Some Biochemical and Haematological Studies on the Methanolic Extract of *Anthocleista Grandiflora* Stem Bark

Odeghe Othuke B

Uwakwe A, A

Monago C.C

Department of Biochemistry
Faculty of Science, PMB 5323
University of Port Harcourt
Rivers State. Nigeria.

Abstract

This research was designed to access the interaction of Anthocleista grandiflora plant and chloroquine in mice induced with Plasmodium berghei with the aim of ascertaining the significance of such interaction in the treatment of malaria infection. A total of 89 mice were used for this study and were divided into six groups. Group 1 uninfected and untreated, Group 2 infected and treated with normal saline, Group 3 infected and treated with 300mg/kg b.wt extract, group 4 infected and treated with 500mg/kg b.wt extract, group 5 infected and treated with 700mg/kg b.wt extract and group 6 infected and treated with chloroquine (5mg/kg b.wt). The infected extract treated animals had significant (P<0.05) difference in PCV, Hb, WBC and platelet count from day 3 to day 14 when compared with the control. The elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and urea in the malaria rats were significantly (p<0.05) decreased.

Keywords: Biochemical, heamatological, *Plasmodium berghei,* malaria and *Anthocleista grandiflora*

1.0 Introduction

Recently, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 7 year period. Traditional medicines have been used to treat malaria for many years and are the source of the two main groups of modern antimalarial drugs (artemisinin and quinine derivatives). These antimalarial drugs were derived from plants and are still effective in treating malaria (Bodeker and Willcox, 2000), Parasitic diseases are of immense global significance as around 30% of world's population experiences parasitic infections. Amongst various parasitic infections, malaria is the most life threatening disease and accounts for an estimated 225 million cases of malaria worldwide in 2009 (WHO, 2011) An estimated 655,000 people died from malaria in 2010 (WHO, 2011), a 5% decrease from the 781,000 who died in 2009 according to the World Health Organization's 2011 World Malaria Report, accounting for 2.23% of deaths worldwide (WHO, 2011).

The most effective strategy for *P. falciparum* infection recommended by WHO is the use of artemisinins in combination with other antimalarials artemisinin-combination therapy, ACT, in order to avoid the development of drug resistance against artemisinin-based therapies. With the onset of drug resistant Plasmodium parasites, new strategies are required to combat the widespread disease.

Plants have always been considered to be a possible alternative and rich source of new drugs. The search for malaria remedies in plants and improved interest in plant drugs by many communities staying in endemic area led to the use of *Anthocleista grandiflora* plant in establishing the scientific basis for the treatment of malaria.
Anthocleista grandiflora, commonly known as the forest fever tree is a large tree of moist forests in the eastern and south-eastern African tropics and the comores. It is a member of the family *Gentianaceae* and a small genus of only 14 species. It is a tall, slender tree up to 30m with a preference for forests in high rainfall areas. The leaves are very large up to 100cm x 50cm and in terminal clusters. It is an evergreen plant typically found at an altitude 0.2, 107cm (0-6, 913 feet) and can be 15-20m tall.

The tree is sometimes epiphytic with auxiliary spines or tendrils, leaves opposite, occasionally alternate, rare venticillate, fascicute or in a whorl, stipinles usually present, often reduced to lines connecting petiole bases. The flowers are in cymes these are often grouped into thyrses; sometimes umbel-like, scorpioid or reduced to single flower bracts usually small. The flowers are usually bisexual and cream coloured. It is not edible as food but possesses root, stems, bark, leaves and flowers which are claimed to have medicinal properties (Palmer and Pitman; 1972).

In Southern Africa, bark decoctions are used traditionally to treat malaria (Palmer and Pitman; 1972). Regionally, preparations of the bark has also found use as an anthelmintic specifically for roundworms (Githers 1949), antidiarrhoeal (Watt and Bieyer-Brandwijk 1962; Mabogo 1990) and to treat diabetes, high blood pressure and venereal diseases (Mabogo 1990). Furthermore, in the north continent, epilepsy is remedied with the aid of the stem bark decoction (Neuwinger 2000).

