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RESEARCH ARTICLE

Inhibitory effect of tannic acid and its derivative (gallic acid) against cisplatin–induced thiobarbituric acid reactive substances (TBARS) production in rat kidney – *in vitro*

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Abstract

Increasing amounts of evidence suggests tannic acid (TA) and its derivative, gallic acid (GA) has beneficial effects against nephrotoxicity. The aim of this study was to investigate the effect of TA and GA (two structurally related phenolic acids) against cisplatin-induced thiobarbituric acid reactive substances (TBARS) production in rat kidney *in vitro*. The effect of both phenolic acids on kidney TBARS level in the presence of cisplatin, an anti-neoplastic drug used in the treatment of many solid-tissue cancers which has its chief side effect in nephrotoxicity, was assessed. Thereafter, the antioxidant properties were also determined. Incubation of the kidney tissue homogenate in the presence of cisplatin (1 mM) caused a significant ($P<0.05$) increase in the TBARS level. However, both phenolic acids caused a significant ($P<0.05$) decrease in the TBARS content of the kidney in a dose dependent manner (0 – 12.5 µM). Nevertheless, TA had a significantly higher ($P<0.05$) inhibitory effect on TBARS production than gallic acid judging by their IC_{50} value. Furthermore, TA possesses stronger antioxidant properties than GA as revealed by their scavenging, chelating and reducing ability. The inhibition of cisplatin–induced thiobarbituric acid reactive substances (TBARS) production in rat kidney by both TA and GA could be due to their antioxidant properties and this provide further insight into the mechanism of action for their nephroprotective properties from previous reported experimental studies. However, the study revealed that hydrolysis of TA reduces its potency.

INTRODUCTION

The use of chemotherapy in the treatment of cancer has opened new possibilities for improvement of the quality of life of cancer patients. Despite its success, treatment with some of the most effective anticancer drugs shows a number of symptoms of direct toxicity. Additionally, many anticancer drugs have been shown to be mutagenic, teratogenic, and carcinogenic in experimental systems. Second malignancies are also known to be associated with several therapeutic treatments [1].

Cisplatin (cis-diamminedichloroplatinum(II), CP) is an antitumor drug commonly used in the treatment of testicular, ovarian, bladder, cervical, esophageal, head and neck and small cell lung cancer [2]. Unfortunately, numerous side effects are related to CP therapy, including ototoxicity, gastrointestinal toxicity, myelosuppression, neurotoxicity and kidney injury [3,4]. Kidneys represent the main route of CP excretion, with proximal tubule cells as a primary

site of CP accumulation [5]. Thus, nephrotoxicity is one of the most serious dose-limiting side effects in CP chemotherapy. The major mechanisms of CP-induced nephrotoxicity include tubular necrosis, oxidative stress, inflammation and apoptosis [3]. Studies using chemiluminescence or electron spin resonance (ESR) have shown that CDDP generates OH radical [1]. A number of strategies have been proposed for the prevention/management of cisplatin-induced nephrotoxicity since there is no specific treatment, with the use of some synthetic drugs been popular. However, these drugs have some associated risks and side-effects [6], hence the need for natural alternatives of plant origin (plant foods/extracts) with little or no side effect.

In recent years, human health has assumed an unprecedented important status. A new diet-health paradigm is everlasting which places more emphasis on the positive aspects of diet. Foods have now assumed the status of functional foods, which should be capable of providing additional physiological benefit, such as prevent or delaying onset of chronic diseases, as well as meeting basic nutritional requirements [7]. Recent reports revealed that a practical approach to the management / prevention of diseases that are associated with oxidative stress is through dietary means, which involves eating foods that are rich in antioxidants [7,8]. Apart from vitamin C and E that are already established food nutrient with antioxidant properties, plant phenolics among them phenolic acids, which are known to be scavengers of various oxygen species have been shown to be more potent [9,10]. The efficiency of phenolic compounds as anti-radicals and antioxidants is diverse and depends on many factors, such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring. For example, Rice-Evans et al. [11] and Joyeux et al. [12] described the advantageous effect of an ortho-hydroxyl substitution in the aromatic ring for the anti-radical and antioxidant activity of phenolic acids.

