In vitro and *In vivo* Evaluation of the Antioxidant Properties of Aqueous Extract of *Andrographis paniculata* Leaves

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Abstract: The *in vitro* and *in vivo* antioxidant property of the aqueous extract of *Andrographis paniculata* leaf was investigated. The aqueous extract was lysophilized and the phytochemicals and minerals present were quantified. *In vitro* antioxidant capabilities were estimated using 2, 2-diphenyl-1-picrylhdrazyl free radical scavenging activity and ferric reducing antioxidant power (FRAP). The calculated IC_{50} and FRAP of ascorbic acid and *A. paniculata* were not significantly different ($p<0.05$). The ascorbic acid equivalent antioxidant capability of the extract was determined to be (112.752 ± 206.737) 10^3mgAA/100g. Twenty eight male albino rats were used for the *in vivo* antioxidant capability studies and were randomly picked into four groups. 250mg/kg, 500mg/kg and 1000mg/kg BW doses were administered daily for 56 days to three groups, while the fourth group received distilled water. After 41 days of administration, five rats in the 1000mg/kg BW dose group had the fur falling off, had increased thirst for water with watery clay coloured feaces and were less active. The effects of the aqueous extracts on the liver malonyldialdehyde (MDA) and glutathione (GSH), serum superoxide dismutase (SOD) and catalase (CAT) activities were estimated. Liver GSH levels were increased significantly ($p< 0.05$) with the 250mg/kg and 1000mg/kg been statistically equal. The MDA concentrations were not significantly reduced ($p< 0.05$) for the 1000mg/kg BW. Serum SOD activities increased significantly ($p< 0.05$) dose dependently, while the CAT activities also increased significantly with the 250mg/kg and 1000mg/kg BW been statistically equal. The histopathological studies of the liver revealed that the aqueous extract of *A. paniculata* conferred protection on the liver at 250mg/kg BW and 500mg/kg BW doses. From the foregoing, it was suggested that the aqueous extract of *A. paniculata* leave possessed high antioxidant property, but toxicity studies will be necessary to determine the safety of the chronic consumption.

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Key words: *Andrographis paniculata;* Antioxidant property; Chronic consumption; Toxicity

1.0 Introduction

Free radicals and other reactive oxygen species (ROS) present in the body are generated both endogenously and exogenously (Arouma, 1994). They are necessary for the body normal metabolism. However, the presence of excess free radicals in the body system could result to oxidative stress and tissue damages (Harman, 1998). Oxidative damages caused by these free radicals to living cells mediate the pathogenesis of many chronic diseases, like atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases (Kappus, 1985: Halliwell and Grootveld, 1987). The free radicals generated in the body are neutralized by the body's natural antioxidant defenses, e.g. glutathione, glutathione peroxidase, catalase, and superoxide dismutase (Aruoma, 1994). However, endogenous antioxidant defenses are not completely efficient and thus, dietary antioxidants are needed to diminish the cumulative effects of oxidative damage caused by excess ROS that remained in our system.

Antioxidants are not only needed by our body to combat ROS, but are also important as food additives. They can be either synthetic or naturally occurring. Synthetic antioxidants have been shown to possess carcinogenic activity, which leads to a need for the replacement of synthetic antioxidant with naturally occurring ones (Madsen and Bertelsen, 1995). Natural antioxidants are shown to be safe and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties.

Andrographis paniculata, an herbaceous plant native to India and Sri Lanka is a member of the plant family, *Acanthaceace*. It is known in the North-Eastern India as 'Maha-tita', literally meaning "king of bitters" in English (Coon and Ernst, 2004). The general uses of *A. paniculata* in folk medicine has been alleged to enhance the immune system, promote digestion, enhance liver, kidney and heart

functions, relieves pain, expel intestinal worms, fight bacteria infections, reduce blood sugar, promote respiratory mucus discharge, anti-malaria, as sedative among others. However, it is been used for conditions as diverse and unrelated as snakebites and diabetes, as well as terminating pregnancies. These conditions are so varied and seemingly unconnected that more recent research has sought to corroborate some of these applications (Panossian *et al.,* 2000). Consumption of the aqueous extracts of *A. paniculata* as tonic to boost the immune system and to prevent, or cure infective, chronic and degenerative diseases is popularly recommended by traditional medicine practitioners (Kulinchencko *et al.*, 2003: Coon and Ernst, 2004: Igwe et al., 2007).