2.0 Materials and Methods

2.1 Plant Collection and Authentication

Fresh stem bark of *Anthocleista grandiflora* was collected in March, 2011, at Choba area, Port Harcourt, Rivers State. The plant specimen was identified and anuthicated by Dr. l.K. Agbagwa, a taxonomist in the dependent of plant science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria.

2.2 Preparation of plant materials

The plant stem barks were sorted to eliminate any dead matter and other unwanted particles. The volcher specimen was thinly on the flat clean tray (to prevent spoilage by moisture condensation) and allowed to dry at room temperature for seven days (Sofowora 1982). The dried plant materials were grounded into curse powder using an electric mill.

The crude extract was prepared by cold maceration technique (O'Neill at el 1985). The plant material was extracted by refluxing 45g of the specimen in 2.5L of methanol (80%) for three consecutive days at room temperature. The extracts were then filtered using cotton and then filtrate was passed through whatman filter paper (No.3, 15cm size with retention down to 0.1um in liquids). The methanol (80%) extract was concentrated in a rotary evaporator (Buchi type TRE121, Switzerland) to a yield of 5.08%. the extracts were kept in a tightly closed bottle on a refrigerator until used for anti-malaria testing. Preliminary qualitative and quantitative phytochemical screening of the plant extract was carried out employing standard procedures (Harbone, 1983)

2.3 Animal and inoculation

A Swiss albino mouse (18-20g) of 72 mice of both sexes of two months old were used for the experiments and was obtained from the University of Nigeria animal house, Nsukka Enugu, Nigeria. The animals were housed in a standard six group each, and acclimatized for a period of twelve days. The animals were housed in wooden cages under standard conditions (ambient temperature, $28.0 \pm 2.0^{o}C$, and humidity 46%, with a 12 hour light/dark cycle), were fed with growers mash. All the mice were allowed free access to food and water ad libitum, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily.

A strain of Plasmodium berghei that was Chloroquine sensitive was gotten from the University of Nigeria, Nsukka, Enugu, Nigeria. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse every 5-7 days. The animals were randomly divided into 6 groups. Each mouse (Group 1-5) except Group 6 (Uninfected) used in the experiment was infected intraperitonally with 0.2ml of infected blood containing about $1x10^{7}$ of *P. berghei berghei* – parasitized erythrocyte per mL on Day 0. Seventy two hours later (day 3) the mice (Group 1-3) were orally administered with *A. grandiflora* extract (300, 500, 700mg/kg/day), Group 4 (Positive control) received 5mg/kg/day chloroquine, Group 5(Negative control) received equal volume of normal saline and Group 6 received distilled water for twelve consecutive days (Day 3 to Day 14)

2.4 Sample collection and analyses

Daily blood films were made from tail blood of all the infected animals (Groups 1-5) and the percentage parasitemia obtained through microscopic determination. The Sahli's hemoglobinometer was used for the haemoglobin estimation of the test animals.

With the same procedure of drug administration and feeding, each mouse was anaesthetized with chloroform vapour and pooled blood from mice in each group, was collected by cardiac puncture on days 0 and 14 into heparinized tubes for haematological studies – packed cell volume (PCV), Haemoglobin (Hb), white blood cell (WBC) counts, platelets counts, neutrophil, lymphocyte and monocyte according to the method described by Dacie and Lewis (1991). Pooled blood samples from animals in each of the groups were also collected into plain tubes to obtain sera for biochemical analysis-urea and serum activities of alanine transaminase (ALT), aspartate transaminase (AST) an alkaline phosphatase (ALP) were measured as described by (Taiwo et al, 2004).

2.5 Statistical Analysis

Results of the study were presented as a mean plus or minus standard error of mean (M ± SEM). Statistical significance was determined by Students paired t-test and one way analysis of variance with multiple comparison tests (Tukey's test) to compare parameters within groups using computer software spss version 17. Data from the test groups were compared with their respective controls and differences at $P<0.05$ were considered significant.

3.0 Results

The phytochemical screening of the methanolic extracts of *A. grandiflora* is depicted in Table 1. The phytochemical results reveal the presence of alkaloids, saponins, terpenes, glycoside, protease inhibitor and flavonoid in the extract.