Tannic acid (TA), a naturally occurring plant polyphenol, is composed of a central glucose molecule derivatized at its hydroxyl groups with one or more galloyl residues (Figure 5). Gallic acid (GA), also known as 3,4,5-trihydroxybenzoic acid, is a monomer or derivative of tannic acid upon hydrolysis (Figure 5). Both TA and GA is one of the most important polyphenolic substances in plants which is present in grapes, different berries, mango, areca nut, walnut and other fruits as well as in several beverages including red wine, beer, coffee, black tea, green tea, and many foodstuffs such as sorghum, black-eyed peas, lentils and chocolate [13,14]. Similar to many phenolic acids, both TA and GA have been shown to possess antioxidant [15,16] anti-inflammatory [16], anti-mutagenic [17,18] and anti-carcinogenic properties [18-20]. In vitro studies revealed that tannic acid inhibited the proliferation of various cancer cell lines and induced cancer cell apoptosis [18-20]. Recently, they have been shown to possess renoprotective potential against cisplatin-induced kidney injury [21,22]. Based on this, it is expedient to compare the protective effect of both TA and GA against cisplatin-induced TBARS production *in vitro* in order to provide further mechanism of action for their beneficial effects against nephrotoxicity and to reveal which phenolic acid is more potent.

Materials and methods

Chemicals

Chemicals such as caffeic acid, chlorogenic acid, thiobarbituric acid (TBA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchase from Sigma-Aldrich, Chemie GmH (Steinheim, Germany), Tris-HCl buffer, sodium dodecyl sulphate (SDS) and acetic acid were procured from BDH Chemical Ltd., (Poole, England). Pharmaceutical grade Cisplatin (CP) under the brand name 'Cytoplatin50' was purchased from Cipla Ltd., India. Except otherwise stated, all other chemicals and reagents are of analytical grade while the water was glass distilled.

Lipid peroxidation assay

Experimental animals

Male Wistar albino rats weighing 190–250 g were purchased from the Central Animal House, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in stainless steel cages under controlled conditions with a 12-hour/12-hour light/dark cycle, 50% humidity, and temperature of 28°C. The rats were allowed *ad libitum* access to food and water. The animals were handled in accordance with NIH Guide for the care and use of laboratory animals.

Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anaesthesia, and the kidney tissue was rapidly dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1:10 w/v) with about 10 up-and-down strokes at approximately 1200 revolutions/min in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayushi Design Pvt. Ltd., New Delhi, India). The homogenate was centrifuged (KX3400C Kenxin International Co., Hong Kong) for 10 minutes at 3000 g to yield a pellet that was discarded and a low-speed supernatant, which was kept for lipid peroxidation assay [23].

Lipid peroxidation and thiobarbibutric acid reactions

The lipid peroxidation assay was carried out by using the modified method of Ohkawa et al. [24]. Briefly, 100 µL of the supernatant fraction was mixed with a reaction mixture containing 30 µL of 0.1M Tris-HCl buffer (pH, 7.4), phenolic acids (0 – 100 µM) and 30 µL of 1mM cisplatin. The volume was made up to 300 µL with water before incubation at 37^{o}C for 1h. The color reaction was developed by adding 300 µL 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing supernatant; this was subsequently followed by the addition of 600 µL of acetic acid/HCl (pH, 3.4) mixture and 600 µL of 0.8% TBA (thiobarbituric acid). This mixture was incubated at 100°C for 1 hour. The TBARS produced were measured at 532 nm and expressed as MDA produced (% control) by using the MDA standard curve (0 – 0.035 mM).

DPPH free radical scavenging ability

The free radical scavenging ability of the phenolic acids against DPPH free radical was evaluated as described by Gyamfi et al. [25]. Briefly, an appropriate dilution of the acids (1 mL) was mixed with 1 mL 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 minutes, and the absorbance was taken at 516 nm. The control was carried out by using 2 mL DPPH solution without the test samples. The DPPH free radical scavenging ability was subsequently calculated as follows:

DPPH scavenging ability (%) = $[(Abs_{Control} - Abs_{Samples})/Abs_{Control}]*100$

Determination of Fe^{2+} chelating ability

The Fe^{2+} chelating ability of the tannic and gallic acids was determined using a modified method of Minotti and Aust [26], with a slight modification by Puntel et al. [27]. Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL of 0.1 M Tris-HCl (pH 7.4), 218 µL saline and the phenolic acids (0 – 10 µM). The reaction mixture was incubated for 5 min, before the addition of 13 µL of 0.25% 1,10 phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated.