Many health derangements like: atherosclerosis, stroke, arthritis, chronic inflammatory diseases, cancer etc, have been linked with oxidative damage from excess free radicals in the body system (Harman, 1998): and since, the need for natural antioxidants to augment the activities of the body's endogenous antioxidants inherent in biological cells can't be overemphasized. Thus, appraising the antioxidant capabilities of the aqueous extract of *A. paniculata* will give important information on the alleged use of the plant as blood tonic, for the prevention of chronic and degenerative diseases associated with oxidative damage. This study, therefore, aimed to provide information on the antioxidants potentials of the aqueous extracts of *A. paniculata in vitro* and in albino rats.

2.0 Materials and methods

2.1 Plant material for analysis

The aerial part of *A. paniculata* was collected from the natural habitat around Airport area in Ilorin, Kwara State. The plant was identified at Forest Research Institute of Nigeria, Ibadan, Oyo State. The leaves were rinsed thoroughly in distil water and dried in the shade for 14 days. The dried leaves were ground to fine powder, using a domestic electric grinder and extracted with water at 37^{o}C. The filtrates were pulled together and centrifuged at 2000rpm for 10 minutes. The supernatant was filtered again and lyophilised using a freeze dryer. The yield of the aqueous extract was 16.28%$^{w}/_{w}$. The dried extract was stored in the desiccators and kept in the dark till when needed.

2.2 Chemicals

All the chemicals and reagents used in the study were of analytical grades from the Bristish Drug House and Sigma Aldrich.

2.3 Phytochemical analysis

The chemical classes of constituent in the plant materials were identified and quantified according to the methods described by Harborne (1973) and Trease and Evans (1983).Oxalate contents were determined by the spectrophotometric methods of Hang and Lantzsch (1983). Determinations were done in triplicates.

2.4 Mineral composition

The sample was incinerated into ash, dissolved in 1 ml of 2M HCl and diluted to 100 ml with de-ionized water. The resulting extract was used for the determination of copper, zinc, iron, calcium and magnesium by the use of an atomic absorption spectrophotometer (Perkin Elmner, USA). Sodium and potassium levels were quantified using flame photometer. Phosphorus was determined as phosphate by the vanadomolybdate colorimetric method (Pearson, 1976). All determinations were done in triplicates.

2.5 In vitro antioxidant capability

2.5.1 DPPH radical scavenging activity assay

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured using the method used by Oboh (2005) with modifications. Different dilutions of the extracts were prepared in triplicate. Then 1 ml of each dilution was added to 2 ml of 0.15mM of DPPH. The mixture was allowed to stand for 30 min before measuring the absorbance at 517 nm. Antioxidant activity
(AA) was expressed as the percentage of DPPH decrease using the equation:

$$AA\ (\%) = \frac{(Acontrol - Asample)}{Acontrol} 100$$

IC_{50} of the extract was determined from the graph of antioxidant activity (%) against amount of extract (mg). Ascorbic acid was used as standard and results were also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid/100 g of fresh plant material with the following equation:

$$AEAC\ (mg\ ascorbic\ acid/100g) = \frac{IC_{50}\ (ascorbic\ acid)}{IC_{50}\ (sample)} 10^{5}$$

2.5.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing property of the extracts was determined using assay described by Yen and Chen (1995). Diluted extracts (1.0ml) were mixed with 2.5 ml of potassium phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide (1g/100ml). The

mixture was incubated at 50°C for 20 min. 2.5 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. Equal volume of distilled water was added to 2.5 ml of the mixture before the addition of 0.5 ml of $FeCl_3$ (0.1 g/100 ml). The procedure was carried out in triplicate and allowed to stand for 30 minutes before measuring the absorbance at 700 nm. The absorbance obtained was converted to gallic acid equivalents in milligrams per gram fresh material (mg GAE/g) using a gallic acid standard curve.

