Immune Modulation Potentials of Aqueous Extract of *Andrographis paniculata* Leaves in Male Rat

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Abstract: The immune modulation potentials of the aqueous extract of *Andrographis paniculata* leaves was investigated. The dry pulverized leaves were extracted with water and lysophilized. Forty male albino rats were randomly picked into four groups. The first group received distilled water, while the other groups were administered daily 250 mg/kg, 500 mg/kg and 1000 mg/kg BW doses for 84 days. Effect of the chronic administration of the extracts on haematological parameters, IL-6, TNF-α, serum levels of bilirubin and uric acid were estimated. The packed cell volume was not significantly changed ($p<0.05$), while haemoglobin and red blood cell were increased significantly ($p<0.05$) only in group four. Dose dependent significant increases ($p<0.05$) were observed in platelet count and erythrocyte sedimentation rate. Mean cell volume was reduced significantly ($p<0.05$), but with no significant differences ($p <0.05$) among the test groups. Mean cell haemoglobin and mean cell haemoglobin concentration showed significant reductions ($p<0.05$) in the group 4 only. The white blood cell and lymphocytes were increased significantly ($p< 0.05$) with group 2 and 3 been statistically equal. Significant reductions ($p< 0.05$) were observed in neutrophil and eosinophils in the group 4 rats only. Monocyte was increased significantly ($p<0.05$) in group 4 only. Dose dependent significant increases ($p<0.05$) were observed in the serum IL – 6 and TNF-α. Total and direct bilirubin decreased significantly ($p<0.05$) in group 2 and 3, while significant increases ($p<0.05$) was shown in group 4. Indirect bilirubin was increased significantly ($p< 0.05$) in group 3 and 4 only. Uric acid was reduced significantly ($p<0.05$) in group 2 and 3, while group 4 showed significant increase ($p<0.05$). The overall result suggested that the chronic consumption of the aqueous extract of *A. paniculata* boosted the immune functions, but the 1000 mg/kg BW dose predisposed to anaemia, possibly multiple myeloma and autoimmunity.
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1.0 Introduction

Medicinal plants are being used in traditional system of medicine from hundreds of years in many countries of the world (Oubre *et al*., 1970). These plants are not only for primary health care, and not just in rural areas of developing countries, but also in developed countries, where modern medicine are predominantly used (Kamboj, 2000). In recent times, the interest in medicinal plants has increased in a great deal due to its therapeutic properties, which is very useful in healing various diseases and the advantage of these plants is being 100% natural (Calixto, 1998). According to the World Health Organization, about 80% of the populations in many third world countries still use traditional medicine for their primary health care, due to poverty and lack of access to modern medicine (De Silva, 1997).

Andrographis paniculata, also known commonly as "King of Bitters," is a member of the plant family, *Acanthaceae.* It is also referred to as the 'bile of earth' since it is one of the most bitter plant that are used in traditional medicine (Coon and Ernst, 2004). The geographical distribution of the plant has led to its traditional use in Ayervedic (Indian), Thai, and Chinese medicine. According to these traditions, *Andrographis* dispels heat (i.e., is antipyretic) and removes toxins, which makes it a good treatment for infectious fever causing diseases. In traditional Chinese medicine, *A. paniculata* is an important cold property herb, used to rid the body of heat and fever.

A. paniculata extracts inhibited the body's inflammatory mechanism and demonstrated not only anti-microbial abilities, but also were instrumental in killing certain tumor cells. In vitro studies have shown that the flavonoid activities supressed the genetic expression of neutrophiils, an inflammatory agent. *In vitro* studies have also indicated that the active chemical in *A. paniculata*, andrographolide, helps to stop the clumping of blood platelets which is the clotting process that can lead to heart attacks (Amroyan *et al*., 1999). It was also suggested to have a major effect activating the general defense functions of the immune system by stimulating the production of antibodies as well as non-specific

immune responses such as increased macrophage phagocytosis, rather than by any direct anti-microbial activity (Amroyan *et al*., 1999).