Determination of reducing power

The reducing property of the acids was determined by assessing their ability to reduce $FeCl_3$ solution as described by Oyaizu [28]. A 500 µL aliquot of the phenolic acids was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 801 × *g* for 10 min. 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing power was subsequently calculated using ascorbic acid equivalent.

Statistical Analysis

Statistical analysis was performed using statistical program for social science (SPSS) 16.0 (SPSS. Inc. Chicago, Illinois, USA). Results were represented as mean ± SD, and the differences were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan's multiple range analysis. A p value < 0.05 was considered to be statistically significant.

Results

As presented in Figure 1, incubation of the kidney homogenate in the presence of cisplatin (1 mM) caused a significant ($P<0.05$) increase in the TBARS content. However, the introduction of both tannic acid and gallic acid (0 – 12.5 µM) caused a dose-dependent significant ($P < 0.05$) decrease in the TBARS level of the cisplatin stressed kidney homogenates with tannic acid (IC_{50} = 8.7 µM) exhibiting higher inhibitory effect than gallic acid (IC_{50} = 17.8 µM) as revealed by their IC_{50} value (Table 1).

Furthermore, the phenolic acids were able to scavenge DPPH free radical in a dose-dependent manner (0 – 100 µM) as shown in Figure 2. However, tannic acid (IC_{50} = 74.6 µM) had a significantly higher scavenging ability than gallic acid (IC_{50} = 105.2 µM) (Table 1).

In addition, both tannic acid and gallic acid exhibited chelating property by their ability to chelate metal such as Fe^{2+} in a dose dependent manner (0 – 10 µM) as shown in Figure 3. However, tannic acid (IC_{50} = 2.5 µM) had better Fe^{2+} chelating ability than gallic acid (IC_{50} = 3.2 µM) (Table 2).

Figure 4 revealed the ferric reducing antioxidant properties of both tannic acid and gallic acid expressed as ascorbic acid equivalent (AAE). The results revealed also that tannic acid (11.1 mmol. AAE/g) had significantly higher ($P < 0.05$) reducing power than gallic acid (3.4 mmol. AAE/g).

TABLE 1: IC_{50} (inhibitor concentration causing 50% inhibition) value of cisplatin induced MDA production, DPPH radical scavenging and Fe^{2+} chelating abilities of gallic and tannic acids

	Gallic acid	Tannic acid
Cisplatin induced MDA production (µM)	$17.8^{a} \pm 2.4$	$8.7^{b} \pm 1.3$
DPPH radical scavenging ability (µM)	$105.2^{c} \pm 5.2$	$74.6^{b} \pm 2.0$
Fe^{2+} chelating ability (µM)	$3.2^{a} \pm 0.10$	$2.5^{b} \pm 0.08$

Values represent mean ± standard deviation of triplicate experiments
Values with the same superscript letter along the same row are not significantly different (P<0.05)