2.6 Experimental animals and procedure

Twenty eight male albino rats (220–240 g), 18–20 weeks old were obtained locally from Oyo Town, Oyo State. The rats were randomly grouped into four, comprising of seven rats per group and were housed in animal care facility at the Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso with 12-hours light/dark cycle. They were fed free standard pellet diet and tap water, and were acclimatized for 8 days before the administration of the aqueous extract of *A. paniculata* was commenced. Calculated doses of the plant extracts (mg/kg body weight of rat) were dissolved in distilled water and stored air tight at 4^0C. Administration was performed orally at 24 hours interval, using metal cannula attached to a 2ml syringe.

Group 1: Control, received 1.5ml distilled water.
Group 2: Test, received 250 mg/kg body weight of *A. paniculata*
Group 3: Test, received 500 mg/kg body weight of *A. paniculata*
Group 4: Test, received 1000 mg/kg body weight of *A. paniculata*

Administration lasted for 56 days, after which the rats were fasted for 12 hours and sacrificed by cervical dislocation and incision was made quickly in the chest region. The heart was pierced and blood was collected for clinical chemistry parameters, and the liver was decapsulated.

2.7 In vivo antioxidant capability

2.7.1 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in the liver was estimated by the method of Fraga *et al*. (1981). 0.5ml of normal saline and 1.0ml of 10% trichloroacetic acid (TCA) were added to 0.5ml of the liver homogenate. The solution was mixed properly and centrifuged at 3000 rpm for 20 minutes. 0.25ml of thiobarbituric acid (TBA) was added to 1.0ml of the supernant. The solution was mixed and boiled for 1 hour at 95^0C, and cooled to room temperature. The absorbance was measured at 532nm.

2.7.2 Determination of reduced glutathione (GSH)

GSH was determined by the method of Ellman (1959). 0.2ml of the liver homogenate was mixed with 1.8ml of EDTA solution. 3.0ml of precipitating reagent (1.67g of meta-phosphoric acid, 0.2g of EDTA disodium salt, 30g NaCl in 1litre of distilled water) was added to the tissue homogenate, mixed thoroughly and left to stand for 5 minutes at room temperature. The solution was later centrifuged at 2000rpm for 10 minutes, and to 2ml of the filterate, 4.0ml of 0.3M disodium hydrogen phosphate solution and 1ml of 5, 5-dithio -bis-2- nitrobenzoic acid (DTNB) reagent were added and read at 412nm.

2.7.3 Determination of serum superoxide dismutase (SOD) and catalase (CAT)

Serum SOD and CAT activities were determined using the standard methods described by Mistra and Fridovich (1972) and Sinha (1971) respectively.

2.8 Statistical analysis

The data were expressed as mean ± S.E.M. Results were analyzed statistically by one-way analysis of variance (ANOVA), followed by the Duncan Multiple Range Test (DMRT) for the pair-wise mean comparison, using the SPSS 14.0 for Window software. *P*-value <0.05 was regarded as statistically significant. Different alphabets were used to denote significantly different means ($p<0.05$).

3.0 Results

3.1 Phytochemicals and minerals estimation

The phytochemicals that were present in the aqueous extract of *A. paniculata* were quantified as depicted in table 1. High levels of alkaloids and saponins were observed (table 1). Table 2 depicts the level of the minerals in the plant extract. The levels of nitrogen, magnesium, iron, sodium, potassium and zinc were reasonably high, compared to others (table 2). However, the presence of lead may be a pointer to toxicity.