Table 1: Phytochemical Screening of Secondary Metabolites of *A. grandiflora* .

Secondary metabolites	Methanolic extract
Alkalod	+
Saponin	+
Terpenes	+
Glycoside	+
Protease Inhibitor	+
Flavonoid	+

Note: + = present, - = absent.

3.1 Effects of methanolic extract of *Anthocleista grandiflora* Stem Bark on Some Haematological Indices of Swiss albino mice.

The results of the haematological parameters showed no significant changes ($P \geq 0.05$) in the uninfected and untreated group as well as those infected but treated with the extract and chloroquine as presented in Table 2. Conversely, the infected and treated with Normal Saline (Negative control) mice showed a significant difference ($P \leq 0.05$) in the progressive development of severe anemia while those infected and treated with extract and chloroquine developed a mild and insignificant anaemia. All the mice in the test groups developed severe and progressive leucocytosis ($P \leq 0.05$) from Day 0 to Day 14 (Table 2).

Table 2 The Effect Of Methanolic Extract Of *Anthocleista grandiflora* Stem Bark On Some Haematological Indices Of Swiss Albino Mice

Haematological Parameter	Mice groups					
	UNITR	**PTNS**	**PT300A.g**	**PT500A.g**	**PT700A.g**	**PTCQ**
Hb (g/dl)						
Day 0	14.22 ± 1.4^a					
Day 3		11.10 ± 0.12^b	$13.09 \pm 0.76^{a,c}$	$13.21 \pm 0.91^{a,c}$	$14.4 \pm .0.99^{a,c}$	14.72 ± 1.47^a
Day 14	13.28 ± 1.80^a	8.91 ± 2.70^b	$13.12 \pm 2.3^{a,c}$	$13.88 \pm 2.4^{a,c}$	$14.31 \pm 2.47^{a,c}$	14.98 ± 1.26^a
PCV (%)						
Day 0	44.67 ± 4.37^a					
Day 3		37.33 ± 0.35^b	$40.3 \pm 2.31^{a,c}$	$43.31 \pm 2.7^{a,c}$	$45.33 \pm 2.9^{a,c}$	$46.67 \pm 4.41^{a,}$
Day 14	42.30 ± 4.4^a	33.73 ± 0.72^b	$41.2 \pm 2.8^{a,c}$	$44.40 \pm 3.0^{a,c}$	$45.62 \pm 3.24^{a,c}$	47.56 ± 4.32^a
WBC($\times 10^9$/l)						
Day 0	7.23 ± 0.92^a					
Day 3		5.97 ± 4.90^b	$6.0 \pm 2.49^{a,c}$	$6.12 \pm 2.53^{a,c}$	$7.07 \pm 2.83^{a,c}$	7.94 ± 2.33^a
Day 14	8.34 ± 1.32^a	4.02 ± 4.95^b	$6.32 \pm 2.34^{a,c}$	$6.87 \pm 0.14^{a,c}$	$7.32 \pm 0.36^{a,c}$	9.13 ± 0.45^a
Platelets ($\times 10^9$)						
Day 0	409.3 ± 126.1^a					
Day 3		645 ± 60.1^b	$638 \pm 97.5^{a,c}$	658 ± 94.68^a	$678 \pm 100.85^{a,c}$	$688 \pm 60.2^{a,c}$
Day 14	423 ± 122.3^a	367 ± 64.3^b	$667 \pm 2.38^{a,c}$	670 ± 93.54^a	$686.5 \pm 94.4^{a,c}$	$699 \pm 64.6^{a,c}$
Neutrophils (%)						
Day 0	14.67 ± 1.24^a					
Day 3		15.33 ± 2.67^b	$17.0 \pm 2.52^{a,d}$	$17.67 \pm 3.3^{a,d}$	$20.67 \pm 7.45^{a,d}$	15.33 ± 1.20^a
Day 14	13.37 ± 1.25^a	14.33 ± 2.72^b	$17.00 \pm 3.23^{a,d}$	$16.34 \pm 2.9^{a,d}$	$22.73 \pm 7.35^{a,d}$	16.43 ± 1.34^a
Lymphocytes (%)						
Day 0	$81.67 \pm 0.88^{a,}$					
Day 3		81.67 ± 2.67^b	$79.50 \pm 3.63^{a,d}$	$76.3 \pm 7.26^{a,d}$	$80.33 \pm 1.2^{a,d}$	82.33 ± 0.88^a
Day 14	84.65 ± 0.97^a	76.33 ± 2.45^b	$78.25 \pm 2.89^{a,d}$	$80.23 \pm 0.47^{a,d}$	$82.67 \pm 8.76^{a,d}$	84.67 ± 0.35^a
Monocytes (%)						
Day 0	3.67 ± 0.67^a					
Day 3		3.00 ± 0.00^b	$2.00 \pm 0.58^{a,d}$	$2.78 \pm 1.34^{a,d}$	$2.47 \pm 1.33^{a,d}$	2.00 ± 0.58^a
Day 14	3.57 ± 1.15^a	3.33 ± 4.32^b	$3.67 \pm 0.57^{a,d}$	$2.67 \pm 2.06^{a,d}$	$2.33 \pm 1.25^{a,d}$	2.56 ± 0.57^a

