Saba et al., 2013; Farshid, Tamaddonfard, Ranjbar, 2013; Nitharwal, Patel, Karchuli, Ugale, 2013).

Gallic acid (3, 4, 5-trihydroxybenzoic acid) has been found as one of the most important polyphenolic compounds in plants (Eslami, Pasanphan, Wagner, Buettner, 2010). Gallic acid and its derivatives have been considered as the natural recipe present in polyphenolic compounds in blackberry, raspberry, mango, areca nut, bearberry, and walnut (Tachibana, Koga, Fujimura, & Yamada, 2004). Furthermore, Li et al. (2005) also reported antiviral, antifungal, anticancer, and antioxidant as some of the biological activities of Gallic acid. Gallic acid and other polyphenolic compounds with two or more phenolic hydroxyl groups in their chemical structure have been shown to have antioxidant effects (Lu, Nie, Belton, Tang, Zhao, 2006). The cardio-, nephro-, radioprotective, and anti-inflammatory effects of Gallic acid has been documented (Nair & Nair, 2013; Umadevi, Gopi, & Vellaichamy, 2013; Priscilla & Prince, 2009). However, since neurotoxicity is one of the side effects of CPA in cancer therapy, the use of antioxidant before or during treatment with CPA may be beneficial. Hence, this study was conducted to investigate the neuroprotective effect of Gallic acid and possible mechanism of action.

MATERIALS AND METHODS

Animal Treatment

Sixty adult male rats weighing approximately (100–145 g) obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were randomly divided into 6 groups of 10 animals per group. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum* and liberally supplied with water.

Group 1 received saline orally. Group 2 received CPA at 100 mg/kg single dose intraperitoneally on day 1. Groups 3 and 4 were treated with Gallic acid (GA) at 60 and 120 mg/kg body weight for 10 days and also received a single dose of CPA

(100 mg/kg) intraperitoneally on day 1. Rats in groups 5 and 6 received GA only at 60 and 120 mg/kg body weight for 10 days respectively.

Care of Animals

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments (PHS, 1996).

Chemicals

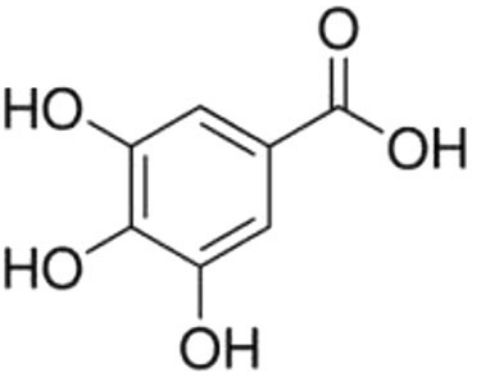
Potassium hydroxide, reduced glutathione (GSH), Trichloroacetic acid, sodium hydroxide, 1, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), xylenol orange, and hydrogen peroxide (H_2O_2), N-(1-naphthyl) ethylenediamine dihydrochloride, cyclophosphamide, 5, 5-Dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of Microsomal Fraction from Brain Tissues

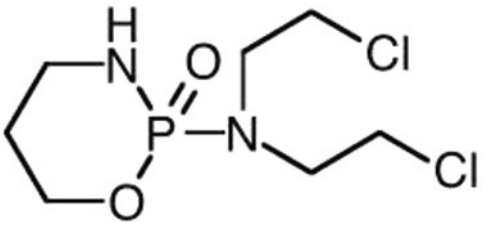
Rats were fasted overnight and sacrificed by cervical dislocation. The brain was removed, cerebrum and cerebellum were separated, rinsed in 1.15% KCl and homogenized in potassium phosphate buffer (0.1 M, pH 7.4), and homogenates were centrifuged at 10,000 g for 20 min to obtain the postmitochondrial fraction (PMF). The supernatants obtained were stored at −4°C until the time of use.

Cerebral and Cerebellar Biochemical Assays

The supernatants from the brain tissues were used for the following biochemical assays. Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 7.2 at 30°C as described by Misra & Fridovich (1972) with slight modification from our laboratory (Schemes 1 & 2) (Oyagbemi *et al.,* 2015). Briefly, 100 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. This preparation prevents



SCHEME 1: Structure of Gallic acid



SCHEME 2: Structure of cyclophosphamide

oxidation of epinephrine and is stable for 4 weeks. 10 μL of cerebral or cerebellar PMF was added to 2.5 ml 0.05M carbonate buffer (pH 10.2) followed by the addition of 300 μL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute. The Catalase (CAT) activity was determined according to the method of Shinha (1972). Reduced GSH was determined at 412 nm using the method described by Jollow, Mitchell, Zampaglione, Gillette (1974). Glutathione-S-transferase (GST) was estimated by the method of Habig, Pabst, Jacoby (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Protein concentration was determined by the method of Lowry, Rosebrough, Farr, Randall (1951). The malondialdehyde (MDA) level was calculated according to the method of Farombi, Tahnteng, Agboola, Nwankwo, Emerole (2000). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$. Glutathione peroxidase activity (GPx) was measured according to Buetler, Duron, & Kelly (1963). Hydrogen peroxide (H_2O_2) generation was determined as described (Woff, 1994). Nitrite content was determined according to the method of Olaleye, Adaramoye, Erigbali, & Adeniyi (2007).

