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ORIGINAL ARTICLE

Toxicological evaluation and *in vitro* assessment of antioxidant and anticholinesterase properties of Andrographis paniculata extract

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ABSTRACT

Andrographis paniculata is a plant herb that is used to treat various disease conditions because of its medicinal properties. This study sought to assess the antioxidant potentials of *A. paniculata in vitro* and evaluate its toxicological effects Wistar albino rats. Aqueous extract of *A. paniculate*, on which the following antioxidant assays, azino-bis-3-ethylbenzo-thiazoline-6-sulfonate radical (ABTS*), 1, 1-diphenyl-2- picrylhydrazyl radical (DPPH*), hydroxyl radical (OH*), ferric reducing antioxidant property (FRAP) and Fe^{2+} chelation were carried out, was prepared. For the toxicological study, the extract was administered orally for 14 days at different doses of 5, 50, 500, and 2000 mg/kg body weight. Afterwards, the liver function markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), total and direct bilirubin], as well as kidney function markers [creatinine, urea, and uric acid] were evaluated. Results obtained from this study showed that *A. paniculata* extract possess antioxidant activities *in vitro* and also revealed that the plant extract is safe at the various doses administered as there was no record of death or any observable sign of skin pathology and also possess antioxidant properties. Therefore, aqueous extract of *A. paniculata* is non-toxic and could be used for medicinal and therapeutic purposes.

KEY WORDS: *Andrographis paniculata*; antioxidant; cholinesterases; toxicological evaluation

Introduction

Medicinal plants have been an integral part of human life in fighting against ailments and various disease conditions in recent years (Chaudhary *et al.,* 2010). It has been estimated that about 80,000 plant species have been identified worldwide and are used as medicinal plants (Joy *et al.,* 1998). These medicinal plants are potential source to alternative medicine (herbal) and could be used to treat health conditions (Kavishankar *et al.,* 2011). People have long histories on the use of medicinal plants for medical purposes and this has been actively promoted (Igoli *et al.,* 2005; Njoroge & Bussmann, 2006). Plant-derived drugs are used in all societies and cultures hence, plants have always played a key role in health care systems around the world. In most of the developed countries, these traditional (herbal) system of medicine are the major forms of healing therapy due to less side effects and, with its availability, economical value and level of effectiveness are socially acceptable (Patil & Gaikwad, 2010).

The world health organization (WHO) has stated that medicinal plants are the most valuable source for deriving various types of drugs (Doughari *et al.,* 2008). However, scientific investigation on medicinal plants has become a risk as it led to interest of common people to capture the healing systems of these plants in the treatment of different diseases (Taylor, 2000). Generally, a substance, though natural, can be toxic depending on dosage administered, therefore, experimental studies are needed to formulate and validate dosage, especially the safety and efficacy of these plants (David *et al.,* 2015). Therefore, evaluating the toxicity of these plants intended for the use is vital for toxicological studies.

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Andrographis paniculata is a medicinal plant used in traditional practice to treat various diseases. Also known as "King of Bitters" or "Kalmegh," *A. paniculata* belongs to the family *Acanthaceae* and is widely distributed in Southern Asia. It is mostly cultivated because of its well-known medicinal value and grows well in most soil types (Latto *et al.,* 2006). The plant has been used in folklore to treat ailments and used as herbal supplements to improve health condition, its leaves and roots are used traditionally for various medical purposes in Asia and Europe (Mishra *et al.,* 2007; Jarukamjorn & Nemoto, 2008). Phytochemical studies have showed that *A. paniculata* contains diverse compounds such as labdane diterpenoid lactones, flavonoids and other compounds and therefore is known to possess broad range of pharmacological effects; anti-inflammatory, radical scavenging, cardiovascular and hepatoprotective potentials (Akbar, 2011; Parixit *et al.,* 2012; Sivakumar & Rajeshkumar 2015). While this plant has been used in traditional folklore because of its medicinal value, there is little or no information on the toxicity or safe dose of the plant. Therefore, this study sought to investigate the acute toxicological profile of aqueous extract of *A. paniculata* in rats.