The data are presented as mean $\pm$ SEM, n=5. Values with superscript a showed a significant ($P \leq 0.05$) difference when compared with the control (normal saline) but values with superscript b showed no significant difference between groups, values with superscript c showed a significant difference and values with superscript d showed no significant difference when compared with the positive control (Chloroquine).

UNITR =Uninfected, untreated mice (Control),
PTNS = Parasitized mice treated with Normal Saline (Negative Control),
PT300A.g = Parasitized mice treated with 300mg/kg *A. grandiflora*,
PT500A.g = Parasitized mice treated with 500mg/kg *A. grandiflora*,
PT700A.g = Parasitized mice treated with 700mg/kg *A. grandiflora*.
PTCQ = Parasitized mice treated with 5mg/kg Chloroquine
Hb = Haemoglobin
PCV = Packed Cell Volume
WBC = White Blood Cell

3.2 Effects of methanolic extract of *Anthocleista grandiflora* Stem Bark on Some Biochemical Indices of Swiss albino mice.

The biochemical investigation result showed a statistical significant ($P \geq 0.05$) difference in the extract and chloroquine infected and treated mice when compared to the negative control (Normal saline) from day 3 to day 14 (Table 3).

In this study, administration of the extract and chloroquine caused a decrease in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). In this investigation plant extract significantly ($p<0.05$) reduced elevated levels of ALT, AST and ALP thus improving renal and hepatic functions.

Table 3.6: Effects of methanolic extract of *Anthocleista grandiflora* Stem Bark on Some Biochemical Indices of Swiss albino mice.

Biochemical Parameter	Mice groups					
	UNITR	PTNS	PT300A.g	PT500A.g	PT700A.g	PTCQ
AST (IU/L)						
Day 0	16.50±2.42[a]					
Day 3		13.00±4.26[b]	13.00±1.23[a,c]	12.5±0.35[a,c]	11.0±1.24[a,c]	9.50±0.34[a]
Day 14	15.00±0.34[a]	13.50±2.91[b]	12.5±0.33[a,c]	12.0±0.37[a,c]	10.5±0.45[a,c]	8.00±0.67[a]
ALT (IU/L)						
Day 0	12.00±1.33[a]					
Day 3		11.00±0.53[b]	13.0±0.34[a,c]	9.50±0.77[a,c]	8.55±1.23[a,c]	8.00±0.36a
Day 14	11.50±0.34[a]	11.50±0.32[b]	10.4±0.57[a,c]	8.50±0.13[a,c]	8.00±0.17[a,c,]	7.50±0.16[a]
ALP (IU/L)						
Day 0	11.35±5.42[a]					
Day 3		11.02±3.72[b]	10.97±2.95[a,d]	10.2±1.23[a,d]	10.0±0.95[a,d]	9.57±2.70[a]
Day 14	11.43±3.33[a]	11.92±0.28[b]	10.8±2.91[a,d]	9.50±2.93[a,d]	9.04±8.04[a,d]	7.98±0.45[a]
UREA ($_M$MOL/L)						
Day 0	7.05±5.21[a]					
Day 3		10.00±0.34[b]	8.50±0.89[a,d]	8.00±2.37[a,d]	7.70±6.73[a,d]	6.50±2.35[a]
Day 14	7.00±2.34[a]	10.75±0.66[b]	7.55±0.25[a,d]	7.85±0.15[a,d]	6.50±2.88[a,d]	4.75±3.15[a]