Statistical Analysis

All values are expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's *t* test. One-way analysis of variance (ANOVA) with Dunnett's posttest was also performed using GraphPad Prism version 4.00.

RESULTS

Cerebral Markers of Oxidative Stress

The results obtained show that CPA treated only rats significantly ($p < .05$) increased cerebral malondialdehyde (MDA) content and H_2O_2 generation (Figures 1 & 2). However, treatment of rats in groups 3 and 4 with GA (60 & 120 mg/kg body weight) significantly ($p < 0.05$) reduced aforementioned markers of oxidative stress.

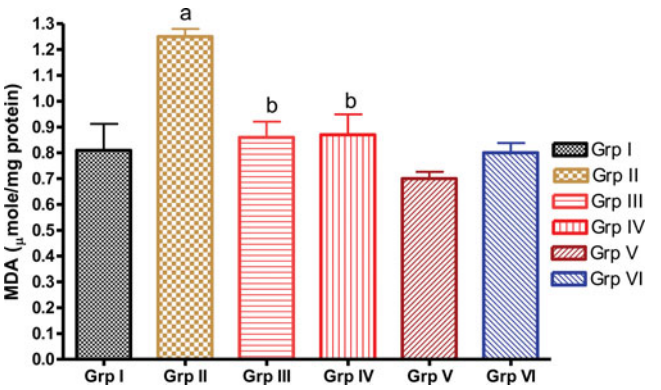


FIGURE 1. The effect of GA on malondialdehyde content of cerebrum. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < 0.05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

Cerebral Nonenzymic and Enzymic Antioxidant Defense System

The cerebral nonenzymic antioxidant (GSH) was markedly increased in CPA treated only rats when compared to the control (Figure 3). In the same vein, the cerebral GSH content in rats treated with GA was not significantly different from the CPA-treated rats and the control. The enzymic antioxidant activities of cerebral CAT, SOD, and GST in CPA-treated rats were significantly reduced compared to the control (Figures 4, 5 & 6). Moreover, the activities of these antioxidant defense systems improved and increased significantly in the cerebral tissues of rats

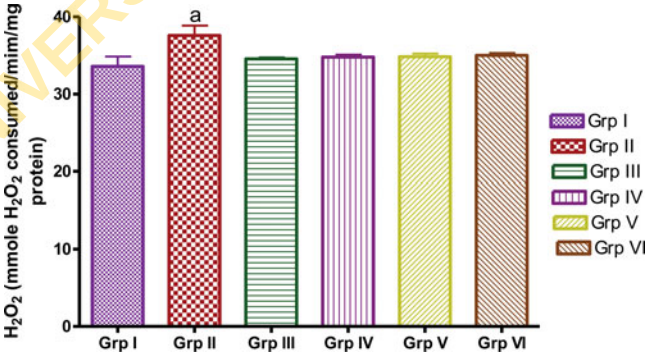


FIGURE 2. The effect of GA on cerebral hydrogen peroxide (H_2O_2) generation. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

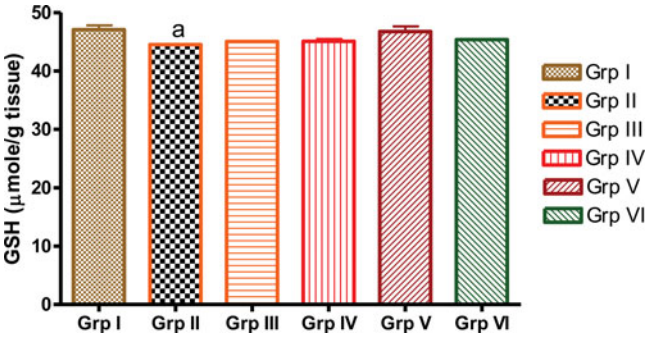


FIGURE 3. The effect of GA on cerebral reduced glutathione (GSH) content. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

treated with GA (60 & 120 mg/kg body weight), respectively, as shown in figures 4, 5 & 6. Interestingly, the cerebral activity of GPx of rats treated with CPA alone was significantly increased compared to the rats treated with GA and the control (Figure 7). The increase in the activity of GPx in rats treated with CPA points to the innate adaptive response to overcome the toxic challenge of CPA.

Cerebral Nitrite Content

In addition, the cerebral nitrite content was significantly increased in CPA treated only rats compared to the control (Figure 8). However, rats treated with GA (60 mg/kg body weight) significantly brought down the nitrite content compared to the CPA-treated rat and the values obtained were comparable to the control (Figure 8).

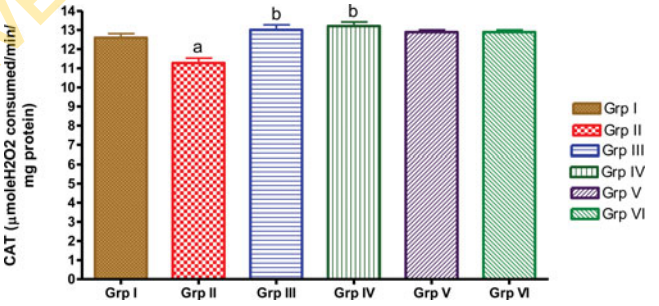


FIGURE 4. The effect of GA on cerebral catalase (CAT) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