Immune deficiency is the root of susceptibility to a variety of infections, and it is the basis of the acquired immune deficiency syndrome (AIDS). Impairments of immune function result in variable clinical symptoms. Immunostimulatory activity of *A. paniculata* is evidenced by increased proliferation of lymphocytes and production of interleukin 2 *in vitro*. *A. paniculata* also enhanced the tumor necrosis factor α production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity (Rajagopal *et al*., 2003). Laboratory tests conducted in Buffalo, New York, also, demonstrated that *A. paniculata* inhibited the growth of human breast cancer cells at levels similar to the drug tamoxifen (Puri *et al*., 1993).

The consumption of the infused aerial parts of *A. paniculata*, alongside with meals (as blood tonic), is being encouraged, because of the medicinal properties alleged by traditional medical practitioners: as it is believed to enhance immune system functions such as production of white blood cells (scavengers of bacteria and other foreign matter), release of interferon, and increase the activity of the lymph system and to prevent or cure infective and degenerative diseases (Tapsell *et al*., 2006). However, if *A. paniculata* does indeed stimulate the immune system, this could lead to a whole host of potential risks, because the immune system is balanced on a knife edge. An immune system that is too relaxed fails to defend the organism from infections and an immune system that is too active attacks healthy tissues, causing autoimmune diseases. A universal immune booster might cause or exacerbate lupus, Crohn's disease, asthma, Graves's disease, Hashimoto's thyroiditis, multiple sclerosis, and rheumatoid arthritis, among other illnesses. Thus, this study was designed to evaluate the effect of the chronic consumption of *Andrographis paniculata* on the immune system of rats.

2.0 Materials and method

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 by Mr. L. T. Soyewo at Forest Research Institute of Nigeria, Ibadan, Oyo State. A specimen of the plant was kept with voucher number (108453) for future reference. 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.

Chemicals

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

2.2 Quantitative assay kits

2.2.2 IL – 6 and TNF – alpha estimation

IL – 6 and TNF – alpha were quantified with rat enzyme linked immuno-sorbent assay (ELISA) kits produced by Ray-Biotech, Inc. U. S. A.

2.2.3 Bilirubin and uric acid estimation

Bilirubin and uric acid were estimated using reagent kits produced by LABKIT, CHEMELEX, S.A. Pol. Ind. Can Castells. C / Industrial 113, Nau J. 08240 Canovelles – Barcelona.

2.3 Laboratory animals

Forty 10–12 weeks old male albino rats of average body weight of 125–140 g were obtained locally from Oyo Town, Oyo State. The rats were housed in animal care facility at the Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso.

2.4 Methods

2.4.1Experimental animals and procedure

The forty male albino rats were randomly grouped into four, comprising of ten rats per group. They 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 10 days before the administration of the aqueous extract of *A. paniculata* was commenced. The cages were cleaned every morning and disinfected 3 days interval. Calculated doses of the plant extracts (mg/kg body weight of rat) were dissolved in distilled water and stored air tight at 4^{0}C. 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 84 days, after which

the rats were fasted for 12 hours and sacrificed by anaesthetia, using di-methyl ether. Incision was made quickly in the chest region and the heart was pierced to collect blood into labelled heparinised bottles and labelled non – anticoagulant bottles.

2.4.2 Haematological analysis

The haematological parameters were analysed by the automated haemology analyzer (SYSMEX K2X1: SYSMEX CORPORATION, JAPAN). The mean corposular haemoglobin, mean corpuscular haemoglobin concentration and mean cell volume were calculated.

2.4.3 Serum interleukin–6 and TNF–α estimation

The serum level of IL-6 and TNF–α was estimated by *in vitro* enzyme linked immunosorbent assay (ELISA) kit, using colourimetric reaction method as instructed in the kit manual with cat # ELR- IL6-001 and ELR - TNF alpha – 001 respectively.

2.4.4 Serum bilirubin estimation

The serum bilirubin levels were estimated by dimethylsulphoxide (DMSO) colourimetric reaction, according to the method as described by (Malloy *et al*., 1937: Kaplan *et al*., 1984).

2.4.5 Uric acid estimation

The serum uric acid levels were estimated by uricase-POD enzymatic colourimetric reaction, according to the method as described by (Fossati *et al*., 1980: Schultz, 1984).