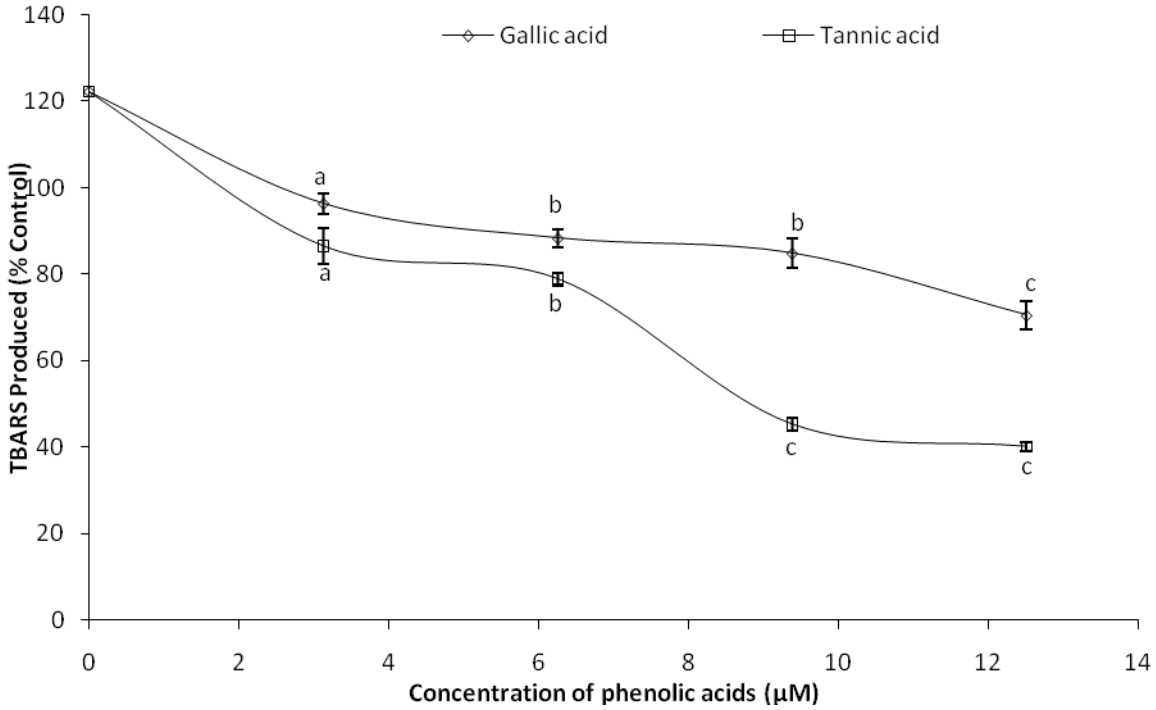

FIGURE 1: Inhibition of cisplatin induced lipid peroxidation in rat kidney by gallic and tannic acids.
Values represent mean ± standard deviation of triplicate experiments
Different letters above each concentration indicate significant differences (P < 0.05).