Materials and methods

Chemicals and reagents

Biochemical assay kits were procured from Randox Laboratories (UK). Trichloroacetic acid (TCA) and sodium acetate were sourced from Sigma Aldrich, Chemie GmbH (Steinheim, Germany), methanol, acetic acid, hydrochloric acid, aluminium chloride, potassium acetate, sodium dodecyl sulphate (SDS), Iron (II) sulphate, and potassium ferrycyanide were sourced from BDH Chemicals Ltd., (Poole, England). All other chemicals and reagents used were of analytical grade and water was glass distilled.

Sample collection and preparation

Fresh *A. paniculata* was gotten from Osogbo, Osun State. Thereafter, the plant was rinsed under running water (tap) and air dried at room temperature to constant weight. It was blended into powder. Aqueous extract of blended sample was prepared by soaking the powdered sample in water (1:20 w/v) as the stock with thorough shaking using the orbital shaker for 4 hours. The mixture was then filtered using Whatman filter paper, after which a concentration of 25 mg/ml was used for *in vitro* analysis. The remaining sample was kept in an air-tight container for further analysis.

In vitro assays

Determination of total phenol content

The total phenol content was determined on the extract using the method reported by Singleton *et al.* (1999). Appropriate dilutions of the extract were oxidized with 2.5 ml 10% Folin Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes (min) at 45°C and the absorbance was measured at 765 nm using a spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard.

Determination of total flavonoid content

The total flavonoid content of the extract was determined using a slightly modified method reported by (Meda *et al.,* 2005). Diluted sample (0.5 ml) was mixed with 0.5 ml methanol, 50 µl of 10 % aluminium chloride ($AlCl_3$), 50 µl of 1 M potassium acetate (CH_3COOK) and 1.4 ml of distilled water. The mixture was then allowed to incubate at room temperature for 30 min. Afterwards, the absorbance of the reaction mixture was measured at 415 nm and then subsequently calculated using quercetin as standard.

Determination of ferric reducing antioxidant property (FRAP)

The reducing properties of the extract was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by (Oyaizu, 1986). The sample (0.20 ml) was mixed with 0.25 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.25 ml 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, and then 0.25 ml 10% trichloroacetic acid (TCA) was added together with 1 ml of water and 0.20 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated using vitamin C as standard.

3.3.4 Azinobis (3-Ethylbenzo-Thiazoline-6-Sulfonate) (ABTS) radical scavenging ability

ABTS (Azino-bis-3-Ethylbenzo-Thiazoline-6-Sulfonate) scavenging ability of the extract was determined according to the method described by Re *et al.* (1999). ABTS* was generated by reacting an ABTS aqueous solution (7 mmol/l) with $K_2S_2O_8$ (2.45 mmol/l) in the dark for 16 hrs and adjusted the absorbance to 0.700 at 734 nm by diluting with distilled water. Then, 0.1 ml of appropriate dilution of each extract was added to 2.0 ml ABTS* solution and absorbance was measured at 734 nm after 15 min incubation in the dark. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

DPPH free radical scavenging ability

The radical scavenging ability of the extract was determined against DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical as described by (Gyamfi *et al.,* 1999). Appropriate dilution of the extract (25–100 µl) was mixed with 1 ml 0.4 mM methanolic solution containing DPPH radicals, the mixture was incubated in the dark for 30 min and the absorbance was taken at 516 nm.

Degradation of deoxyribose (Fenton's / Hydroxyl radical reaction)

The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell & Gutteridge, 1981. Freshly prepared aqueous extract (0–100 µl) was added to a reaction mixture containing 120 µl, 20 mM deoxyribose, 400 µl, 0.1 M phosphate buffer (pH 7.4), 40 µl, 20 mM hydrogen peroxide and 40 µl, 500 µM $FeSO_4$, and the volume was made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8% TCA (trichloroacetic acid), this was followed by the

addition of 0.4 ml of 0.6% TBA solution. The reaction mixture was incubated for a second time in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer.