The data are presented as mean ± SEM, n=5. The Values obtained with superscript a showed significant ($P \geq 0.05$) difference when compared with control and values with superscript b showed no significant difference within groups, values with subscript c showed no significant difference and values with superscript d showed significant difference when compared to the positive control (Chloroqine)

UNITR =Uninfected, untreated mice (Control),
PTNS = Parasitized mice treated with Normal Saline (Negative Control),
PT300A.g = Parasitized mice treated with 300mg/kg A. grandiflora,
PT500A.g = Parasitized mice treated with 500mg/kg A. grandiflora,
PT700A.g = Parasitized mice treated with 700mg/kg A. grandiflora.
PTCQ = Parasitized mice treated with 5mg/kg Chloroquine
AST = Aspartate transaminase, ALT = Alanine transaminase and ALP = Alkaline phosphatase

4.0   Discussions and Conclusion
4.1   Discussion

In this study, the extract and chloroquine produced a significant positive ($p < 0.05$) difference on the hematological (PCV or haematocrit), hemoglobin (Hb), white blood cells (WBC), platelets, lymphocyte, neutrophils and monocyte when compared with the negative control (Table 4). The slight increase in hematological values demonstrated an improvement in disease progression (Chang and Stevenson, 2004; Weatherall et al., 2002). Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters (Ajagbonna *et al.*, 1999). Tona et al., (2001) revealed that the PCV of malaria parasite infected rodents as measured by haematocrit in the range of 43 to 44%, and this is similar to the observation discovered in *A. grandiflora* stem bark research.

Anaemia is a fairly common problem encountered in malaria. Czaja et al, 2002 reported that anaemia condition can be characterized by a decrease in the level of circulating Hb, less than 13g/dl in male and 13g/dl in females and this is in agreement with this present study which makes it a potential source for the treatment of anaemia. Also, there was a decrease in Hb and PCV in all the infected and those treated with normal saline (negative control) animal groups.

This was obvious from day 3 postinfection in the infected groups and it persisted more significantly in the infected treated animals with the normal saline till day 14 (Table 2). The observed anaemia in *P. berghei* infected mice may be due to RBC destruction caused either by parasite multiplication or by spleen reticuloendotelial cell action (Chinchilla et al., 1998).

This decrease may also be due to multiple causes, of which repeated haemolysis of infected red cells is the most important. The haemolysis may be due to non-immune destruction of parasitized red cells in case of high parasitemia or immune mediated destruction of parasitized as well as non-parasitized red cells because the changes in the red cell antigen structure brought about by the parasitic invasion stimulate the production of antibodies against the red cell. This triggers immunemediated red cell lysis. In addition, the growing parasite consumes and degrades the intracellular proteins which are mainly hemoglobin (Gavigan *et al.,* 2001). This may account further for the decrease in Hb. These decreases however were considerably reversed in the infected extract-treated and infected chloroquine-treated groups on day 14 post-infection. This suggests that the extract may have some stimulatory effect on the production of red blood cells (erythropoiesis). This might have contributed to the increase in Hb and PCV observed in the infected extract-treated group on day 14 postinfection (Table 3). The observed increase in WBC in the infected animal groups on day 3 post-infection may result from stimulation of the immune system of the animals to fight the malaria parasites (Table 4). White blood cells function mainly to fight infection, defend the body by phagocytosis against invasion by foreign organisms, and to produce, transport and distribute antibodies in the immune response.

