

Immunochemical Status and the Effect of Raw Liquid Extract of *Vernonia Amygdalina* on Acute Phase Protein (Albumin, Fibrinogen), Total Bile Acids and Lactate Dehydrogenase in *Plasmodium Spp* Infected Patients in Some Herbal Homes of Rural Nigeria

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Abstract Background to the Study: Pathophysiology of Plasmodium infection and the metabolism of the phytochemicals of raw liquid extract of *Vernonia amygdalina* used in the traditional treatment of Plasmodium spp infection in Saki-East Local Government area of Oyo state -Nigeria involve the hepatocytes, which could bring about biochemical alterations in the plasma level of albumin, fibrinogen, Lactate dehydrogenase (LDH) and Total Bile Acids (TBA). **Aim and Objective:** This work was designed to determine the effect of the raw extract of *Vernonia amygdalina* used in the treatment of *Plasmodium spp* infection on acute phase protein (albumin, fibrinogen), Total bile acids and Lactate dehydrogenase. **Materials and Method:** Forty five 45 (72.6%) that were HIV, HBsAg and anti-HCV seronegative female-23 (51.1%); male-22 (48.9%) aged 21-48 years were recruited and studied out of the sixty two (62) patients infected with *Plasmodium spp* based on the exclusion and inclusion criteria from 5 herbal homes in Saki-East Local government area of Oyo-state-Nigeria. Giemsa-thick film staining technique was used for Plasmodium detection including estimation of plasmodium parasite density and Viral immunochemical serology for anti-HIV, anti-HCV and HBsAg was carried out in the 62 subjects initially recruited was determined by ELISA and Immunoblotting. These tests were also employed in recruiting the normal control subjects (n=50). Plasma albumin, fibrinogen, Total Bile Acids and Lactate dehydrogenase were analyzed in the control and Plasmodium infected subjects before and after treatment with the raw extract of *Vernonia amygdalina*. **Results:** There was a significantly lower mean values of fibrinogen, LDH, Parasite density in the control Plasmodium non-infected subjects (264±10.6 mg/dl; 253 ±13.1 U/L; 0 µL) than the values (438±10.1 mg/dl; 302±18 U/L; 490±10.0 /µL) obtained in the plasmodium infected subjects before treatment with p<0.05. There was also a significantly lower mean values of fibrinogen and parasite density in the control Plasmodium non-infected subjects (264±10.6 mg/dl; 0/µL) than the values obtained in the Plasmodium infected patients after treatment with the extract (358±10.2 mg/dl; 205±15/µL) with p<0.05. However there was a significantly higher mean values of Fibrinogen, TBA, LDH, and Parasite density of 438±10.1 mg/dl, 13±1.8 µmol/L, 302±18 U/L and 490±10/µL respectively in the Plasmodium infected subjects before treatment than the values of these parameters obtained in the same subjects after the treatment (358±10.2 mg/dl; 10.1±1.5 µmol/L; 264±12 U/L; 205±15/µL) with p<0.05. The results obtained also showed a lower significant difference in the plasma value of albumin in the Plasmodium infected subjects before treatment (3.6±0.3 g/dl) than the value obtained in the same subjects after treatment (4.1±0.2 g/dl) with p<0.05. The immunochemical status of the Plasmodium infected patients revealed: 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or anti-HCV which include: 9.7% (6) HIV, 4.8% (3) HBV and 3.23% (2) HCV while 8.1% (5) of them were detected to be alcoholics and 1.6% (1) was found to be under analgesic therapy using paracetamol. **Conclusion:** There was a significant biochemical alterations in the concentration of plasma acute phase proteins (fibrinogen, albumin), Lactate dehydrogenase, Total Bile acids and parasite density in Plasmodium infected patients before the traditional treatment with extract of *Vernonia amygdalina* which was restored almost to normal after the treatment supporting the use of *Vernonia amygdalina* as an effective anti-plasmodia agent. The result obtained also revealed the immunochemical status of the Plasmodium infected patients as: 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or anti-HCV which include: 9.7% (6) HIV, 4.8% (3) HBV and 3.2% (2) HCV.