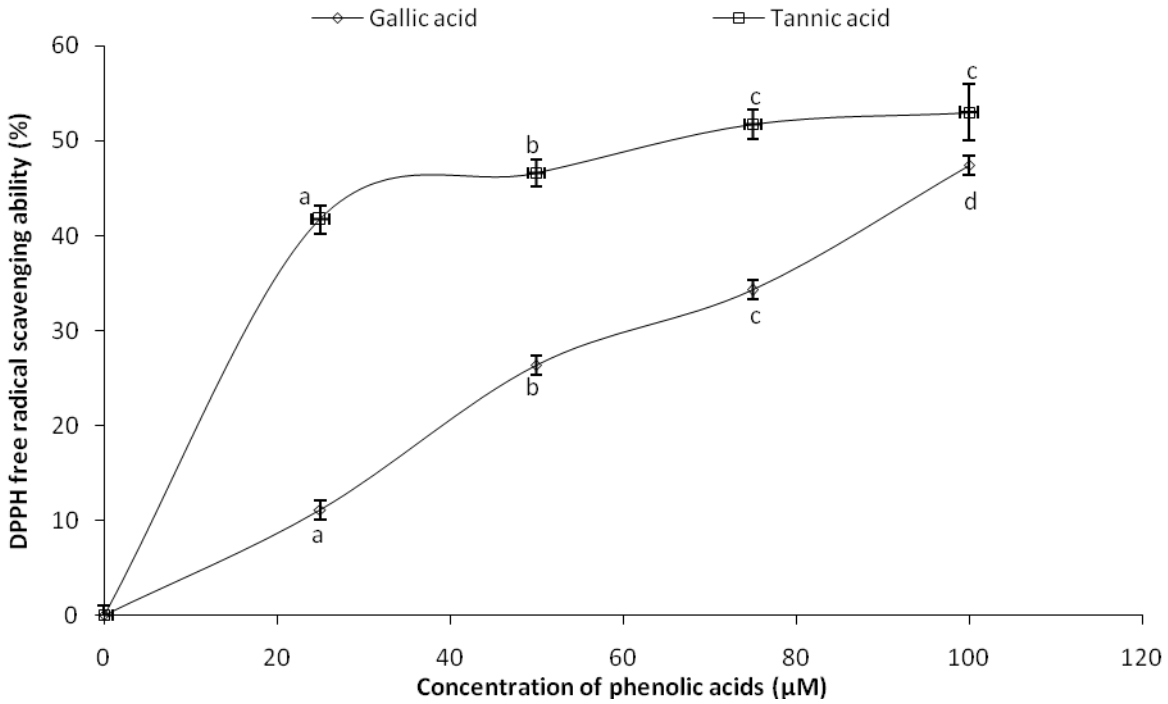

FIGURE 2: DPPH free radical scavenging ability of gallic and tannic acids
Values represent mean ± standard deviation of triplicate experiments
Different letters above each concentration indicate significant differences ($P < 0.05$).

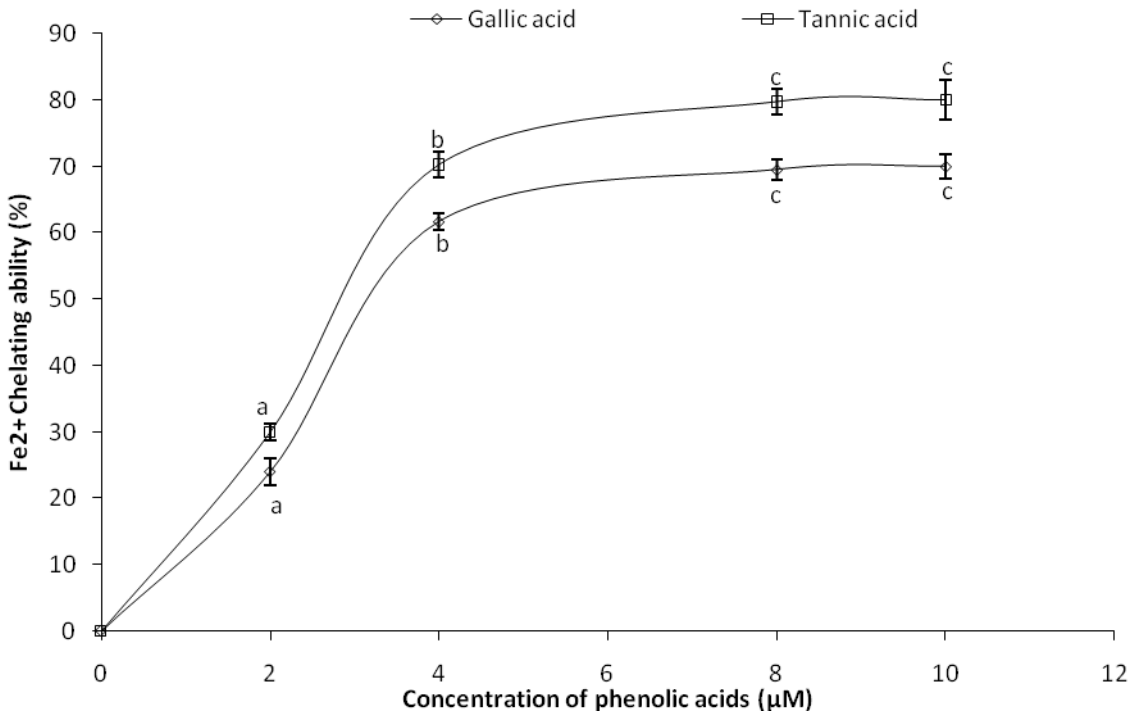

FIGURE 3: Fe^{2+} chelating ability of gallic and tannic acids
Values represent mean ± standard deviation of triplicate experiments
Different letters above each concentration indicate significant differences ($P < 0.05$).