Determination of iron (Fe^{2+}) chelating ability

The Fe^{2+} chelating ability of the extract was determined using a modified method described by (Puntel *et al.,* 2005). 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 extract (20–100 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1, 10-phenanthroline (w/v). The absorbance of the mixture was taken at 510 nm in a spectrophotometer.

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) activities

The Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) activities were determined using the colorimetric method of Ellman *et al.* (1961). The AChE and BChE of brain tissue homogenate were determined in a reaction mixture containing 0.1 M phosphate buffer (pH 8.0), 0.33 M of 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB), aqueous extract (concentrations ranging from 50–150 µl) and tissue supernatant. After incubation at room temperature for 20 min, the enzyme was pre-incubated for 2 min. The reaction was initiated by adding the substrate (acetylthiocholine iodide and butyrylthiocholine iodide) for AChE and BChE, respectively. The absorbance change over a period of 3 min was monitored at 412 nm.

Thiobarbituric acid reactive species (TBARS) assay

An aliquot of 100 µl of the supernatant was incubated for 1 h at 37 °C in the presence of the aqueous extract (concentrations range from 20–100 µl), with and without the pro-oxidant; ferrous sulphate ($FeSO_4$). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) were determined as described by the method of Ohkawa *et al.* (1979) accepting that the buffer of color reaction has a pH of 3.4. The color reaction was developed by adding 300 µl of 8.1% SDS (sodium dodecyl sulfate) to the supernatant mixture, followed by sequential addition of 500 µl acetic acid (pH 3.4) and 500 µl 0.8% of thiobarbituric acid (TBA). The mixture was then incubated at 100°C for 1 h. TBARS produced were measured at 532 nm. Malondialdehyde (MDA) was used as standard and TBARS produced was reported as MDA equivalent.

HPLC- DAD characterization of Andrographis paniculata

Andrographis paniculata at a concentration of 15 mg/ml was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C_{18} column (4.6 mm × 250 mm × 5 µm particle size). The mobile phase was water with 1% formic acid (v/v) (solvent A) and HPLC grade acetonitrile (solvent B) at a flow rate of 0.7 ml/min and injection volume 50 µl. The composition gradient was: 5% solvent B reaching 15% at 10 min; 30% solvent B at 20 min, 65% solvent B at 30 min and 98% solvent B at 40 min, followed by 50 min at isocratic elution until 55 min. At 60 min the gradient reached the initial conditions again, following the method described by Boligon *et al.* (2015). The sample and mobile phase were filtered through 0.45 µm membrane filter (millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the acetonitrile: water (1:1, v/v) at a concentration range of 0.030–0.500 mg/ml. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid; 327 nm for caffeic acid; and 366 nm for quercetin, apigenin and orientin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 700 nm).

Animals

Adult Male Albino Wistar rats weighing between 150–220 g obtained from the animal holding facility of University of Ilorin were used for this study. The animals were maintained at a constant temperature on a 12 hrs light/dark cycle with free access to food and water ad libitum. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

Acute toxicity test

Toxicological evaluation was carried out to determine how toxicity of the plant extract. After acclimatization, a total of 25 adult male Wistar rats were randomly allocated into 5 groups containing 5 rats per group each. Doses at different concentrations (5, 50, 500, 2000 mg/kg body weight [BWT]) of the sample was administered orally once to the animals and observed for 14 days.

Experimental design

Groups: 1. Control received distilled water; 2. 5 mg/kg bwt of *A. paniculata* extract; 3. 50 mg/kg bwt of *A. paniculata* extract; 4. 500 mg/kg bwt of *A. paniculata* extract; 5. 2000 mg/kg bwt of *A. paniculata* extract.