2.5 Stastitical analysis

This research work was a completely randomised design (CRD). Results analyses were performed using Prism 3.00 software. The results were expressed as mean ± standard deviation of 3 - 8 replicates where appropriate. Results were subjected to one way analysis of variance (ANOVA) to test the effect of each dose level on the parameter under investigation at 5% degree of freedom. The Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant treatment dose at 5% level of significance. P-value <0.05 was regarded as statistically significant and denoted by alphabets.

3.0 Results

The results were presented in tables. The values were expressed as mean ± standard deviation of at least 5 replicates and alphabets were used to depict significantly different ($p<0.05$) mean value.

3.1 Physical examinations of rat

Administration of the aqueous extract of *A. paniculata* to albino rats recorded the death of one rat at the 500mg/kg BW dose after 71 days and partial paralysis and/ mortality of three rats at 1000mg/kg BW dose after 50 days. The rats of the 1000mg/kg BW dose group were often less active after administration and consumed more water than the other dose groups. After 32 days of administration, four rats in this group had their fur dropping off, the feaces were watery with the colour faded and the eyes were very red and budged out. However, one of the three paralyzed rats did not die till the end of the experiment. Figure 1 depicts the pictures of the rats in the each group.

3.2 Haematological analyses

Results of the haematological estimations were presented in table 1. The administration of the extract had no significant effects ($p < 0.05$) on the PCV of the test groups (table 1). The Hb count and RBC count were increased significantly ($p < 0.05$) for the group 4 rats only. The platelets counts were increased significantly ($p < 0.05$) dose dependently in the test groups. Dose dependent significant increases ($p < 0.05$) were also observed in the ESR of the test groups (table 1).

3.3 Red cell indices

The results of the erythrocyte indices (MCV, MCH and MCHC) are depicted in table 2. The MCV was reduced significantly ($p < 0.05$) in the test groups (2, 3, and 4) with no significant difference among the test groups. The MCH and MCHC showed significant reductions ($p < 0.05$) in the group 4 rats only (table 2).

3.4 White blood cell count

Results of leukocytes estimations were shown in table 3. The WBC and lymphocytes (L) count were increased significantly ($p < 0.05$) in the test groups with group 2 and 3 been statistically equal. The result of the granular leukocytes (neutrophil, monocytes, basophil and eosinophils) are presented in table 3. Significant reduction and increase ($p < 0.05$) were observed respectively in the neutrophil and monocyte counts in the group 4 rats only. The eosinophils count was reduced significantly ($p < 0.05$) in group 2 and 3 rats, while significant increased ($p < 0.05$) was observed in group 4 rats. Basophils were not detected in group 3 rats, while two rats in group 2 and three rats in group 1 showed basophil count with no tandard deviation. However, basophils were estimated in five rats of group 4.

3.5 Serum cytokines

The effect of the extract on serum cytokines (IL

- 6 and TNF – α) levels were presented in table 4. Dose dependent significant increases ($p < 0.05$) were observed in the serum levels of IL - 6 and TNF – α in the test groups.

3.6 Serum bilirubin and uric acid

The effect on the administration of the aqueous extract on serum bilirubin and uric acid were depicted in table 4. The total bilirubin concentrations was significantly increased ($p<0.05$) in the group 4, while group 2 and 3 showed significant reductions ($p<0.05$). Direct bilirubin concentrations decreased significantly ($p< 0.05$) in group 2 and 3, while significant increase ($p< 0.05$) was shown in group 4 (table 4). The indirect bilirubin levels were increased significantly ($p< 0.05$) in group 3 and 4 only. The uric acid concentrations were reduced significantly ($p< 0.05$) in group 2 and 3, while significant increase ($p < 0.05$) was shown in group 4 (table 4).