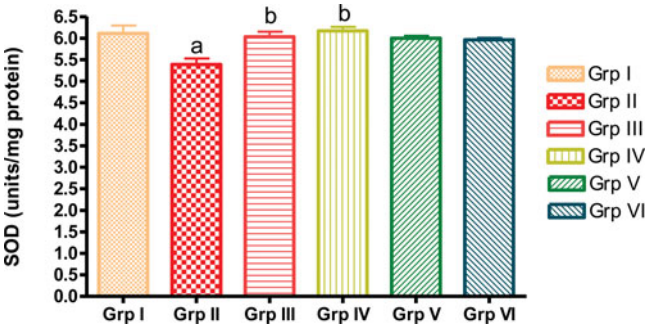


FIGURE 5. The effect of GA on cerebral superoxide dismutase (SOD) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

Cerebellar Markers of Oxidative Stress

Our findings show that the cerebellar malondialdehyde (MDA) contents and H_2O_2 generation increased significantly ($p < .05$) in CPA treated only rats when compared to the control (Figures 9 & 10). However, pre-treatment with GA (60 & 120 mg/kg body weight) significantly ($p < .05$) reduced the cerebellar TBARS and H_2O_2 contents. The cerebral nonenzymic antioxidant (GSH) was markedly increased in CPA treated only rats when compared to the rats pre-treated with GA and the control (Figure 11).

Cerebral Nonenzymic and Enzymic Antioxidant Defense System

From this study, the increase in the cerebellar GSH content was not significantly different from the control values and better than that of the CPA-treated only

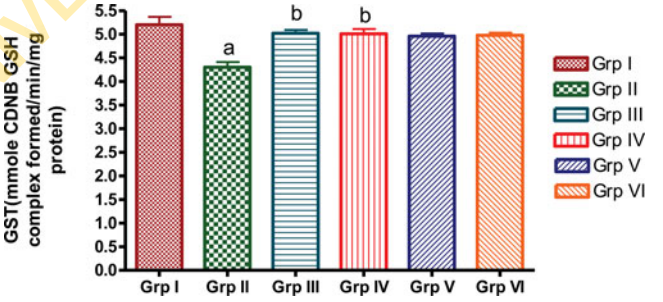


FIGURE 6. The effect of GA on cerebral glutathione-S-transferase (GST) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

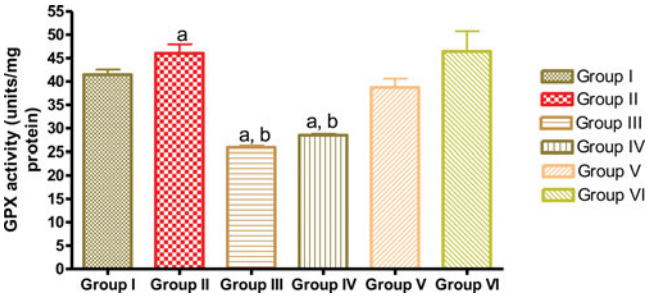


FIGURE 7. The effect of GA on cerebral glutathione peroxidase (GPx) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

rats (Figure 11). The activities of the cerebellar enzymic antioxidant system (CAT, SOD and GST) in CPA-treated rats were significantly reduced compared to the rats in control group and those treated with GA, respectively (Figures 12, 13 & 14). Furthermore, the activities of these antioxidant enzymes increased significantly in the cerebellar tissues of rats treated with GA (60 & 120 mg/kg body weight), respectively, as indicated in Figures 12, 13 and 14. Our data show that activity of GPx in the cerebellum of rats treated with CPA only significantly increased ($p < 0.05$) compared to the rats treated with GA and the control (Figure 15). The result is similar to what was obtained in the cerebrum.

Cerebellar Nitrite Content

Also, the cerebellar nitrite content was significantly increased in rats treated with CPA compared to the control (Figure 16). However, rats treated with GA (60 &

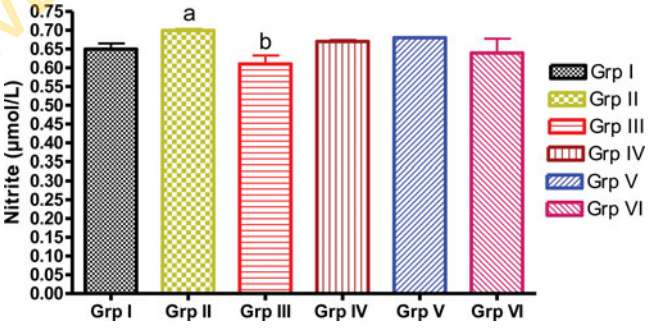


FIGURE 8. The effect of GA on cerebral nitrite content. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

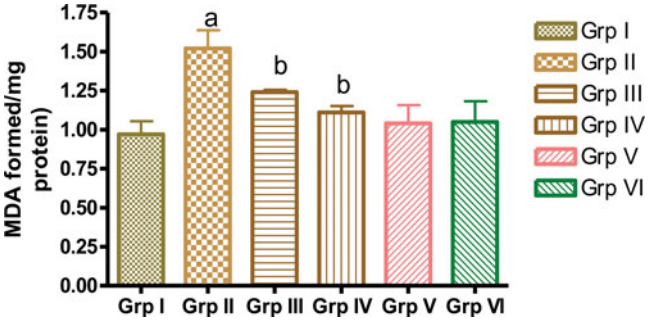


FIGURE 9. The effect of GA on malondialdehyde content of cerebellum. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

120 mg/kg body weight) significantly reduced the nitrite content compared to the CPA treated rats.