Biochemical analysis

After 14 days of monitoring, the rats fasted overnight and were euthanized by cervical dislocation and blood samples were collected by cardiac puncture for biochemical analyses. Biochemical assays such as aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), billirubin, urea, uric acid and creatinine were carried out on plasma using RANDOX laboratory kits based on the principle first described by Reitman & Frankel (1957). Liver and Kidney were isolated and rapidly placed on ice and weighed. The tissues were homogenized in sodium phosphate buffer (pH 7.4) using a Teflon glass homogenizer with about 10 up and down strokes at approximately 1200 rev/min. The homogenates were then centrifuged at 3000 rpm for 10 minutes and clear supernatants obtained were used for other biochemical assays such as lipid peroxidation, total protein, total thiol and non-protein thiol content.

Statistical analysis

Results of replicate readings were pooled and expressed as mean ± standard deviation (SD), and the mean was

Table 1. Total phenolic and flavonoid contents of *A. paniculata* extract.

Parameters	Values
Total phenol (GAE/g)	2.95±0.01
Total flavonoid (QE/g)	2.26±0.03

Results are expressed as mean±standard deviation of replicate experiment (n=3).
Key:GAE- Gallic acid equivalent, QE- Quercetin equivalent.

Table 2. HPLC composition of *A. paniculata*

Compounds	Concentration (mg/g)
Gallic acid	1.18±0.01
Caffeic acid	2.03±0.02
Orientin	7.15±0.01
Quercetin	3.97±0.01
Apigenin	2.06±0.03

Results are expressed as mean±standard deviation of three determinations.

compared using one-way analysis of variance (ANOVA) followed by Tukey test, using GraphPad Prism 5.0. The significance level was taken at $p<0.05$.

Results

The results of total phenol and total flavonoid contents of aqueous extract of *A. paniculata* as presented in Table 1 are 2.95±0.01 GAE/g and 2.26±0.03 QE/g respectively. FRAP and ABTS scavenging ability of aqueous extract of *A. paniculata* (Table 2) was carried out and the results are 6.67±0.41 mg AAE/g and 40.05±0.05 µmol TEAC/g respectively.

DPPH radical (DPPH*) scavenging ability (Figure 1A) and hydroxyl radical (OH*) scavenging ability (Figure 1B) of aqueous extract of *A. paniculata* revealed that the extract was able to scavenge DPPH* and OH* with increase in concentration of the extract. Iron chelating ability