On day 3 postinfection, there was a decrease in WBC in all the infected groups. This may imply a reduction in the ability of the mice to resist the infection (Yakubu *et al.,* 2007). However, WBC in the infected extract treated and infected chloroquine-treated groups were higher than in the infected treated animals with normal saline. This suggests a boost in the immune system by the extract and reference drug. On day 14 post-infection, there was a continued decrease in WBC in the infected treated group with normal saline which correlates with a high parasitaemia and other derangements as a result of the infection. The significant increase in WBC displayed by infected-extract treated and infected chloroquine treated groups indicates an improved ability of the mice to combat the infection as a result of treatment. The results also show that chloroquine at 5mg/kg/day is equally effective in prevention of anaemia due to its anti-protozoan effect in infected mice The boost in the level of WBC in the uninfected extract-treated group (day postinfection) showed a significant decline a week after treatment was withdrawn (day 14 post-infection). This implies that the increase was induced by extract and the effect is reversible (Table 2).

On day 3 post-infection, there was a decrease in the level of platelets in the infected groups (Table 2). Like red blood cells, platelets are nuclear and discoid; they measure 1.5–3.0 μm in diameter. The body has very limited reserve of platelets, so they can be rapidly depleted (Wagner and Burger, 2003).

Decreased platelet count is also fairly common in malaria and may result from sequestration of platelets in the spleen (Horstmann *et al.,* 1981). By day 14 post-infection, there was a continuous drop in the level of platelets in the infected untreated group as a result of continued infection. The infected extract-treated and infected chloroquine-treated groups showed a significant increase in platelet count compared to the infected treated group with normal saline, still suggesting a stimulatory effect of the extract on platelet production. The increase in the WBC count is an indication of the ability of the extract to stimulate increase in the production of the cells of the immune system. Also, when there is a significant increase in the white blood cell counts of the treated albino mice, this could signify toxicity of the drug on the bone marrow of the mice, the increase in the white blood cell counts indicates a pathological condition. It is noteworthy, however that all the infected mice treated or untreated developed leucocytosis. The leucocytosis may be an indication of enhanced granulopoiesis and lymphocytosis as cellular and humoral responses, respectively to the protozoan infection (Jubb et al., 1995).

Lymphocytes are the main effector cells of the immune system (Mcknight et al., 1999). The increase in the lymphocytes in this study may affect the effector cells of the immune system. The significant increase in the neutrophils by the extract could possibly suggest the ability of the extract to enhance blood component to phagocytose. The biochemical investigation result showed a statistical significant (P≥0.05) difference in the extract and chloroquine infected and treated mice when compared to the negative control (Normal saline) from day 3 to day 14(Table 3).

Recent studies have indicated the existence of a strong correlation between hepatic injury and oxidant stress in experimental animals treated with antituberculosis drugs (Tasduq et al, 2005, Attri et al; 2001). Biochemical tests related to the hepatocellular integrity can be checked to follow hepatocellula integrity and liver injury. Serum activities of AST and ALT were employed to access liver status because these enzymes can be used to differentiate between liver and heart disease (Nicholas and Lewis, 1989). In this study, administration of the extract and chloroquine caused a decrease in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). Determination of the activity of transaminase enzyme can provide valuable confirmatory or suggestive values (Dufour et., 2000). Smith et al, 1998 also reported an increase in ALT in oral acetaminophen-induced hepatotoxicity in rats indicating a biochemical evidence of significant liver damage.

Under state of stress, damage to liver, kidney and other organs may occur and these enzymes may be liberated into the blood. (Zikic et al., 2001). This oxidative stress could be as a result of the reaction of reactive species with cellular antioxidants which may cause antioxidant depletion (Jeong, 1999, Timbrel et al; 1980). Potent antioxidant and free radical scavenging activities of flavonoids (Hillwel, 1994) could counteract the free radical generation responsible for induced malarial disorder, and may contribute to the very high potency of A grandiflora plant extract. Also heterogeneous phytoconstituents of crude extracts have been reported to have synergistic effect (Mazunder *et al*., 2005). Biochemical tests related to the hepatocellular integrity can be checked to follow hepatocellula integrity and liver injury. Serum activities of AST and ALT were employed to access liver status because these enzymes can be used to differentiate between liver and heart disease (Nicholas and Lewis, 1989). In this study, administration of the extract and chloroquine caused a decrease in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). In this investigation plant extract significantly (p<0.05) reduced elevated levels of ALT, AST and ALP thus improving renal and hepatic functions. Also, severe malaria may be characterized by the elevated excretion of urea whose concentration may be five times higher than the normal value (Lehninger,1998). This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun *et al.,* 2006).