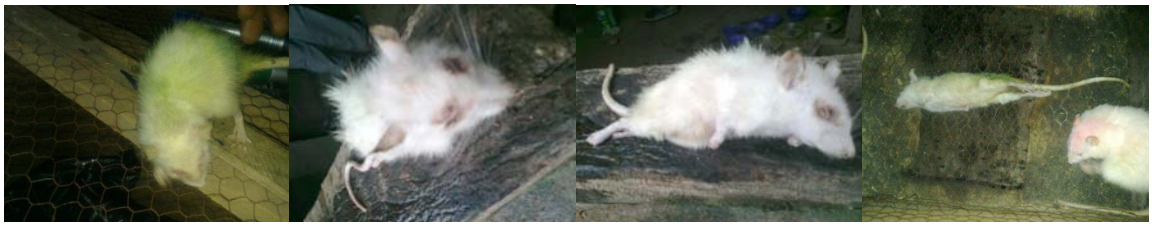

Figure 1: Photograghs of Four Rats in the 1000 mg/kg BW dose group

Table 1: Effect of Aqueous Extract of *A. paniculata* on Haematological Parameters in Male Albino Rats

	Control	250 mg/kg BW	500 mg/kg BW	1000 mg/kg BW
PCV (%)	41.5 ± 1.6432 [a]	39.4 ± 0.5477[a]	40.7 ± 1.2042 [a]	42.4 ± 1.5166 [a]
HBC (g/dl)	12.917 ± 0.7111[a]	13.058 ± 1.3465[a]	13.018 ± 0.9192[a]	11.094 ± 0.6236[b]
RBC (10^{12}) /L	7.2167 ± 0.2316[a]	7.552 ± 0.8081[a]	7.988 ± 0.5608[a]	8.878 ± 0.4006[b]
PLATELET(µl)10^3	334 ± 12.1119[a]	358.8 ± 7.5630[b]	384.2 ± 18.7082[c]	427.6 ± 20.5971[d]
ESR (mm^3/hr)	2.8 ± 0.4472[a]	3.6 ± 0.5477[b]	4.2 ± 0.4472[c]	6.8 ± 0.8367[d]

Table 2: Effect of Aqueous Extract of *A. paniculata* on Erythrocyte Indices in Male Albino Rats

	Control	250 mg/kg BW	500 mg/kg BW	1000 mg/kg BW
MCV (fL)10^{15}	56.952 ± 3.2970[a]	52.039 ± 2.7083[b]	50.951± 2.3360[b]	47.759 ± 2.5111[b]
MCHC (g/L)	3.112 ± 0.1704[a]	3.322 ± 0.1091[a]	3.294 ± 0.1596[a]	2.617 ± 0.1938[b]
MCH (10^{-12})	0.179 ± 0.0027[a]	0.173 ± 0.0062[a]	0.168 ± 0.0072[a]	0.125 ± 0.0177[b]

Table 3: Effect of Aqueous Extract of *A. paniculata* on White Blood Cells in Male Albino Rats

	Control	250 mg/kg BW	500 mg/kg BW	1000 mg/kg BW
NUETROPHIL (%)	48.4 ± 2.9664[a]	51.8 ± 2.8636 [a]	52.4 ± 2.0736 [a]	32 ± 4.6368 [b]
WBC 10^9 (/L)	3.46 ± 0.6025[a]	4.7 ± 0.3674 [b]	5.5 ± 0.3701 [b]	8.84 ± 0.7668 [c]
LYMPHOCYTE (%)	18 ± 3.1623[a]	24.4 ± 2.0736 [b]	27.1 ± 4.1473 [b]	53.4 ± 5.5946 [c]
EOSINOPHIL (%)	2.5 ±0.5774[a]	1.25 ± 0.5774 [b]	1.33 ± 0.5774 [b]	3.5 ± 0.5774 [c]
BASOPHIL (%)	1.00 ± 0.0	1.00 ± 0.0	0.00 ± 0.0	2.25± 0.5
MONOCYTE (%)	3.6 ± 1.1401[a]	3.8 ± 1.0954[a]	4.2 ± 1.7886[a]	5.6 ± 1.140 [b]

Table 4: Effect of Aqueous Extract of *A. paniculata* on Serum IL-6, TNF-α, Bilirubin and Uric acid in Male Albino Rats