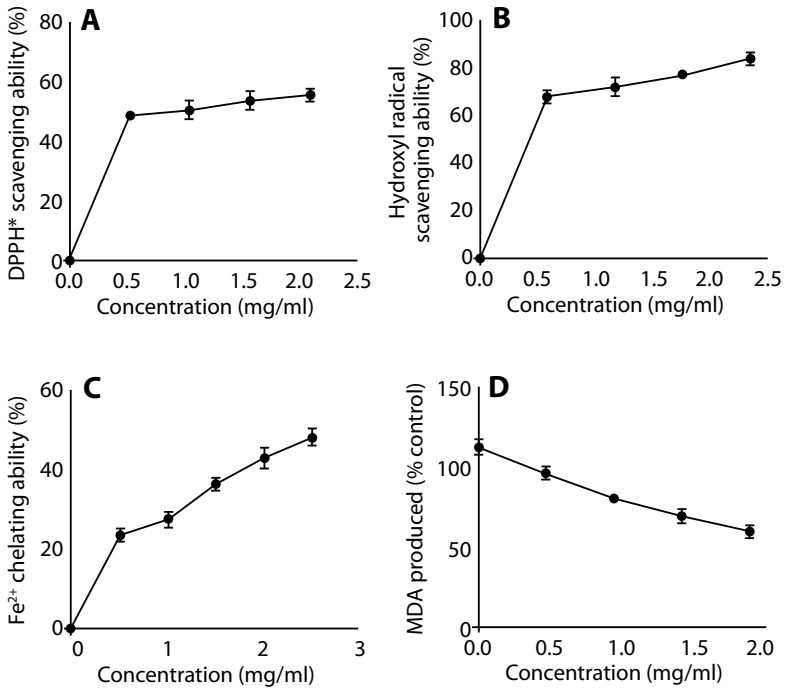

Figure 1. *In vitro* antioxidant properties of *A. paniculata* aqueous extract as typified by (A) DPPH radical scavenging ability, (B) hydroxyl radical scavenging ability, (C) Fe^{2+} chelating ability, and (D) inhibition of Fe^{2+}-induced lipid peroxidation (n=3).

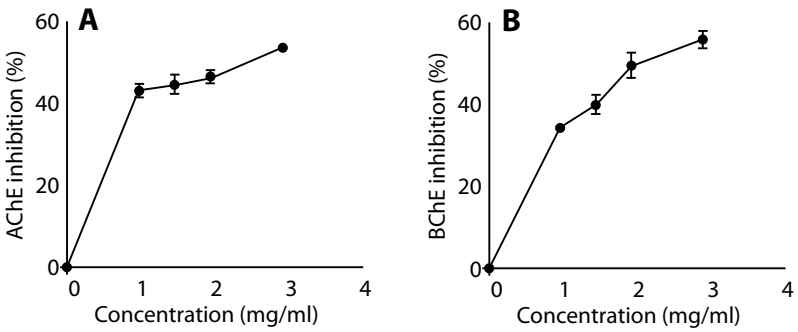

Figure 2. Effect of *A. paniculata* aqueous extract on (A) acetylcholinesterase (AChE), and (B) butyrylcholinesterase activity *in vitro* (n=3).

of *A. paniculata* (Figure 1C) revealed that the extract was able to chelate Fe^{2+} in concentration dependent manner.

Presented in Figure 2A is the result of the effect of aqueous extract of *A. paniculata* on AChE activity. It was observed that the activity of the enzyme was inhibited with increase in concentration of the aqueous extract. Similarly, in Figure 2B aqueous extract of *A. paniculata* was able to inhibit the activity of the enzyme (BChE) in concentration-dependent manner. The inhibitory effect of aqueous extract of *A. paniculata* aqueous extract on iron-induced lipid peroxidation (Figure 1D) shows that the extract inhibited MDA produced with increase in concentration.

HPLC composition of *A. paniculata* (Table 3) revealed phytochemicals present in the plant such as gallic acid, quercetin, orientin, caffeic acid and apigenin.

Table 4 depicts the effect of aqueous extract of *A. paniculata* on liver function parameters in Wistar rats. Administration of the extract showed no significant effect on plasma ALT, AST and ALP levels at the various doses when compared to control. However, an increase in albumin (ALB), total bilirubin and direct bilirubin levels in the group that received 2000 mg/kg AP were observed when compared to the control group. Similarly, the effect of aqueous extract of *A. paniculata* on kidney function test (Table 5) revealed that administration of the extract led to in increase in plasma urea and uric acid levels in the group that received 2000 mg/kg AP when compared to the control. However, no significant difference ($p>0.05$) was observed between other treated groups. Also, there was no significant difference ($p>0.05$)

Table 3. Ferric reducing antioxidant property (FRAP) and ABTS radical scavenging ability of *A. paniculata* aqueous extract

Parameters	Values
FRAP (mg AAE/g)	6.67±0.41
ABTS (µmol TEAC/g)	40.05±0.05

Results are expressed as mean±standard deviation of replicate experiment (n=3). Key: AAE- Ascorbic Acid equivalent, TEAC- Trolox equivalent antioxidant capacity