3.2 In vitro antioxidant capability

The result of the *in vitro* antioxidant property of the aqueous extract of *A. paniculata* is shown table 3. The *in vitro* antioxidant properties of the plant extract were comparable with those of the standard (ascorbic acid). The calculated IC_{50} values of the aqueous extract (3.623 ± 0.302) 10^{-3} mg/ml and that of ascorbic acid (4.085 ± 0.166) 10^{-3} mg/ml were not significantly reduced ($p<0.05$), while the determined AEAC of the extract was ($112.752 \times 10^3 \pm 206.737$) mgAA/100g. Also, the abilities of the extract to reduce ferric acid showed that the

antioxidant power of ascorbic acid and the extract were not significant reduced ($p<0.05$).

Table1. Phytochemicals Quantified in Aqueous Extract of *A. paniculata*.

Phytochemical	***A. paniculata* (%)**
Alkaloids	7.823 ± 0.051
Tannin	0.018 ± 0.010
Oxalate	2.231 ± 0.037
Flavonoids	0.321 ± 0.002
Phenol	0.025 ± 0.004
Cyanogenic glycosides	0.004 ± 0.001
Saponins	2.429 ± 0.011
Cardenolides	0.010 ± 0.003

Values are means of three determinants.

Table 2. Minerals Quantified in Aqueous Extract of *A. paniculata*

Mineral	***A. paniculata* (%)**
Lead	0.149 ± 0.006
Phosphorus	0.683 ± 0.004
Magnesium	1.479 ± 0.003
Copper	0.349 ± 0.009
Calcium	1.622 ± 0.033
Iron	2.727 ± 0.021
Nitrogen	2.418 ± 0.007
Zinc	1.275 ± 0.013
Selenium	0.120 ± 0.003
Sodium	2.018 ± 0.042
Potassium	1.239 ± 0.015

Values are means of three determinants

3.3 In vivo antioxidant capability

Administration of the aqueous extract of *A. paniculata* to albino rats recorded no mortalities. However, the rats of the 1000mg/kg BW dose group (IV) were often less active after administration and consumed more water than the other dose groups. After 41 days of administration, five of the seven rats in this group had their fur dropping off, the feaces were watery with the colour faded (very light brown) and the eyes were very red and budged out.

The *in vivo* antioxidant capability determination showed significant increases ($p<0.05$), (table 4), in the serum superoxide dismutase (SOD) activities of the test groups, in a dose dependent manner, but the 500mg/kg BW and 1000mg/kg BW doses were statistically equal. The CAT activities, also, increased significantly, but with no significant differences between the 250mg/kg BW and 1000mg/kg BW (table 4). The liver GSH levels were increased significantly in a dose dependent manner, but with no significant difference ($p<0.05$) in the 250mg/kg BW and 1000mg/kg BW. Liver lipid peroxidation products estimation (table 4), showed that the malonyl dialdehyde (MDA) levels was not reduced significantly in 1000mg/kg BW, while significant reductions were observed in the other dose groups ($p<0.05$).

It is worthy of note that the 1000mg/kg BW dose reduced the serum SOD and CAT activities, and the liver GSH levels to the 250mg/kg BW dose, although, not significantly different ($p<0.05$). Thus, the 1000mg/kg BW dose might be potentially toxic, because the 500mg/kg BW dose did not show such trend. However, the foregoing results suggested that the aqueous extract of *A. paniculata* possessed high antioxidant properties and might prevent the risk of oxidative stress.

3.4 Histopathology of Liver

Histopathological study on the liver of rats (figures 5-8), revealed moderate hepatic fatty degeneration and hyperplasia in the control rat, while the integrity of the hepatocytes of the 250mg/kg BW and the 500mg/kg BW dose groups were not significantly compromised. The defects or inflammation in the hepatocytes of the 1000mg/kg BW rat were significant to the 250mg/kg BW and 500mg/kg BW dose groups. Non-significant < 25%, Mild > 25%, Moderate < 50%, and Severe > 50% (of defect or inflammation of hepatocytes).