	Control	250 mg/kg BW	500 mg/kg BW	1000 mg/kg BW
IL-6 (pg/ml)	434 ± 42.1901[a]	640 ± 35.3553[b]	752 ± 41.2311[c]	1196 ± 41.5933[d]
TNF –α (pg/ml)	324 ± 15.1658[a]	450 ± 31.6228[b]	570 ± 46.9042[c]	910 ± 44.3847[d]
Total Bilirubin (mg/dl)	1.006 ± 0.1137[a]	0.649 ± 0.1154[b]	0.9097± 0.0605[c]	1.345 ± 0.0941[d]
Direct Bilirubin (mg/dl)	0.526 ± 0.1720[a]	0.201 ± 0.0188[b]	0.343 ± 0.0182[c]	0.646 ± 0.0469[d]
Indirect Bilirubin(mg/dl)	0.480 ±0.1134[a]	0.449 ± 0.022[a]	0.566 ± 0.1002[b]	0.699 ± 0.0772[c]
Uric Acid (mg/dl)	4.53 ± 0.4982[a]	3.50 ± 0.2675[b]	3.99 ± 0.1170[c]	6.69 ± 0.1889[d]

4.0 Discussion

The volume of red blood cells in whole blood (PCV) was not affected by the chronic administrations of the extract (table 1). However, the extract might have induced anaemia at 1000mg/kg BW dose as observed in the haemoglobin count and increased number of red blood cell (RBC) per liter of whole blood (table 1). The dose dependent increase in the platelet count in the study disconcor with the anaemic capability of the extract (Topley, 1998), and might suggest possible adverse effect on blood metabolism like leukaemia, multiple myeloma, bone marrow infiltration etc (Janeway *et al.*, 1997). This observation might be dis-advantageous in vascular endothelial tissues, as increased blood platelets are implicated in atherosclerosis. The increase in the (ESR), which is the rate at which RBC sediment on their own weight per unit time, supported the salient anaemic capability of the extract (table 1).

The reduction in the mean cell volume signifies that the size of the RBC were reduced, indicating microcytic anaemia due to either iron deficiency anaemia and/ anaemia of chronic disease (Topley, 1998). Reduction of the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) at the 1000mg/kg BW dose (table 2), supported the possibility of the extract predisposing the consumer to iron deficiency anaemia and or, microcytic hypochromic anaemia (Vasudevan and Sreekumari, 2000).

The aqueous extract of *A. paniculata* could have erythrocytes building capability due to the level of iron, as previously reported by Oyewo *et al*. (2010). However, the presence of lead may suggest possible toxicity to the liver because no safe level of lead in blood has been established. More so, the incorporation of iron into protoporphyrin ring in haem biosynthesis is inhibited by lead, which could result to acquired porphyria, hypochromic-microcytic anaemia and hyperuricemia (Jeremy *et al*., 2001: Champe *et al*., 2005).

The extract elucidated a boost of the white blood cell and lymphocyte (table 3). White blood cells (leukocytes) are involved in fighting infection and clearing off damaged or dead cells and tissues in body (Jeremy *et al*., 2001). However, excessive count of white blood cells (WBC) are implicated in arthritis, trauma, uraemia, leukamia, myeloproliferative disorder, haemorrhage, myocardial infaction, inflammation, tissue necrosis, stenuous excercise and acute infections (Vasudevan and Sreekumari, 2000).

Reduced WBC count are reported in viral, bacteria and parasitic infections, hypersplenism, anaemias, bone marrow infitration and anaphylatic shock (Tracey and Cerami, 1994). The sharp increase in WBC and lymphocyte counts (lymphocytosis) at the 1000 mg/kg BW dose requires carefully examination, as the increase may be due to any of the aforementioned conditions. Reduction in the blood level of neutrophils (neutropenia) at the 1000 mg/kg BW dose (table 3) may indicate leukaemia, neoplasia, tissue damage, anaphylaxis, malignant disease, splenomelay, megaloblastic anaemia etc (Topley, 1998). The decrease in the blood eosinophil level at the the 250 mg/kg BW and 500 mg/kg BW doses may reduce the possibilities of parasitic infections, malignancies, lymphomas, connective tissue diseases and allergic reactions, while increase in eosinophil blood level at the 1000 mg/kg BW dose could predispose to these conditions.