Table 4. Effect of various doses of *A. paniculata* aqueous extract on liver function markers.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
AST (U/l)	10.17±1.21	6.07±0.08*	8.42±2.41	9.47±1.05	9.84±1.32
ALT (U/l)	12.98±0.51	3.07±0.51*	7.16±0.89*	10.43±0.64	12.74±1.15
ALP (U/l)	250.4±15.7	142.8±30.1*	185.1±21.80*	229.7±10.5	241.4±24.10
ALB (mg/dl)	3.70±0.37	4.01±0.23	4.13±0.12	4.27±0.17	4.35±0.04
Total bilirubin (mg/dl)	1.29±0.10	0.77±0.07*	0.93±0.06	1.09±0.04	1.31±0.07
Direct Bilirubin (mg/dl)	0.46±0.03	0.25±0.06*	0.35±0.03	0.39±0.02	0.49±0.04

Results are expressed as mean±standard deviation (n=5). *$p<0.05$ versus control group. KEY- Group 1: Control; Group 2: 5 mg/kg *A. paniculata*, Group 3: 50 mg/kg *A. paniculata*; Group 4: 500 mg/kg *A. paniculata*; Group 5: 2000 mg/kg *A. paniculata*

Table 5. Effect of various doses of *A. paniculata* aqueous extract on kidney function markers.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Creatinine (mg/dl)	0.34±0.03	0.09±0.05*	0.17±0.10*	0.25±0.01	0.32±0.14
Urea (mg/dl)	13.51±0.46	7.74±0.93*	10.05±0.69	12.35±0.71	13.67±0.23
Uric acid (mg/dl)	4.06±0.15	1.40±0.22*	2.56±0.20	3.38±0.20	4.32±0.08

Values are expressed as mean±standard deviation (n=5). *$p<0.05$ versus control group. KEY- Group 1: Control; Group 2: 5 mg/kg *A. paniculata*; Group 3: 50 mg/kg *A. paniculata*; Group 4: 500 mg/kg *A. paniculata*; Group 5: 2000 mg/kg *A. paniculata*

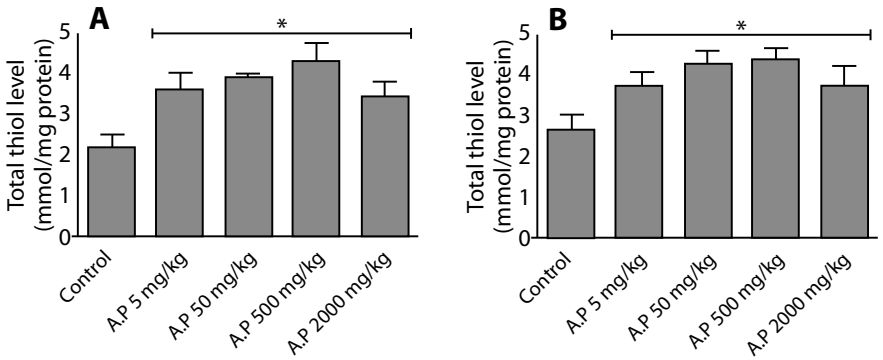

Figure 3. Effect of *A. paniculata* aqueous extract administration on (A) liver, and (B) kidney total thiol level. Bar represent mean ± standard deviation (n=5). *$p<0.05$ versus control group.

observed in plasma creatinine levels of treated groups as compared to control.

Figure 3A shows the effect of administration of various doses of *A. paniculata* on total thiol levels in the liver of Wistar rats. The administered doses resulted in increase of total thiol levels and were significantly different when compared to the control group. As shown in the result, the various doses administered increased non-protein thiol levels. However, groups that received 5, 50 and 500 mg/kg AP were significantly different as compared to the control. No significant difference was observed between the control group and the group that received 2000 mg/kg AP ($p>0.05$).

Presented in Figure 3B is the result of the effect of administration of various doses of *A. paniculata* on total thiol levels in the kidney of Wistar rats. It was observed that the doses of aqueous extract of *A. paniculata* administered resulted to an increase in total thiol levels across all treated groups in relation to control group. Also, the effect of administration of various doses of *A. paniculata* on non-protein thiol levels in the kidney of Wistar rats caused an increase in non-protein thiol levels when compared to the control group.