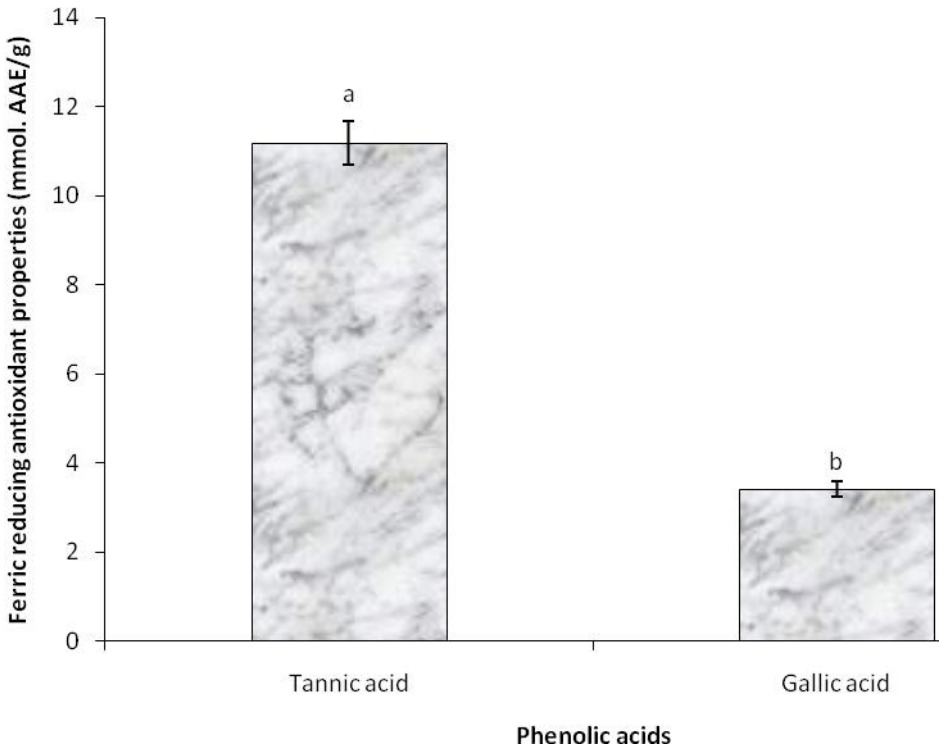

FIGURE 4: Ferric reducing antioxidant property (FRAP) of gallic and tannic acids
Values represent mean ± standard deviation of triplicate experiments
Bars with different letters indicate significant differences ($P < 0.05$).

A. **B.**

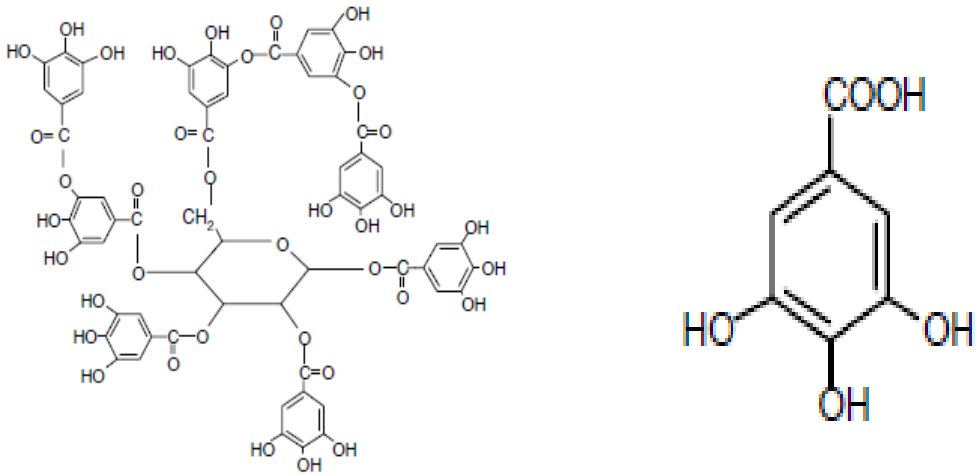

FIGURE 5: Chemical structures of used phenolic acids (A) Tannic acid and (B) Gallic acid

Discussion

Oxidative stress plays a critical role in the pathophysiology of CP-induced kidney injury [21]. Lipid peroxidation is a hallmark of oxidative stress in the cell [29,30]. Lipid peroxidation is another process that produces many pathological events in the cells and the organism of man [29-32]. This process, causing damage to unsaturated fatty acids, tends to decrease membrane fluidity and leads to many other pathological events. Previous studies showed increased lipid peroxidation in the kidneys after CP administration which was markedly attenuated by natural antioxidants, including hesperidin, carnosic acid, apocynin and cannabidiol [33-36].