Keywords: *Vernonia amygdalina*, treatment, *Plasmodium spp* infection, acute phase protein, Total bile acids, Lactate dehydrogenase

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1. Introduction

The leaves of *Vernonia amygdalina* are commonly used in Nigeria as vegetable and for the traditional treatment of some disease conditions including malaria caused by *Plasmodium vivax*, *ovale*, *malariae* and *falciparum*. The common name of *Vernonia amygdalina* (VA) is bitter leaf. It is a shrub or small tree of 2 – 5 m that belong to the family Asteraceae. It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste. It is a domestic plant in Nigeria known as 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo and 'Chusa-diki' in Hausa. The phytochemicals in bitter leaf reported by Ugwoke *et al.*, [1] include carbohydrates, saponins, alkaloids, tannins, proteins and steroid occurred in very high concentration (+++), while flavonoids and glycosides occurred in high concentration (++). The concentration of resins was low (+). Acidic compounds and oils were not observed (-). Similarly, the chemical analysis revealed the presence of fat, protein, carbohydrate, fibre, ash and moisture, necessary for human growth and development. *Vernonia amygdalina* has been found useful in ethno medicine. Phytochemicals such as oxalates, phytates and tannins have also been reported by [2,3,4] as well as flavonoids [2,5,6] *Vernonia amygdalina* extracts have been shown to exhibit profound ethno medical and pharmacological properties viz, anti-diabetic, antimalarial, antihelminthic and antibiotic properties [7]

The phytochemicals in *Vernonia amygdalina* which include tannins are astringent, bitter tasting plant polyphenols that bind and precipitate proteins. Tannins may be employed in traditional treatment as antidiarrheal, antitumour and antihaemorrhoidal [8]. They have anti-inflammatory and reproductive effects [9]. They have been reported to have antioxidant, antimalarial and antimicrobial activities [10]. Alkaloids are poisonous, neurotoxic and are used as analgesics, antimalarials [11], CNS stimulants and used to treat erectile dysfunction [12]. Phenolics- some phenols are germicidal and are used in formulating disinfectant, they are antioxidants [13] and have antimalarial activity [14]. Flavonoids are polyphenolic compounds, they have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumour and antioxidant activities [15]. They have been shown to have immune-stimulating, anti-tumour and aphrodisiac effects [16].

Saponins are glycosides with distinctive foaming characteristics. They inhibit or kill cancer cells without killing normal cells. Saponins can bind cholesterol and also function as natural antibiotic for plants. Glycosides are anti-toxin binding the poison. Triterpenes used as pesticides, anti-allergy, anti-inflammatory, anti-tumour and antimalarial activities [17,18]. Steroids- important in growth and development. They have cholesterol lowering effects (lowering cholesterol absorption in intestines) [19].

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase

proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response). Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, mannose-binding protein, complement factors, ferritin, ceruloplasmin, Serum amyloid A and haptoglobin. Others give negative feedback on the inflammatory response, e.g. serpins. Alpha 2-macroglobulin and coagulation factors affect coagulation, mainly stimulating it. This pro-coagulant effect may limit infection by trapping pathogens in local blood clots [20].

Fibrinogen is a soluble plasma glycoprotein that is synthesized by the liver and is the most abundant clotting factor in plasma. Fibrinogen is one of the positive acute phase proteins, and its plasma levels commonly increase in acute-phase conditions. It causes blood clotting to trap invading microbes in blood clots [20].

Fibrinogen is often used to assess whether or not fibrinogen levels are adequate to allow normal blood clotting, to determine if an individual has an inherited deficiency, to evaluate unexplained or prolonged bleeding and to evaluate the risk of both cardiovascular and heart disease. Pathological changes in the concentration of Fibrinogen are found in many disease states [21]. Decreased levels are associated with Hypofibrinogenemia, Afibrinogenemia, Disseminated Intravascular Coagulation (DIC), Liver diseases, viral hepatitis and Fibrinogenolysis. Increased Fibrinogen concentration is seen in chronic inflammatory illness, malignancy, surgery, trauma and cardiovascular disorders [21].

Serum albumin proteins, which are the most abundant proteins in the clear, fluid portion of blood (serum), typically decrease during inflammation. Albumin helps the body tissues maintain the pressure necessary for proper distribution of body fluids. If the pressure inside and outside of body tissues is not equal, it can potentially lead to tissue damage. Healthy individuals typically have 3.4-5.4 grams of albumin per deciliter of serum. Albumin is a negative acute-phase protein which their plasma levels decrease in inflammation. The decrease the concentration of albumin may be used as marker of inflammation. The physiological role of decreased synthesis of negative acute phase proteins such as albumin is generally to save amino acids for producing "positive" acute-phase proteins more efficiently to destroy or inhibit growth of microbes and give negative feedback on the inflammatory response [21].