Discussion

Phenols and flavonoids are important constituents of plants that play a major role in scavenging free radicals and also take part in antioxidant activity because of the presence of hydroxyl group in these phytochemicals (Kinnari *et al.,* 2016). The antioxidant potential of plant can be observed when the plant is able to scavenge free radicals, chelate metals and inhibit lipid peroxidation. Radicals are the major reactive oxygen species that cause biological damage, which results to lipid peroxidation (Valko *et al.,* 2006). Results from this study (*in vitro*) showed that aqueous extract of *A. paniculata* exhibited ABTS*, DPPH* and OH* scavenging ability in a concentration dependent manner making it a potent antioxidant. This is in agreement with the work of Oboh *et al.* (2018). The plant's ability to scavenge free radicals may be attributed to the capability of the extract to readily donate hydrogen ion to free radicals in order to make it stable (Krishna-Kumar *et al.,* 2012).

Similarly, the ability of a plant to chelate Fe^{2+} makes a good feature of antioxidant mechanism so as to prevent Fe^{2+} from initiating lipid peroxidation and oxidative stress through catalyzed reactions (Dastmalchi *et al.,* 2007; Uttara *et al.,* 2009). This present study showed that aqueous extract of *A. paniculata* was able to chelate iron (Fe^{2+}) and also exhibited iron reducing property in concentration dependent manner. This result also supports previous studies (Adefegha *et al.,* 2015).

The cholinergic system (AChE and BChE) is known to play a role in cognitive processes. Availability of cholinesterase may lead to increase neurotoxicity of plaques and neurofibrillary tangles (NFTs) in the brain allowing it to be vulnerable to neurodegenerative diseases such as Alzheimer's disease, Parkinson disease *etc.* (Darvesh *et al.,* 2010; Montine *et al.,* 2012). These enzymes hydrolyze the breakdown of butrylcholine (BCh) and acetylcholine (ACh) to acetate and choline. Inhibition of cholinesterase has been accepted as a therapeutic approach in the treatment and management of neurodegenerative diseases in order to increase the availability of ACh and BCh in neurotransmission (Woo *et al.,* 2011; Verma *et al.,* 2014). Results in this study showed that aqueous extract of *A. paniculata* inhibited the activities of AChE and BChE in concentration dependent manner. This suggest that the plant extract could possess neuroprotective properties and thus improve cognition. This agrees with previous studies (Nwanna *et al.,* 2016) where the neuroprotective effect of some commonly consumed vegetables was evaluated. The inhibitory effect of the extract on AChE and BChE could be directly or indirectly related to availability of compounds such as quercetin and gallic acid, compounds with known anticholinesterase properties (Adefegha *et al.,* 2018).

Thiobarbituric acid reactive species (TBARS) assay is a simple and reliable flourometric technique widely used to measure malondialdehyde (MDA) levels in plasma, serum and tissue homogenates (Giera *et al.,* 2012). Lipid peroxidation is a marker for oxidative stress, which is linked to production of reactive oxygen species involved in oxidative damage of cell structures (Dianzani & Barrera, 2008). Plants with reduction power indicate good electron donors and may reduce oxidized intermediates of lipid peroxidation, acting as antioxidants in the body (Chanda & Dave, 2009). In this study, aqueous extract of *A. paniculata* showed inhibitory activity on $FeSO_4$ induced lipid peroxidation. It was observed that the plant extract inhibited MDA produced with increase in concentration of the extract. This finding supports previous studies (Saliu & Olabiyi, 2016). These inhibitory effects of the extract could attribute to the antioxidant properties and scavenging ability of the plant, mainly radical scavenging and metal chelating abilities (Oboh *et al.,* 2018).