Table 3. *In vitro* Antioxidant Activity of Aqueous Extract of *A. paniculata*

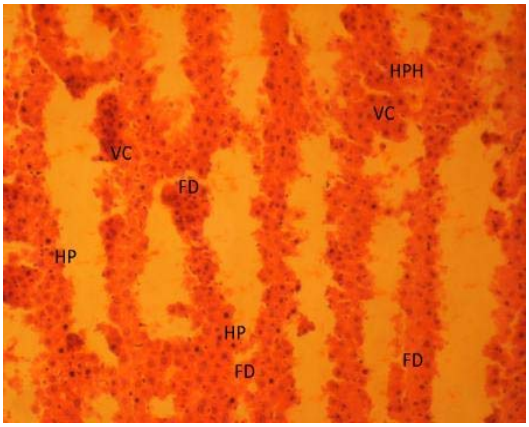
	Ascorbic acid	***A. paniculata***
IC_{50} (mg/ml) 10^{-3}	4.085 ± 0.166 [a]	3.623 ± 0.302 [a]
AEAC (mgAA/100g) 10^3		112.752 ± 206.737
FRAP (mg GAE/g)	14.312 ± 0.270 [a]	12.821 ± 0.753 [a]

Values are means of three determinants and values with different superscripts are significantly different ($p<0.05$).

Table 4. Effect of Extract on Liver MDA and GSH, and Serum SOD and CAT

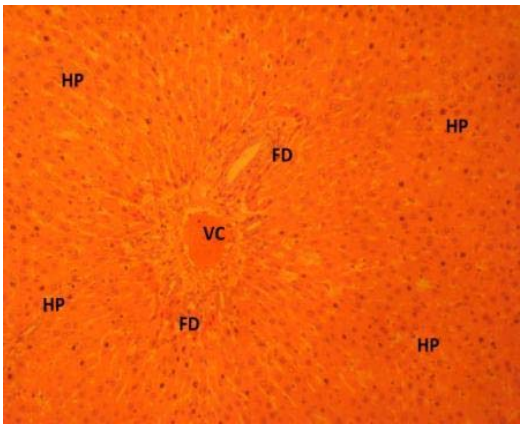
Group	**MDA (per mg of protein)**	**GSH (µg/mg of protein)**	**SOD (% inhibition)**	**CAT(µmol/min/ml)**
I	3.813 ± 0.416 [a]	50.211 ± 4.501[a]	53.65 ± 8.127 [a]	1.065 ± 0.089 [a]
II	1.589 ± 0.262 [b]	62.734 ± 2.338[b]	61.45 ± 4.296[b]	1.433 ± 0.170[b]
III	1.072 ± 0.402 [c]	68.423 ± 3.678[c]	69.11 ± 4.970 [c]	1.889 ± 0.104 [c]
IV	3.501 ± 0.597 [a]	58.566 ± 4.142[b]	67.03 ± 5.001[c]	1.302 ± 0.229 [b]

Values are means of six determinants and values with different alpabets are significantly different ($p<0.05$).



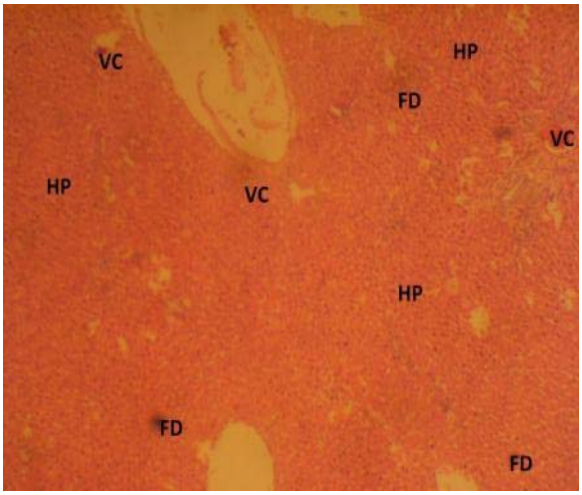
MAG X 100

Figure 5. Photomicrograph of the Liver of an Albino Rat in the Control Group. HP (hepatocytes), VC (venus congestion), HPH (hepatic hyperplasia), FD (fatty degeneration). Moderate hepatic necrosis, hepatic hyperplasia, severe vascular congestion and severe multi-foci fatty degeneration were seen in the hepatocytes.