Cerebral and Cerebellar Weight

Figures 17 & 18 show that the cerebral and cerebellar weight did not differ significantly ($p > .05$) from the CPA-treated rats and the control. Also, the cerebral and cerebellar weight of rats treated with GA or those that received GA alone did not differ significantly ($p > .05$) from the CPA treated rats.

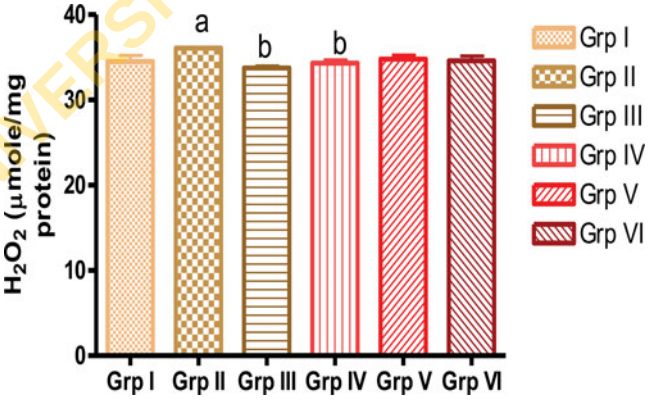


FIGURE 10. The effect of GA on cerebellar hydrogen peroxide (H_2O_2) generation. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

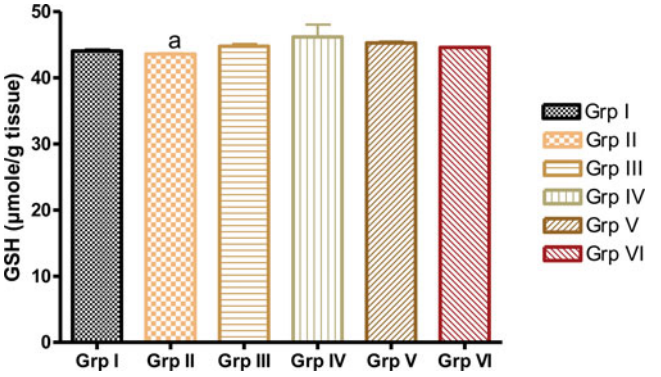


FIGURE 11. The effect of GA on cerebellar-reduced glutathione (GSH) content. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

DISCUSSION

Data obtained from our findings showed that CPA induced excessive production of MDA and H_2O_2 generation both in cerebrum and the cerebellum of rats treated with CPA alone. From this study, treatment with GA was able to ameliorate and quench free radicals generated by CPA. This study has demonstrated that GA has a chemopreventive effect on the neurotoxicity induced by CPA through free radical scavenging activity and improvement of the antioxidant defense system. Oboh, Akomolafe, & Adetuyi (2010a); Oboh and Ogunruku (2010b) confirmed that CPA

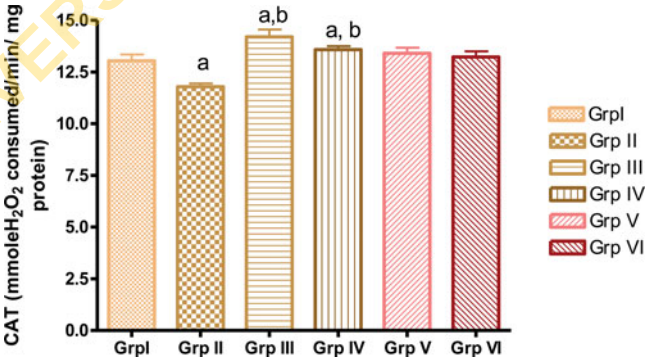


FIGURE 12. The effect of GA on cerebellar catalase (CAT) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

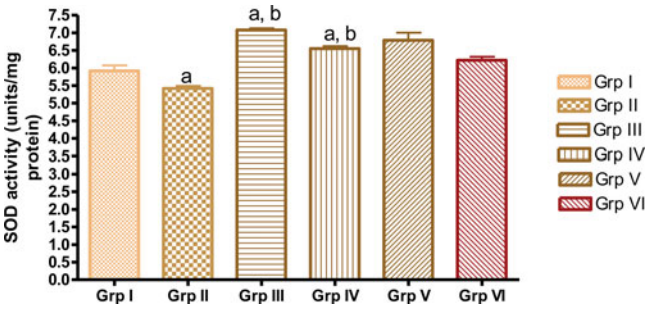


FIGURE 13. The effect of GA on cerebral superoxide dismutase (SOD) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

induced-oxidative stress and neurotoxicity in the rat and the protective role of phytochemicals.

Lipid peroxidation is a common result of oxidative stress in tissues and is expressed as the level of TBARS. Furthermore, the oxidation process of polyunsaturated fatty acid is measured in the form of TBARS formation as an index of lipid peroxidation (Pamplona, 2011; Bae, Oh, Rhee, & Yoo, 2011). According to this study, treatment with CPA resulted in the significant elevation of markers of oxidative stress.