HPLC characterization of *A. paniculata* revealed that orientin and quercetin are the most abundant phytochemicals present and could be responsible for the plant's antioxidant property. Orientin is a flavonoid that has been shown to possess wide range of pharmacological activities such as antioxidant, anti-ageing and anti-inflammatory effects (An *et al.,* 2012; Sun *et al.,* 2013). It is a phenolic compound that can be found in peels and juice of passion fruit and also in millet (Brazier-Hicks *et al.,* 2009). The phenol groups present in the structure of orientin could be responsible for the antioxidant property. Quercetin (3, 3, 4, 5, 7-pentahydroxyflavone) is a flavonol that is majorly found in vegetables and fruits, it represents a major class of polyphenols (Del Rio *et al.,* 2013). Quercetin have been reported to possess biological and pharmacological activities due to its effects on inflammation, infections and cardiovascular diseases (Kelly, 2011; Russo *et al.,* 2012). Quercetin is known to be a strong antioxidant and a potent scavenger of reactive oxygen species due to the catechol group in the B ring and the OH group

at positions 3 and 5 of the heterocyclic ring (Boots *et al.,* 2008; Kim *et al.,* 2013). Therefore, it is important at this point to infer that the observed antioxidant activities of the extract could be attributed to the presence of compounds such as orientin and quercetin.

Toxicology test is usually done to provide information on the toxic effect of a substance and it also helps to decide doses of the substance for animal studies. Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) as well as albumin (ALB) and bilirubin levels in the plasma/serum have been described as useful markers in clinical evaluation as they give information on the state of pathological changes in the liver (Ashafa *et al.,* 2010). Elevated levels of plasma/serum AST and ALT activities could be a result of damage to plasma membrane, which could lead to leakage of these enzymes from the hepatocytes (Fahmy *et al.,* 2014). The results from the effect of administration of *A. paniculata* aqueous extract on plasma liver function in this study revealed that there was no significant (p>0.05) increase in plasma ALT, AST and ALP activities as compared to the control. This suggests that oral administration of the plant extract at doses of 5–2000 mg/kg had no hepatotoxic effect in relation to the control.

Creatinine, urea and uric acid are also markers used for kidney functions to determine the extent of renal damage (Sunmonu *et al.,* 2006). They are end products passed into the blood stream to be excreted by the kidney, so elevated levels of these products in the blood could indicate renal dysfunction (Oloyede & Sunmonu, 2009). The effect of administration of *A. paniculata* aqueous extract on plasma kidney function showed that there was significant (p<0.05) increase in urea, creatinine and uric acid levels at dose 2000 mg/kg when compared to the control. This result is similar to the report of Ashafa *et al.* (2010).

Thiols (-SH) are non-enzymatic antioxidants that play several roles in biological systems by protecting against reactive oxygen, nitrogen species, and removal of reactive electrophiles (Flora *et al.,* 2008). Thiols are of two types; total thiol and non-protein thiol, the level of these enzymes could be affected by many conditions such as oxidative stress (Rosas-Diaz *et al.,* 2015; Soysal *et al.,* 2017). A decrease in thiol concentration has been linked to several cellular dysfunction, membrane permeability and energy production (Shipounova *et al.,* 2010; Nakamura & Lipton, 2010). This study revealed that the various doses of *A. paniculata* administered increased thiol levels (thiol and non-protein) in the liver in relation to the control. Also, it was observed that *A. paniculata* was able to increase the total thiol and non-protein thiol levels in the kidney of Wistar rats when compared to the control during the toxicological evaluation.

Conclusion

In conclusion, results from this study revealed that aqueous extract of *A. paniculata* possess antioxidant properties by inhibiting lipid peroxidation and scavenging DPPH, ABTS and OH radicals *in vitro*. Also, toxicology evaluation of the extract did not result in any death of the animals and no pathological changes was observed. Therefore, oral administration of aqueous extract of *A. paniculata* up to 2000 mg/kg BWT is considered safe. However, further studies on the plant's toxicity should be carried out.

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