The blood basophil levels observed in this study, though not tested statistically, might indicate that the 1000 mg/kg BW dose of the extract could predispose to myeloproliferative disorders and some allergies. The increase observed in the blood monoctye level (table 3) in the 1000 mg/kg BW dose support the immune boosting capabilities of the aqueous extract of *A. paniculata,* but may suggest possible chronic myelomonocytic leukaemia (Tracey and Cerami, 1994).

The dose dependent increases in the serum IL-6 and TNF-α in the study strongly indicate the immune modulation potential of the aqueous extract of *A. paniculata*. IL-6 and and TNF-α are important cytokines involved in the differentiation and proliferation of the immune cells (Janeway *et al*., 2001). IL-6 is secreted by monocytes /macrophages, fibroblasts, endothelial cells, keratinocytes, mast cells, T cells and many tumor cell lines to stimulate

immune response to trauma, skin burns or other tissue damage leading to inflammation and fever. It is one of the mediators that are released very early in an injury process (Murtaugh *et al*., 1996). IL-6 stimulates the acute phase reaction, which enhances the innate immune system and protects against tissue damage (Abbas *et al*., 1997). It increases the synthesis of the two major acute phase proteins, C-reactive protein (CRD), which increases the rate of phagocytosis of bacteria and serum amyloid A (SSA), by regulating the transcription of their genes. IL-6 has major effects on haematopoiesis, thrombopoiesis and appears to be a growth factor of malignant cells (Roitt, 1991). Increased levels of IL-6 in blood correlate with increased synthesis of fibrinogen, erythrocyte sedimentation rate, secretion of glucocorticoids, and the activation of the complement, the B cells and the clotting cascade, and decreased blood albumin (Castell *et al*., 1989: Kushner *et al*., 1990: Abbas *et al*., 1997).

IL-6 has been reported to have positive and negative actions on metabolic responses in liver, adipose tissue, and skeletal muscle. It is relevant to many disease processes, in which elevated serum or plasma levels may occur in different conditions including such as diabetes (Kristiansen and Mandrup-Poulsen, 2005), systemic lupus erythematosus, sepsis, lymphoid malignances, multiple myeloma, autoimmune diseases, lymphomas, AIDS, alcoholic liver disease, Alzheimer disease and in organ infections or transplant rejection (Abbas *et al*., 1997: Tackey *et al*., 2004). IL-6 stimulates energy mobilization in the muscles and fat tissues, which leads to increased body temperature. IL-6 in particular is thought to worsen the symptoms of autoimmune diseases and fibromyalgia (Febbraio and Pedersen, 2005). Interleukin-6 has been found to act as a growth factor in several tumors and some viruses also use IL-6 to replicate. Interleukin-6 also causes calcium to be released from bone, promoting osteoporosis (Tracey and Cerami, 1994). Moreso, elevated levels of IL-6 may be associated with an increased risk of atherosclerosis, heart attack and stroke (Dubiński and Zdrojewicz, 2007), prostate cancer (Smith *et al*., 2001), and rheumatoid arthritis (Nishimoto, 2006). Advanced or metastatic cancer patients have higher levels of IL-6 in their blood, hence, there is interest in developing anti-IL-6 agents as therapy against many of these diseases (Barton, 2005: Smolen and Maini, 2006). Finally, if acute – phase response, inflammation and fever are not controlled they become detrimental, leading to sepsis and shock.

TNF-α is produced by activated macrophages and other cell types including T and B cells, NK cells, LAK cells, astrocytes, endothelial cells, smooth muscle cells and some tumor cells (Aggarwal and Reddy, 1994). The primary role of TNF-α is in the regulation of immune cells. TNF- α is also able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication.

Most organs of the body appear to be affected by TNF-α, which has both growth stimulating and inhibiting properties. TNF- α induces neutrophil proliferation during inflammation and also induces neutrophil apoptosis upon binding. It aids in maintaining homeostasis by regulating the body's circadian rhythms. It also promotes the replacement of injured cells and senescent tissues by stimulating fibroblast and stimulates immune response to bacterial, fungal, viral and parasitic infections, as well as, necrotic cells (Janeway, 1997). TNF- α also induces the secretion of acute phase protein and increases the vascular permeability to recruit macrophages, neutrophils and CD_8 T killer cells. It causes the blood clotting so as to contain the infection and is known to cause the apoptosis of tumor cells, but it over expression could promote the growth of the tumor cells (Locksley *et al*., 2001).