Bile acids are synthesized in the liver as a breakdown product of cholesterol and secreted into the gall bladder. They are released into the small intestine where they solubilize dietary lipids such as cholesterol, aiding their absorption. Bile acids are reabsorbed from the portal blood by hepatocyte extraction and re-excreted into bile, passing through the enterohepatic circulation several times before final excretion [22]. The measurement of bile acids in serum is a sensitive indicator of liver function. Fasting serum bile acids can be used in the diagnosis and prognosis of liver disease. Levels rise in many liver diseases, for example hepatitis and liver sclerosis [22]. Abnormal levels in fasting patients or immediately after a

meal can be used to detect liver disease and damage, impaired liver function, intestinal dysfunction and perhaps a gall bladder blockage. Bile acid measurement may detect some forms of liver disease earlier than standard liver tests because bile acids levels correspond to liver function, rather than liver damage [23].

Lactate dehydrogenase is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease [24].

A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD⁺ and back.

Lactate dehydrogenases exist in four distinct enzyme classes. Each one acts on either D-lactate (D-lactate dehydrogenase (cytochrome)) or L-lactate (L-lactate dehydrogenase (cytochrome)). Two are cytochrome c-dependent enzymes. Two are NAD (P)-dependent enzymes. This article is about the NAD (P)-dependent L-lactate dehydrogenase. LDH is a protein that normally appears throughout the body in small amounts. Many cancers can raise LDH levels, so LDH may be used as a tumor marker, but at the same time, it is not useful in identifying a specific kind of cancer. Measuring LDH levels can be helpful in monitoring treatment for cancer. Noncancerous conditions that can raise LDH levels include heart failure, hypothyroidism, anemia, and lung or liver disease [25].

Tissue breakdown releases LDH, and therefore LDH can be measured as a surrogate for tissue breakdown, e.g. hemolysis. Other disorders indicated by elevated LDH include cancer, meningitis, encephalitis, acute pancreatitis, and HIV. LDH is measured by the lactate dehydrogenase (LDH) test (also known as the LDH test or Lactic acid dehydrogenase test). Comparison of the measured LDH values with the normal range help guide diagnosis [26].

This work is therefore designed to determine the effect of raw extract of *Vernonia amygdalina* used in the treatment of Plasmodium spp infection on acute phase protein (albumin, fibrinogen), Total bile acids and Lactate dehydrogenase

2. Materials and Methods

2.1. Materials

2.1.1. Study Area

The study was carried out in Saki-East Local government area of Oyo state- Nigeria. It shares border with the Republic of Benin and area in Kwara state- Nigeria, Oorelope, ATISBO local government areas at the northern part of Oyo state, Nigeria.

2.1.2. Study Population

Sixty two (62) Plasmodium infected patients that visited 5 herbal homes between April and June, 2014 in Saki-East local government area that were tested positive of Plasmodium infection classified into female (n= 37); male: (n=25) aged 21 to 48 years were recruited for this work. Forty-five (45) of the Sixty-two including Female (n=23) and Males (n=22) were finally studied based on the

inclusion and the exclusion criteria because 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or anti-HCV considering their viral immunochemical profile, 8.1% (5) of them were detected to be alcoholics and 1.6% (1) was found to be under analgesic therapy using paracetamol. None of the subjects was jaundiced as at the time of sample collection. Fifty (50) age-matched non-alcoholics or under any drug that could affect the liver function and apparently healthy anicteric-subjects that tested negative to Plasmodium, HIV, HCV and HBV infection were studied as normal control subjects including Female-25: Male-25.

2.1.3. Sample Size

All the sixty two patients that visited 5 selected herbal homes in Saki-east Local government area of Oyo state, Nigeria between April and June, 2014 were considered for the study. Forty five 45 (72.6%) that were HIV, HBsAg and anti-HCV seronegative female-23 (51.1%); male-22 (48.9%) were recruited out of the sixty two (62) patients infected with *Plasmodium spp* and studied based on the exclusion and inclusion criteria.

2.1.4. Case Selection Procedure/s

Inclusion Criteria

Anicteric Plasmodium infected patients that were HIV, HBsAg and anti-HCV seronegative, non-alcoholics who were not under any therapeutic management that could cause liver dysfunction such as administration of paracetamol, contraceptives, alcohol that aged 21-48 years were included in the study.

Exclusion Criteria

1. Plasmodium infected patients that were HIV, HbsAg and anti-HCV seropositive were excluded.

2. Icteric Plasmodium infected HIV, HBsAg and anti-HCV seronegative patients aged 21 - 48 years were not recruited for the study.