ROS are produced continuously as natural by-products of the normal metabolism of oxygen and can cause oxidative damage to biomolecules resulting in loss of protein function, deoxyribonucleic acid (DNA) cleavage, or lipid

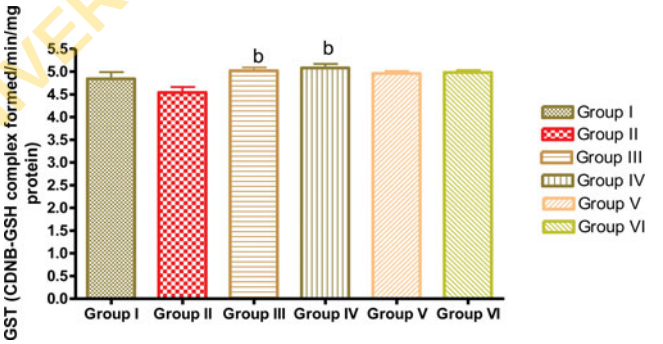


FIGURE 14. The effect of GA on cerebellar glutathione-S-transferase (GST) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

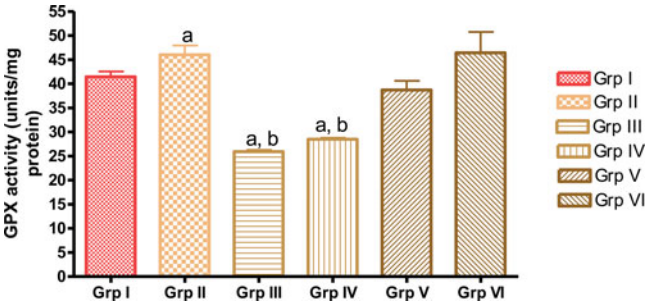


FIGURE 15. The effect of GA on cerebellar glutathione peroxidase (GPx) activity. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

peroxidation which culminates in oxidative stress with resultant cell injury or death (Gopalakrishnan, Nash, Velayutham, Villamena, 2012; Dikalov, 2011). It reported that overproduction of reactive oxygen species (ROS) can result in oxidative stress, a pathophysiological process that can damage cell structures and induce cancer, cardiovascular disease, atherosclerosis, hypertension, diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, and ageing (Shyu, Chang, Yeh, Sheu, & Chou, 2014). Reduction in lipid peroxidation in the pancreatic tissue of rat intoxicated with streptozotocin or decreased malondialdehyde levels of the liver, brain, and kidney of the aged mice that were administered with GA has been reported, respectively (Li et al., 2005; Punithavathi, Prince PSM, Kumar, & Selvakumari, 2011).

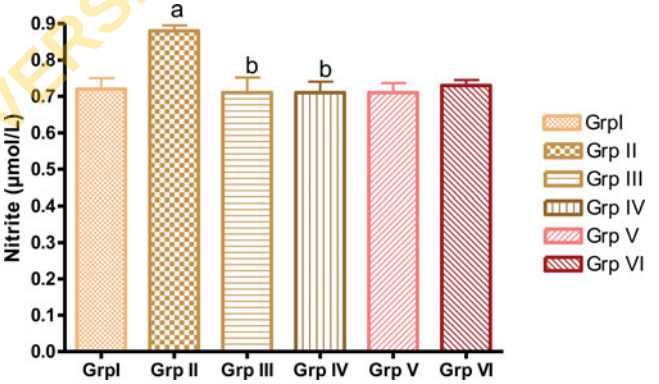


FIGURE 16. The effect of GA on cerebellar nitrite content. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

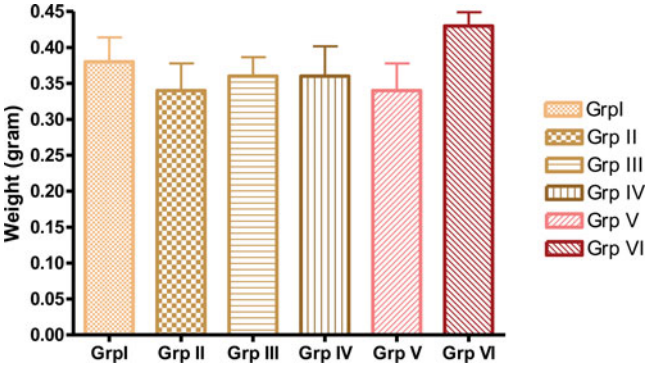


FIGURE 17. The effect of GA on cerebellar weight. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

In the present study, we found that GA ameliorated CPA-induced oxidative stress in the rat brain (cerebrum and cerebellum). Neuroprotective activities of GA against 6-hydrodopamine auto-oxidation induced apoptosis in human SH-SY5Y cells, and amyloid beta protein induced toxicity in cultured rat cortical neurons have been reported. The ability of GA to quench free radical production from the toxic metabolite of CPA demonstrated its awesome antioxidant capacity (Lu et al., 2006; Ban et al., 2008).