Over-production of TNF-α, however, has been implicated as playing a role in a number of pathological conditions, including cachexia, septic shock, and autoimmune disorders. Dysregulation and, in particular, overproduction of TNF-α have been implicated in a variety of human diseases, as well as cancer (Locksley *et al*., 2001). High blood levels of TNF- α correlate with increased risk of mortality (Rink and Kirchner, 1996). TNF-α is believed to mediate pathogenic shock (sepsis) and tissue injury associated with endotoxemia. High blood levels of TNF-α increased the risk of heart disease by 79 percent and of heart failure by 121 percent (Cesari *et al*., 2003). When TNF- α production increases to the extent that it escapes the local infection, or when the infection enters the blood stream, sepsis occurs. Sepsis shock results to fever, falling blood pressure, myocardial suppression, dehydration, acute renal failure and finally, respiratory arrest. Excess production of TNF- α in the body reduces blood volume, albumin level and neutrophil (systemic edema), body organs fail and death ensues.

Prolong over-production of TNF- α leads to the loss of the anti-tumor activiy, due to polymerization of TNF- α molecules and the shedding of the receptors. The symptoms of prolonged TNF- α production are anorexia, net catabolism (lipid and protein), weight loss, hepatosplenomegaly, insulin resistance, endothelial activation, anaemia, illness such as cancer and AIDS (Tracey and Cerami, 1994). Recent studies have demonstrated that IL-6 and TNF-α are stronger predictors of cardiovascular disease than C-reactive protein. In the health, aging

and body composition study, people with the highest IL-6 levels were two to five times more likely to have a heart attack, stroke or other cardiovascular episode than those with the lowest levels (Cesari *et al*., 2003). Prolong over productions of IL-6 and TNF- α, lead to sepsis shock and organ failure, and ultimately, paralysis and death (Dubiński and Zdrojewicz, 2007).

The significant reduction in the serum total bilirubin at 250mg/kg BW dose (table 4) could be adduced to enhanced haem metabolism and bile excretion, while the 500mg/kg BW dose also enhanced haem metabolism and bile excretion, but not significantly. However, the dose of 1000mg/kg BW possibly caused imbalance in haem metabolism and excretion, which resulted to the increase in the serum total bilirubin level. The dose dependent significant increase in serum total bilirubin among the test groups may be due to increased anaemia or increased red blood cell degradation, biliary stricture (benign or malignant) and chronic liver disease (Vasudevan and Sreekumari, 2000: Champe *et al*., 2005). Increased total bilirubin causes jaundice, which is characterized by the yellowing of eyes and the skin (Friedman *et al*., 1996). The significant reduction in the serum direct bilirubin concentrations at 250mg/kg BW and 500mg/kg BW suggested the possibility of the aqueous extract of *A. paniculata* to protect and enhance hepatocytes and biliary functions at the doses. However, the increase in the serum direct bilirubin at the 1000mg/kg BW dose, suggested possible biliary obstruction and hepatotoxicity, which could lead to obstructive jaundice, haemolytic jaundice and hepatocellular jaundice (Vasudevan and Sreekumari, 2000: Champe *et al*., 2005).

The non significant reduction in the serum indirect bilirubin at 250mg/kg BW dose may suggest that the aqueous extract of *A. paniculata* did not cause significant anaemia , possibly haemolytic anaemia (Vasudevan and Sreekumari, 2000). Dose of 500mg/kg BW indicated possible predisposition to haemolytic anaemia that was strongly supported by the 1000mg/kg BW dose. Thus, it was logical to infer that the 1000mg/kg BW dose could predispose to obstructive jaundice (increased serum direct bilirubin), haemolytic jaundice (increased serum indirect bilirubin and hepatocellular jaundice (increased serum indirect and direct bilirubin) (Vasudevan and Sreekumari, 2000: Jeremy *et al*., 2001). However, the 500mg/kg BW dose could possibly predispose to haemolytic jaundice only. More so, the possibilities of obstructive and or hepatocellular jaundice in the 1000mg/kg BW dose group was strongly supported by the faded colouration of the rat feaces during administration of the aqueous extract of *A. paniculata*. This was probably due to hepatocellular damage or the obstruction of the secretion of conjugated bilirubin into bile by the liver, which led to the diffusion (leakage) of conjugated bilirubin into the serum.