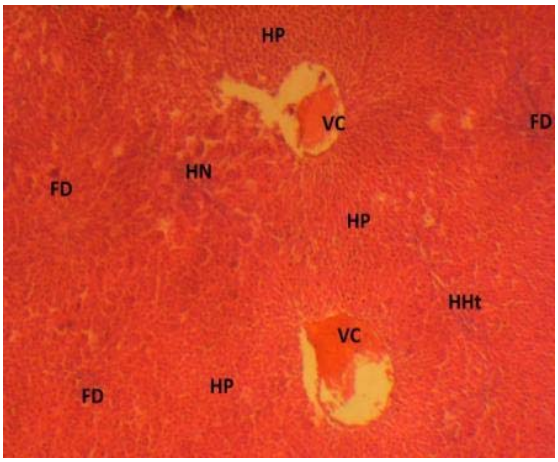
MAG X 100

Figure 6. Photomicrograph of the Liver of an Albino Rat Representing the 250 mg/kg BW Dose Group. HP (hepatocyte), FD (fatty degeneration), VC (venus congestion). Normal hepatocytes with non significant foci venus conjestion and mild fatty degeneration.



MAG X 100

Figure 7. Photomicrograph of the Liver of an Albino Rat Representing the 500 mg/kg BW Dose Group. HP (hepatocytes), FD (fatty degeneration) and VC (venus congestion). Normal hepatocytes with portal triad, mild venus conjestion and mild fatty degeneration.



MAG X 100

Figure 8. Photomicrograph of the Liver of an Albino Rat Representing the 1000 mg/kg BW Dose Group. HP (hepatocyte), VC (venus congestion), HPH (hepatic hyperplasia), FD (fatty degeneration) and HN (hepatic necrosis). Moderate fatty degeneration, mild venus congestion, moderate localized hepatic necrosis and hepatic hypotrophy.

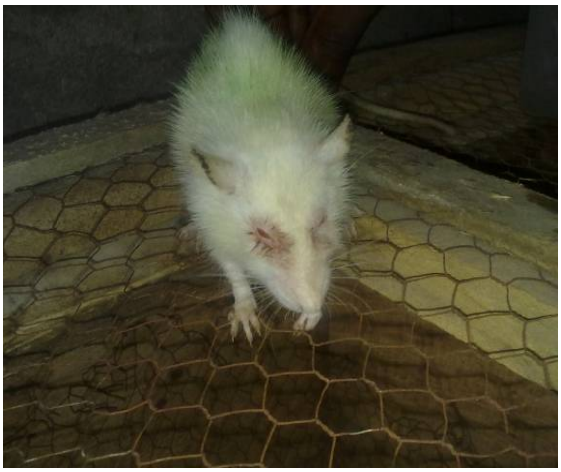


Figure 9: The Photograph of a Rat with Eyes Defect and Dropping Fur in the 1000mg/kg BW Dose Group.

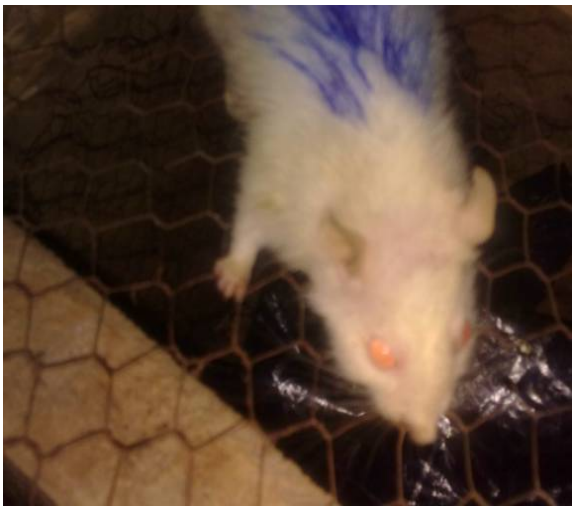


Figure 10: The Photograph of a Rat with Swollen Red Eyes in the 1000mg/kg BW Dose Group.