The depletion of intracellular nonenzymic antioxidant defense system (GSH) by CPA treatment also contributed significantly to the induction of oxidative stress

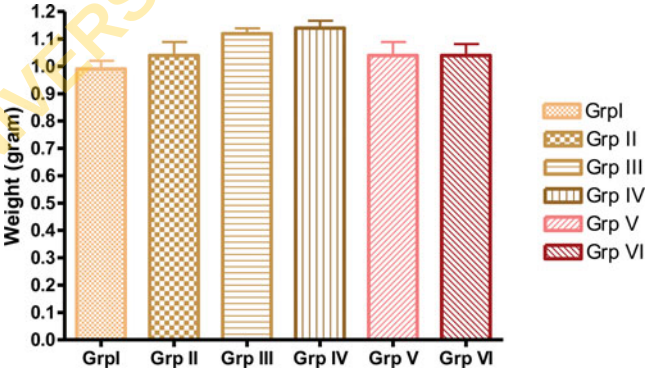


FIGURE 18. The effect of GA on cerebral weight. Values are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($p < .05$) when compared with control (Grp I), whereas superscripts (b) indicates significant difference ($p < .05$) when compared with CPA treated only (Grp II). Grp I (Control), Grp II (CPA treated only), Grp III (CPA+ 60 mg/k GA), Grp IV (CPA+120 mg/kg GA), Grp V (60 mg/kg GA only) & Grp VI (120 mg/kg GA only).

and excessive accumulation of lipid peroxidation products both in the cerebrum and the cerebellum. GA treatment inhibited depletion of GSH by CPA in both regions of the brain. We could, therefore, speculate that GA might be involved in the improvement of the GSH recycling system during oxidative stress. Gallic acid has been shown to prevent memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats via its antioxidant capacity (Mansouri et al., 2013). Hence, physiological functions of both cerebrum and cerebellum that might have been disrupted and or impaired in this study were preserved with the treatment of GA. It has been demonstrated that treatment with GA led to an increase in GSH levels and a reduction in oxidized glutathione (Kim, 2007). However, it is well known that the GSH/oxidized glutathione (GSSG) ratio enhancement by GA is enhanced with the addition of the hydroxyl groups (Requejo, Hurd, Costa, & Murphy, 2010). Since GSH is a major naturally-occurring brain antioxidant and sulfhydryl groups are found associated with proteins and as components of small molecules that participate in the tissue antioxidant redox pool (Requejo et al., 2010 & Hansen, Roth, Winther, 2009; Halliwell & Gutteridge, 2007), we, therefore, propose that the brain antioxidant defense system was compromised in rats treated with CPA.

In this study, CPA treatment significantly inhibited SOD, CAT, and GST in the cerebral and cerebellar tissues. The inhibition of this first line of defense against free radical generation and oxidative stress was reversed by treatment with GA in a dose-dependent manner. This therefore confirms the chemopreventive and neuroprotective effect of GA. The use of GA as a natural antioxidant has been extensively worked on and reviewed elsewhere (Singh, Singh, & Bhatti, 2014; Abarikwu, Akiri, Durojaiye, & Alabi, 2014; Nabavi et al., 2013a; Nabavi et al., 2013b). CAT is a ferric heme protein that catalyses the decomposition of hydrogen peroxide to water and oxygen. It has been suggested that CAT activity may be decreased secondarily to CAT saturation during the breakdown of free radicals and hydrogen peroxide or the inhibition of CAT by these free radicals (Eraslan, Saygi, Essìz, Aksoy, Gul, & Macit, 2007). SOD removes the anion superoxide by accelerating the rate of its dismutation to hydrogen peroxide, which is a ROS to nontoxic product (Halliwell, 2001). Hence, elevated SOD/CAT ratio may result in an increase of H_2O_2 concentration or increased rate of H_2O_2 production and may lead to lipid and protein oxidation, resulting in increased neuronal damage (Halliwell, 2001).

Antioxidant activity of GA is shown to depend on the phenolic hydroxyl groups of the molecule present in it (Lu et al., 2006). The aforementioned aberration observed in antioxidant enzyme activities of CAT, SOD & GST, TBARS, and GSH levels in both regions of the brain were normalized with GA (60 & 120 mg/kg body weight) compared to CPA-treated rats. The presence of a hydroxyl group on the GA molecule may be responsible for the antioxidant activity of GA derivatives and may, therefore, explain the protective mechanism against CPA-induced oxidative stress and neurotoxicity. The cerebrum is responsible for memory and the cerebellum for balance, motor control, attention, language, emotional functions, etc. Hence, GA as a potent antioxidant holds promise as a novel drug molecule against neurodegenerative disease. In addition, induction of ROS and oxidative stress has been associated with neurodegenerative disease (Mansouri et al., 2013). We also observed a significant increase of nitrite concentrations in cerebral and cerebellar

regions of the brain indicating that reactive nitrogen species (RNS) were elicited in the rats treated with CPA. The RNS generated was quenched by GA treatment by significantly reducing nitrite contents in GA-treated rats compared to CPA alone. Therefore, both ROS and RNS production in the brain was quenched by GA in this study.

It is interesting to note that GPx activity increased significantly in CPA-treated rats. The increase in the activity of this antioxidant enzyme should not be miss-interpreted for the absence of toxicity and organ damage. We speculated that mRNA that codes for the GPx might be upregulated in an attempt to avert the toxicity induced by CPA treatment. We, therefore, speculate that these regions of the brain might show some level of adaptation to toxic damage and hence avert neuronal degeneration.

CONCLUSION

Taken together, we demonstrate for the first time that CPA administration contributes to the induction of oxidative stress in rat brain both in the cerebrum and the cerebellum and that treatment with GA reversed the oxidative status by increasing the antioxidant defense system and reducing lipid peroxidation. Therefore, the study suggests that GA could be used as a potential phytochemical that could mitigate neurodegenerating disease and the neurotoxicity associated with chemotherapeutic agent CPA. In addition, food supplements with inclusion of GA might be lending a helping hand in the fight against the avalanche of side effects that accompany the use of CPA. Combining all, chemotherapeutic agents with structure/function similarities to GA could be of potential benefit to the pharmaceutical industries as an adjuvant in chemotherapy with little or no side effects.