The disruption in the secretion of conjugated bilirubin into bile would lead to the decrease in the enterohepatic circulation and more urobilinogen is excreted in the urine (responsible for the dark colouration), while less conjugated bilirubin is secreted into the gut during lipid metabolism. Thus, the level of urobilin and stercobilin in the feaces are decreased, so the feaces would appear clay coloured (Vasudevan and Sreekumari, 2000: Champe *et al*., 2005).

The significant decrease in the serum uric acid concentration (hyporuricemia) observed at 250mg/kg BW and 500mg/kg BW doses of the aqueous extract of *A. paniculata* implied that there were reduced tissues degradation (turn over due to trauma and high rate of catabolism as in starvation), low possibilties of growing malignant tissues (lymphomas, polycethemia, leukaemia), renal injury or failure, lead poisoning and gout (Jeremy *et al*., 2001: Champe *et al*., 2005). Low levels of uric acid in the blood are seen much less commonly than high levels and are seldom considered cause for concern (Aringer and Graessler, 2008). Interestingly, uric acid has been shown to be a very strong reducing agent *in vivo*. It has comparable antioxidant capabilities as ascorbic acid and is often used as a biomarker for oxidative stress (Glantzounis *et al*., 2005: Baillie *et al*., 2007).

In humans, over half the antioxidant capacity of blood plasma comes from uric acid. However, like other strong reducing substances such as ascorbate, uric acid can also act as a pro-oxidant particularly at elevated levels (Proctor, 1970: Cutler, 1984). Thus, it is unclear whether elevated levels of uric acid in diseases associated with oxidative stress such as stroke and atherosclerosis are a protective response or a primary cause (Becker, 1993). Therefore, the increase in serum uric acid concentration between the 250mg/kg BW and 500mg/kg BW doses (table 4) may suggest a boost in the antioxidant system. On the other hand, a high uric acid level may not cause problems in all cases, but some people develop gout, kidney stones or kidney failure. A high uric acid level may appear prior to the development of high blood pressure, heart disease or chronic kidney disease, but it's often unclear whether high uric acid level is a direct cause or merely an early warning sign of these conditions (Heinig and Johnson, 2006). The significant increase in the serum uric acid levels at the 1000 mg/kg BW dose may suggest possible risk of increased tissue degradation (turn over due to trauma and high rate of catabolism as in starvation), high possibilties of growing malignant tissues (lymphomas, polycethemia, luekaemia), renal injury

or failure, lead poisoning and gout (Jeremy et al., 2001: Champe *et al*., 2005). More so, the probabilty of developing other aforementioned disease conditions associated with hyperuricemia would be increased at the dose level. From the overall findings of this study, it was logical to make the submission that the administration of the aqueous extract of A. paniculata at the 1000 mg/kg BW possibly predisposed to multiple myeloma due to the marked increases in IL-6 and TNF- α levels, whose symptoms includes: anaemia, bone pain and tenderness, hyperuricaemia, nerve damage, neutropenia, hypoalbuminaemia, thrombocytopenia and renal diseases (Vasudevan and Sreekumari, 2000).

5.0 Conclusion

The aqueous extract of *A. paniculata* presented a boost in the immune system of rat at all the dose levels studied. The boost of the immune system recorded was attributed to the increase in the serum TNF- α and IL-6 levels. However, the possibilities of anaemia indicated by the reduced haemoglobin count, increased platelet count and erythrocyte sedimentation rate, reduced erythrocyte indices and neutrophil count of the test groups, especially at the 1000mg/kg BW dose was strongly supported by the increased serum levels of IL-6 and TNF- α, which also suggested the autoimmune capability of the chronic consumption of the extract. To explain clearly the metabolic effects of increased serum levels of IL-6 and TNF-α, studies is currently on to evaluate the effect of the chronic administration of the aqueous extract of *A. paniculata* on glucose utilization, lipid and protein profiles in male albino rats.

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