4.0 Discussion

Phytochemicals are secondary plant metabolites and have different functions, including strength, pollination, defense against predators etc, while some are simply waste products (Ibegbulem *et al*., 2003). In animals, some of these phytochemicals exhibit pharmacological activities (Trease and Evans, 1983). Plants that contain alkaloids, flavonoids and saponins in substantial quantities might have good hypoglycemic and hypocholesterolemic activities (Price *et al*., 1987; Khanna, 2002; and Igwe *et al*., 2007). The phenolic compounds are known for their free radical scavenging ability and may, thus, enhance the activities of endogenous antioxidant enzymes (Yoshiki *et al*., 1998; Hu *et al*., 2002; and Tsao and Akhtar, 2005). Saponins have been shown to protect biological cells against lipid peroxidation and also enhance the activities of antioxidant enzymes (Hu *et al*., 2002: Tirtha *et al*., 2007). The aqueous extract of *A.paniculata* contains substantial quantities of saponins, which inferably means that the extract will have antioxidant capabilities.

Minerals like copper, zinc, selenium, manganese and iron may enhance the activities of antioxidant enzymes, because these elements are cofactors for such enzymes. Thus, *A. paniculata* will have antioxidant capabilities, because it contains appreciable quantities copper, zinc and iron. Also, the quantity of sodium, potassium and magnesium observed in the aqueous extract may help among other areas, in the maintenance of the osmotic pressure, water distribution and body pH. This could explain why the plant is believed to prevent oedema, kidney problems and oliguria (Claxito *et al*., 1998). However, the presence of lead may suggest possible toxicities, because no safe level of lead in blood has been established.

The *in vitro* antioxidant capacity of the extract was measured by determining the antioxidant activity against free radicals of DPPH and the capability of the extract to inhibit the colour of DPPH to 50% is measured in-terms of IC_{50}. The results of the DPPH free radical scavenging activity of the aqueous extract of *A. paniculata*, which were comparable with those of ascorbic acid, supported the possibilities that the plant extracts may have good antioxidant capabilities (Lim and Murtijama, 2008). Fe^{3+} to Fe^{2+} transformation is an indication of reductive ability. The strong ferric reducing antioxidant power exhibited by the aqueous extract of *A. paniculata*, supported the antioxidant potential of the plant extract.

A certain level of free radicals is essential for a good health, as they are involved in fighting infections, repair works within cells, the contraction of smooth muscles in the blood vessels etc. However, the over production of free radicals could cause an imbalance between oxidation and antioxidation, thereby leading to inactivation of enzymes, cancers, heart diseases, oxidative stress, cell damage etc.(Carando *et al*., 1999). Although, there are no proof yet that the ingestion of antioxidants prolong life span or cure any disease, but growing evidences strongly suggest they can prevent the deleterious effect of the prolonged exposure of cells free radicals (Corder *et al*., 2002). However, natural antioxidants have been shown to be safer than synthetic antioxidants and also, possess antiviral, anti-inflammatory, anti-cancer, anti-mutagenic and hepatoprotective properties (Madsen and Bertelsen, 1995).

Cells have a number of mechanisms to protect themselves from the toxic effects of ROS. These include free radical scavengers and chain reaction terminators enzymes, like SOD, CAT and GSH peroxidase (Proctor and McGinness, 1986). Inhibition of these protective mechanisms or the reduction in their activities would result in enhanced sensitivity of the cells to free radical-induced cellular damage, due to accumulation of superoxide ions and hydrogen peroxide. SOD removes superoxide ions (O_2^-) by converting it to hydrogen peroxide (H_2O_2), which could be rapidly converted to water and oxygen by CAT (Halliwell *et al*., 1992).