4.2 Conclusion

Decreased activities of these enzymes showed that the integrity of hepatocytes was normal, preventing the release of intracellular enzymes into the systemic circulation. The reduction in AST, ALT. ALP and Urea concentrations may be suggestive side effects of the drug which may inhibit some cardiovascular disease, platelet aggregation, phagocytosis and many others (Isaac, 1992). Determination of the activity of transaminase enzyme can provide valuable confirmatory or suggestive values (Dufour et., 2000). Smith et al, 1998 also reported an increase in ALT in oral acetaminophen-induced hepatotoxicity in rats indicating a biochemical evidence of significant liver damage.

Acknowledgement

References

Ajaiyeoba, E., Falade, M., Ogbole, O., Okpako L. and Akinboye, D. (2006). In vivo antimalarial and Cytotoxic properties of Annona senegalensis extract. *African Journal of Traditional, Complementary and alternative Medicine, 3(1): 137 – 141*

Attri, S., Rana, S., Vaiphei, K., Katyal, R., Sodhi, C., Kanwar, S. and Singh, K. (2001): Protective effect of Nacetylcysteine in isoniazid-induced hepatic injury in growing rats. Indian Journal of Experimental Biology 39: 436-440.

Bodeker, G. and Willcox M.L. (2000) Conference report: the first international meeting of the Research Initiative on Traditional Antimalarial Methods (RITAM). *J Alt Compl Med* 6:195-207

Chang, K.H and Stevenson, M.M. (2004). Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria, *International Journal of. Parasitology*, 34: 1501-1516

Chinchilla, M., Guerrero, O.M., Abarca, G., Barrios, M. and Castro, O. (1998). An *in vivo* model to study the anti-malaric capacity of plant extracts. *Review Biological. Tropics,* 46(1): 1-7.

Czaja, A. J. and Freeze, D. K. (2002). Diagnosis and treatment of autoimmune hepatitis. *Hepatology*, 36:479-497.

Dacie, J.V. and Lewis, S.M. (1991). In Practical Haematology 7th edition ELBS with Churchill Livingstone. Longman group UK 1991. pp. 5 - 82

Dufour, D.R., Lott, J.A., Nottle F.S. and seff, L.B. (2000). Diagnosis and monitoring of hepatic injury in performance characteristics of laboratory tests. *Clin. Chem.. 46: 2027-2049.*

Gavigan, C.S., Dalton, J.P. and Bell, A. (2001) The role of aminopeptidases in haemoglobin degradation in *Plasmodium falciparum*-infected erythrocytes. *Molecular Biochemistry Parasitology,* 117(1): 37-48.

Githens, T.S, (1949). Drug plants of Africa. African handbooks: 8. University of Pennsylvania Press, Philadelphia, USA

Harborne, J. B. and Williams, C. A. (2000): Advances in flavonoid research since 1992. *Phytochemistry 55: 481–504.*

Hilwell, B., 1994. Free radicals, antioxidants and humandisease: Curiosity, cause or constipation?. *Lancet* 344: 721-724.

Horstmann, R.D., Dietrich M., Bienzle, U. and Rasche, H. (1981) Malaria-induced thrombocytopenia. *Ann Haematol* 42(3): 157 -164.

Isaac, C. E., Litov, R., Marie, P. and Thomas, H. (1992). Addition of lipids to infant formular produces antiviral and antibacterial activity. *J. Nutr. Biochem. 3: 304-308.*

Iwalokun, B.I., Alibi-Sofunde, J.A., Odunala, T. Magbagbeola, O.A. and Akinwande, A.I. (2006). Hepatoprotective and anti-oxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *Journal Medicine Food,* 9(4): 526-530.