In this present study, incubation of the kidney tissue homogenate in the presence of cisplatin (1 mM) caused a significant ($P<0.05$) increase in the TBARS content (Figure 1). This result agrees with a recent work reported by Oboh et al. [37] where cisplatin has been shown to induce lipid peroxidation in rat tissue. In addition, Pabla et al. [38] and Jiang et al. [39] reported CP-induced renal cell apoptosis both under *in vitro* and *in vivo* conditions. The increased lipid peroxidation in the presence of cisplatin could be attributed to the fact that it can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive OH•, which is formed from H_2O_2 through the Fenton reaction. However, both phenolic acids caused a significant ($P<0.05$) decrease in the TBARS content of the kidney in a dose dependent manner. Nevertheless, as revealed by their IC_{50} (inhibitor concentration causing 50% inhibition of TBARS level) value, tannic acid had a significantly higher ($P<0.05$) inhibitory effect than gallic acid (Table 1).

Furthermore, the mechanisms through which the phenolic acids prevent cisplatin-induced lipid peroxidation were subsequently evaluated by determining their antioxidant properties. Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing free radicals produced in the body [40]. Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action [41]; hence, the ability of the phenolic acids to scavenge DPPH free radical was assessed in this study. This is based on a model system whereby antioxidant capacity is measured by the ability to donate a hydrogen atom thereby neutralizing DPPH radicals. DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule [42]. The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging [42]. The result presented in Figure 2 revealed that both gallic and tannic acids scavenge DPPH radical in a dose dependent manner. However, judging by their IC_{50} value (Table 1), tannic acid had a significantly ($P<0.05$) higher DPPH radical scavenging ability than gallic acid. The higher radical scavenging ability of tannic acid could be due to the number of hydroxyl groups more than gallic acid with only three hydroxyl groups bonded to the aromatic ring in an ortho position as shown in Figure 5. Rice-Evans et al. [11] reported that the position and number of hydroxyl group in phenolic compounds influence their antioxidant properties.

Furthermore, the ability of antioxidants to chelate and deactivate transition metals, prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalysed reaction [43]. Iron (Fe), an essential metal needed for normal cellular physiology is present in biological systems bound to several protein moieties such as heamoglobin, ferritin etc. It may also exist in free forms in which it is able to participate in Fenton reaction with hydroxyl radical and Fe^{3+} as products. Chelation of such transition metals such as Fe is regarded as a preventive antioxidant mechanism. Our result revealed that both phenolic acids were able to chelate Fe^{2+} in a dose-dependent manner. However, judging by their IC_{50} value (Table 1), tannic acid had a significantly ($P<0.05$) higher Fe^{2+} chelating ability than gallic acid. This Fe^{2+} chelating ability of the acids is of immense importance in the protective ability of antioxidant phytochemicals against oxidative stress, as this will make free Fe biologically unavailable thereby inhibiting its role in activating free radicals. However, the Fe^{2+} chelating ability of the phenolic acids could be attributed to the presence of the following functional groups: -OH and -COOH in a favourable structure–function configuration [44,45].

Reducing power is a novel antioxidation defence mechanism; the two mechanisms available to affect this property are by electron transfer and hydrogen atom transfer [46]. This is because the ferric-to-ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the Ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants [47]. The result revealed that tannic acid had significantly higher ($P<0.05$) reducing power than gallic acid. Since the antioxidant activity of phenolics is mainly due to their redox properties, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [11]. The higher reducing power of the tannic acid agrees with the other antioxidant properties carried out in Figure 3 and 4. This confirm that the functional groups such as the number of the hydroxyl group influence their antioxidant property as previously reported by Rice-Evans et al. [11].

Conclusion

In conclusion, both TA and GA inhibited cisplatin–induced thiobarbituric acid reactive substances (TBARS) production in rat kidney in a dose-dependent manner in vitro. This inhibitory effect could be due to their antioxidant properties as typified by their DPPH radical scavenging, Fe^{2+} chelating and reducing abilities. Furthermore, this study provide further insight into the mechanism of action for their nephroprotective properties from previous reported experimental studies and confirm their antioxidant potential. However, tannic acid possesses better antioxidant properties than gallic acid which could be due to the number of functional groups present indicating that hydrolysis affects its potency.

Conflict of Interests

The authors declare that they have no competing interests.

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