It is well documented that these two antioxidant enzymes are hepatocellular biomakers in assessing liver damage (Chottopadhyay *et al*., 2005). Antioxidant enzymes have cofactors that enhance their activities: selenium in glutathione peroxidase, zinc, iron and copper in SOD (McCord and Fridovich, 1983: Tainer *et al*., 1988). The significant increases observed in the SOD and CAT activities in the study support the results of the *in vitro* antioxidant capabilities and that of the mineral analysis. That is, the corresponding increases in SOD and CAT activities signify that the aqueous extract of *A. paniculata* has efficient protecting mechanisms in response to free radicals. However, the 1000mg/kg BW dose was less efficient in enhancing the H_2O_2 detoxifying activities of CAT, compared to the 500mg/kg BW.

Glutathione (GSH) is widely distributed in most cells. It is an intracellular reductant and plays major roles in enzyme catalysis, metabolism and transport. The enzyme, glutathione reductase, converts oxidized GSH to reduced GSH (Leeuwenburgh and Ji, 1995). Reduced GSH protects cells against free radicals, peroxides and other toxic compounds, by neutralizing them. Thus, reduced GSH enhances the antioxidative process and also, prevents the formation of disulphide bonds (SH) between the membranes of SH

groups (Vasudevan and Sreekumari, 2000). A large reserve of reduced GSH is present in the hepatocytes and red blood cells. Deficiency of GSH within cells can lead to tissue disorders and injury in organ like the liver, lungs and the muscle (Leeuwenburgh and Ji, 1995). The aqueous extract of *A. paniculata* demonstrated a good means of recovering reduced GSH, by the observed increase in the liver GSH level. The liver GSH result explained the results of the serum SOD and CAT activities, in which the 1000mg/kg BW increased significantly, the serum SOD, but not the CAT activity, compared to the 250mg/kg BW. That is, the ROS detoxification process of SOD and CAT was not efficiently balanced at 1000mg/kg BW dose. The 500mg/kg BW dose detoxified free radicals better than the other dose levels and would thus, reduce the severity or the risk of oxidative stress.

Oxidative stress has been linked strongly to the initiation and progression of tissue lipid peroxidation. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death (Arteel, 2003). It is a result of excess ROS generated in the cell. Malonyl dialdehyde (MDA) is one of the end products in the lipid peroxidation process (Kurata *et al.*, 1993). The liver is involved in the removal of wastes and zenobiotics from the blood and the detoxification of foreign substances through cytochrome P_{450}, thereby generating free radicals. MDA will always be present, even in healthy individuals, but in very minute quantity. Although, the liver was not challenged prior to administration of the plant extract, but the MDA levels of the liver were significantly reduced for the 250mg/ kg BW and 500mg/kg BW dose group only. The 500mg/kg BW dose equipped the liver better in neutralizing the generated free radicals in the cell, as supported by the liver MDA quantification. However, the non significant reduction observed with the 1000mg/kg BW may suggest possible toxicity at the dose level.

The photomicrographs confirmed that the extract did protect the liver at the administered dose, except the 1000mg/kg BW dose. Thus, the aqueous extract of *A.paniculata* has an efficient protective mechanism in response to ROS and may be associated with decreased risk of oxidative stress and free radical mediated tissue damage and diseases.

5.0 Conclusion

The overall results revealed that the aqueous extract of *A.paniculata* leaf has high antioxidant capabilities, and this could explain why the plant is allegedly consumed as tonic, to prevent degenerative diseases and maintain a healthy life. Since the extract conferred protections on the liver, it might be a good remedy in hepatotoxic conditions and in protecting the liver against diseases caused by oxidative stress, by enhancing the activities of cellular antioxidant defenses. However, the chronic consumption of the plant extract at 1000mg/kg BW dose was potentially toxic, as supported by the histopathological study on the liver. Further studies regarding the effect of the aqueous extract of *A.paniculata* leaf on serum glucose, protein and lipid metabolisms, and possible tissues toxicity is currently in progress.

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Disclosure Statement

"No competing financial interests exist".

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