ACKNOWLEDGMENTS

The authors want to acknowledge the technical assistance of Mr. Agboola Olalekan Samson of the Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

ABOUT THE AUTHORS

Ademola Adetokunbo Oyagbemi, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry, and Pharmacology, University of Ibadan, Nigeria, **Temidayo Olutayo Omobowale**, Faculty of Veterinary Medicine, Department of Veterinary Medicine, University of Ibadan, Nigeria, **Adebowale Bernard Saba AB**, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria, **Ebunoluwa Racheal Olowu**, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria,

Racheal Omolola Dada, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria, **Akinleye Stephen Akinrinde**, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.

REFERENCES

Abarikwu SO, Akiri OF, Durojaiye MA, Alabi AF. Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes. J Steroid Biochem Mol Biol. 2014;143C:49–60.

Asiri YA. Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues. Oxid Med Cell Longev. 2010;3:308–316.

Bae YS, Oh H, Rhee SG, Yoo YD. Regulation of reactive oxygen species generation cell signaling. Mol Cells. 2011;32:491–509.

Ban JY, Nguyen HT, Lee HJ, Cho SO, Ju HS, Kim JY, *et al*. Neuroprotective properties of gallic acid from Sanguisorbae radix on amyloid beta protein (25–35)-induced toxicity in cultured rat cortical neurons. Biol Pharm Bull. 2008;31:149–153.

Buetler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–888.

Chabra A, Shokrzadeh M, Naghshvar F, Salehi F, Ahmadi A. Melatonin ameliorates oxidative stress and reproductive toxicity induced by cyclophosphamide in male mice. Hum Exp Toxicol. 2014;33:185–195.

Dikalov S. Cross talk between mitochondria and NADPH oxidases. Free Radic Biol Med. 2011;51:1289–301.

Eraslan G, Saygi S, Essìz D, Aksoy A, Gul H, Macit E. Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocyanidin and N-acetylcysteine against exposure to cyfluthrin in mice. Pestic Biochem Physiol. 2007;88:43–49.

Eslami AC, Pasanphan W, Wagner BA, Buettner GR. Free radicals produced by the oxidation of gallic acid: an electron paramagnetic resonance study. Chem Cent J. 2010; 4:15.

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by Kolaviron a-*Garcina kola* seed extract. Food Chem Toxicol. 2000;38:353–541.

Farshid AA, Tamaddonfard E, Ranjbar S. Oral administration of vitamin C and histidine attenuate cyclophosphamide-induced hemorrhagic cystitis in rats. Indian J Pharmacol. 2013;45:126–129.

Gopalakrishnan B, Nash KM, Velayutham M, Villamena FA. Detection of nitric oxide and superoxide radical anion by electron paramagnetic resonance spectroscopy from cells using spin traps. J Vis Exp. 2012;66:e2810.

Habig WH, Pabst MJ, Jacoby WB. Glutathione-S-transferase activity: The enzymic step in mercapturic acid formation. J Biol Chem. 1974;249:130–139.

Halliwell B, Gutteridge J. Measurement of reactive species. In: Halliwell B, Gutteridge J, (eds). Free Radicals in Biology and Medicine. Oxford: Oxford University Press; 2007. pp. 268–340.

Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging. 2001;18:685–716.

Hansen RE, Roth D, Winther JR. Quantifying the global cellular thiol-disulfi de status. Proc Natl Acad Sci USA. 2009;106:422–442.

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis; protective role of GSH & evidence for 3, 4 bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11:151–169.

Kim SH, Lee IC, Baek HS, Shin IS, Moon C, Bae CS, Kim SH, Kim JC, Kim HC. Mechanism for the protective effect of diallyl disulfide against cyclophosphamide acute urotoxicity in rats. Food Chem Toxicol. 2014;64:110–118.

Kim YJ. Antimelanogenic and antioxidant properties of gallic acid. Biol Pharm Bull. 2007;30:1052–1055

Li L, Ng TB, Gao W, Li W, Fu M, Niu SM, Zhao L, Chen RR, Liu F. Antioxidant activity of Gallic acid from rose flowers in senescence accelerated mice. Life Sci. 2005;**77:**230–240.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–275.

Lu Z, Nie G, Belton PS, Tang H, Zhao B. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of Gallic acid derivatives. Neurochem Int. 2006;48:263–274.

Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Farbood Y, Sarkaki A, Bavarsad K. Gallic acid prevents memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats. Pharmacol Biochem Behav. 2013;111:90–96.

Misra HP, Fridovich I. The role of superoxide anoin in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;217:3170–3175.

Nabavi SF, Habtemariam S, Sureda A, Hajizadeh MA, Daglia M, Naba SM. In vivo protective effects of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress in rat erythrocytes. Arh Hig Rada Toksikol. 2013a;64:553–559.

Nabavi SM, Habtemariam S, Nabavi SF, Sureda A, Daglia M, Moghaddam AH, *et al*. Protective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress in rat's kidney. Mol Cell Biochem. 2013b;372:233–239.