Jeong, H.G. (1999): Inhibition of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury. *Toxicology Letters* 105: 215-22.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (1995). Pathology of DomesticAnimals 3rd edition, Academic Press Inc. New York.

Lehninger, A.L., 1998. Principles of Biochemistry. CBS Publishers and Distributors   Pvt. Ltd., India,pp: 531-535.

Mabogo, D.E.N. 1990. The ethnobotany of the Vhavenda. Unpublished M.Sc thesis. University of Pretoria

Mazunder, U.K., M. Gupta and Y. Rajeshhwar, 2005. Antihyperglycemic effect and antioxidant potential of *Phyllantus niruri* (Euphorbiaceaea).in streptozotocininduceddiabetic rats. Eur. *Bull. Drug Res*. 13:15-23.

Mcknight, D.C., Mills, R.G., Bray, J.J. and Crag, P.A. (1999). Human Physiology, 4th ed. Churchill Livingstone, p 290-294.

Neuwinger, H.D. 2000. African traditional medicine. A dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart Pp 45.

Nicholas, C.P. and S. Lewis, 1989. Clinical concept of enzymology In: Fundamentals of Enzymology. 2nd Edition. . Oxford University press. New York, pp: 450-477.

O'Neill, M. J., Bray, D. H, Boardman, P. and J. D. Phillipson. 1985. Plants as source of antimalarial drugs. Part 1. In vitro test method for the evaluation of crude extracts from plants. Planta Med.61:394-398

Palmer, E. and Pitman, N. 1972. Trees of southern Africa. Volume 3. Cape Town: pp. 1845-1847.

Smith, G.S., Nadig, D.E., Kokoska, E.R., Solomon, H., Tiniakos, D.G. and Miller, T.A. (1998). Role of neutrophils in hepatotoxicity induced by oral acetaminophen administration in rats. *J Surg Res*;80:252-8.

Sofowora, S. 1982. Medical plants and Traditional Medicine in Africa, John Wiley and Sons Ltd. New York, p214

Taiwo, V.O., Afolabi, O.O. and Adegbuyi, O.A. (2004). Effect of *Thevetia peruviana* seed cake based meal on the growth, haematology and tissue of rabbits. *Tropical and subtropical Agroecosystems* 4: 7 - 14.

Tasduq, S.A., Peerzada, K., Koul, S., Bhat, R and Johri, R.K. (2005): Biochemical manifestations of antituberculosis drugs induced hepatotoxicity and the effect of silymarin. Hepatology Research 31: 132-135.

Timbrell, J. A. (1991). Principle of biochemical toxicology, 2nd Ed. (taylor and francis Eds.): 369.

Timbrel, J.A., Mitchell, J.R., Snodgrass, W.R. and Nelson, S.D. (1980): Isoniazid hepatotoxicity: The relationship between covalent binding and metabolism in vivo. American Journal of Pharmacology and Experimental Therapeutics Vol. 23.(2). Pp 364-369.

Tona, L., Mesia, K., Ngimbi, N.P., Chrimwami, B., Okond'ahoka, O., Cimanga, K., Bruyne, T., Apers, S., Hermans. N., Totte, J., Pieters, L. and Vlietinck, A. J. (2001). Invivoantimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri'*, *Annal Tropical Medicine Parasitology,* 95(1): 47- 57.

Wagner, D.D. and Burger, P.C. (2003) Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol 23: 2131-2137.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962. The medicinal and poisonous plants of southern and eastern Africa. E. & S. Livingstone Ltd., Edinburgh and London.

Weatherall, D.J., Miller, L.H., Baruch, D.I., Marsh, K., Doumbo, O.K., Casals-Pascual, C. and Roberts, D. J. (2002). Malaria and the red cell, Hematology, *American Hematology* 35:57.

World Health Organisation (2011). World malaria report summary. Geneva

Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2007) Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacology Magazine*, 3(9): 34-38.

Zikic, R.V., Stajn, A.S., Paulovic, S.Z., Ogajanovic, B.J. and Saicic, Z.S. (2001). Activity of superoxide dismutase and catalase in Erythrocytes and plasma transaminases of goldfish exposed to Cadmium. *Physio. Rev. 50:105-111.*