Nagi MN, Al-Shabanah OA, Hafez MM, Sayed-Ahmed MM. Thymoquinone supplementation attenuates cyclophosphamide-induced cardiotoxicity in rats. J Biochem Mol Toxicol. 2011;25:135–142.

Nair GG, Nair CK. Radioprotective effects of gallic acid in mice. Biomed Res Int. 2013;2013:953079. doi: 10.1155/2013/953079.

Nitharwal RK, Patel H, Karchuli MS, Ugale RR. CP-induced oxidative stress, genotoxicity, as well as hepatotoxicity. Chemoprotective potential of Coccinia indic against cyclophosphamide-induced toxicity. Indian J Pharmacol. 2013;45:502–507.

Oboh G, Akomolafe TL, Adetuyi AO. Inhibition of cyclophosphamide-induced oxidative stress in brain by dietary inclusion of red dye extracts from sorghum (Sorghum bicolor) stem. J Med Food. 2010a;13:1075–1080.

Oboh G, Ogunruku OO. Cyclophosphamide-induced oxidative stress in brain: protective effect of hot short pepper (*Capsicum frutescens* L. var. abbreviatum). Exp Toxicol Pathol. 2010b; 62:227–233.

Olaleye SB, Adaramoye OA, Erigbali PP, Adeniyi OS. Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats. World J Gastroenterol. 2007; 13(38):5121–6.

Oyagbemi AA, Omobowale TO, Akinrinde AS, Saba AB, Ogunpolu BS, Daramola O. Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. Environ Toxicol. 2015;30:1235–1243.

Pamplona R. Advanced lipoxidation end-products. Chem-Biol Interact. 2011;192:14–20.

PHS (PUBLIC HEALTH SERVICE). Public health service policy on humane care and the use of laboratory animals. US Department of Health and Humane services, Washington, DC, 1996; pp. 99–158.

Priscilla DH, Prince PS. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem Biol Interact. 2009;179:118–124.

Punithavathi VR, Prince PSM, Kumar R, Selvakumari J. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. Eur J Pharmacol. 2011;650:465–471.

Requejo R, Hurd TR, Costa NJ, Murphy MP. Cysteine residues exposed on protein surfaces are the dominant intramitochondrial thiol and may protect against oxidative damage. FEBS J. 2010;**277:** 1465–1480.

Roy SS, Chakraborty P, Bhattacharya S. Intervention in cyclophosphamide induced oxidative stress and DNA damage by a flavonyl-thiazolidinedione based o rganoselenocyanate and evaluation of its efficacy during adjuvant therapy in tumour bearing mice. Eur J Med Chem. 2014;73:195–209.

Saba Khan S, Parvez S, Chaudhari B, Ahmad F, Anjum S, Chaudhari B, Raisuddin S. Ellagic acid attenuates bleomycin and cyclophosphamide-induced pulmonary toxicity in Wistar rats. Food Chem Toxicol. 2013;58:210–219.

Shinha KA. Colorimetric assay of Catalase. Anal. Biochem. 1972;47:389–394.

Shyu KG, Chang CC, Yeh YC, Sheu JR, Chou DS. Mechanisms of ascorbyl radical formation in human platelet-rich plasma. Biomed Res Int. 2014;2014:614506. doi: 10.1155/2014/614506.

Singh JP, Singh AP, Bhatti R. Explicit role of peroxisome proliferator-activated receptor gamma in gallic acid-mediated protection against ischemia-reperfusion-induced acute kidney injury in rats. J Surg Res. 2014;187:631–639.

Song J, Liu L, Li L, Liu J, Song E, Song Y. Protective effects of lipoic acid and mesna on cyclophosphamide-induced haemorrhagic cystitis in mice. Cell Biochem Funct. 2014; 32:125–132.

Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol. 2004;11:380–381.

Tasdemir S, Tasdemir C, Vardi N, Ates B, Taslidere E, Karaaslan MG, *et al*. Effects of ozone therapy on cyclophosphamide-induced urinary bladder toxicity in rats. Clin Invest Med. 2013;36:E9–17.

Türk G, Ceribaşi AO, Sakin F, Sönmez M, Ateşşahin A. In conclusion, CP-induced lipid peroxidation leads to structural and functional damage, as well as apoptosis, in spermatogenic cells of rats. Both LC and EA protect against the development of these detrimental effects. Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide-induced testicular lipid peroxidation and apoptosis. Reprod Fertil Dev. 2010;22:587–596.

Umadevi S, Gopi V, Vellaichamy E. Inhibitory effect of gallic acid on advanced glycation end products induced up-regulation of inflammatory cytokines and matrix proteins in H9C2 (2–1) cells. Cardiovasc Toxicol. 2013;13:396–405.

Viswanatha Swamy AH, Patel UM, Koti BC, Gadad PC, Patel NL, Thippeswamy AH. Cardioprotective effect of Saraca indica against cyclophosphamide induced cardiotoxicity in rats: a biochemical, electrocardiographic and histopathological study. Indian J Pharmacol. 2013;45:44–48.

Woff SF. Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. Methods Enzymol. 1994;233:182–189.

Yuan D, Wang H, He H, Jia L, He Y, Wang T, *et al*. Protective effects of total flavonoids from Epimedium on the male mouse reproductive system against cyclophosphamide- induced oxidative injury by up-regulating the expressions of SOD3 and GPX1. Phytother Res. 2014;28:88–97.