3. Anicteric Plasmodium infected HIV, HBsAg and anti-HCV seropositive patients aged 21 -48 years were not included in the study.

4. Plasmodium infected HIV, HBsAg and anti-HCV seronegative patients but were alcoholics, and or those that have initiated antimalarial therapy or therapy like administration of paracetamol, contraceptives, alcohol that could cause liver dysfunction aged 21-48 years were excluded from the study.

Blood Sample

Five (5) milliliter of blood was collected into lithiumheparinized bottle from each of the test subjects after an overnight fasting before and after the administration of raw *Vernonia amygdalina* extract for the estimation of Plasmodium parasite density, LDH, HIV, HBsAg and anti-HCV tests, Albumin and Total Bile Acids. Another 5 ml of blood was also collected from each of the subjects into specimen bottles containing Sodium citrate anticoagulant for the estimation of Fibrinogen before and after the administration of the raw *Vernonia amygdalina* extract. The same volume of sample of blood was also collected from each of the control subjects.

The raw extract of *Vernonia amygdalina*

The leaves of the *Vernonia amygdalina* were plucked and confirmed at the Oyo State Agricultural Development Programme Headquarter in Saki-Nigeria. The leaves were squeezed for the crude extract of the liquid content by the

traditional healers without the addition of water. About 60 ml of this was administered to each of the patients for average of nine (9) days and until the malaria symptoms disappear.

2.2. Methods

a. Estimation of Total Bile Acids was carried out on the plasma samples of the subjects using Randox reagent kit. The manufacturer's instruction was strictly followed.

Principle: Two reactions are combined in this kinetic enzyme cycling method. In the first reaction bile acids are oxidised by 3- α hydroxysteroid dehydrogenase with the subsequent reduction of Thio-NAD to Thio-NADH. In the second reaction the oxidised bile acids are reduced by the same enzyme with the subsequent oxidation of NADH to NAD. The rate of formation of Thio-NADH is determined by measuring the specific absorbance change at 405 nm. (Abbreviations: NADH, NAD, Thio-NADH, Thio-NAD).

b. Screening for HIV Antibodies HIV screening were carried out using Immuno chromatographic kit (Chembio HIV 1 and 2 STAT-PAK). Positive samples were further confirmed by Western blot/ Immunoblotting using Immunoetics Qualicode TM HIV 1 and 2 kit.

c. Screening for HbsAg by Enzyme- Linked Immunosorbent Assay (ELISA) The ELISA kit from BIORAD Monolisa HBsAg ULTRA EIA92430 Marnes 49 American Journal of Biomedical Research La-Coquette-France was used. ELISA was done according to the manufacturer's instruction. The Optical density OD was read at 450/620 to 700 nanometre. The cut off value was determined by the mean of negative control + 0.05 (0.08). The test is valid if all values of negative control are lower or equal to 0.08 and Positive control was over 0.08 or equal to 1.0. A test sample is considered negative if the ratio value of sample: cut off value is lower than 1.0 and positive if equal to or greater than 1.0.

d. Screening for HCV Antibody by ELISA ELISA kit from DIA PRO Diagnostic Bioprobes 20099 Sesto San Giovanni (Milano)-Italy was used. ELISA was done according to the manufacturer's instruction The Optical density OD is read at 450/620 to 700 nanometre. The cut – off value is calculated as follows: NC (negative control) +350= cut-off (C), Calibrator mean value=0.540, S/C=1.4 (where S= sample and C- cut off). S/C = higher than 1.1. Any sample with a ratio value of sample /cut off less than 0.9 was considered negative and if higher than 1.1 is positive.

e. Plasma Lactate Dehydrogenase was estimated in the subjects using reagent kit of Randox. The manufacturer's instruction was followed strictly.

Principle: The LDH method measures the oxidation of L-lactate to pyruvate with simultaneous reduction of nicotinamide adenine dinucleotide (NAD). The change in absorbance at 340 nm due to the appearance of reduced NAD (NADH) is directly proportional to the LDH activity, since other reactants are present in non-rate limiting quantities and is measured using a dichromatic (340, 383 nm) rate technique.

f. Fibrinogen estimation was carried out using the reagent kit of RANDOX. The manufacturer's instruction was strictly followed.

Principle: The Fibrinogen assay is based on the Clauss method. In the presence of a high concentration of Thrombin the time required for clot formation in diluted plasma is inversely proportional to Fibrinogen concentration. The clotting time is read at 415 nm.

g. Albumin estimation was carried out using COBAS C111 Auto-Chemistry Analyzer with the reagent kit of ROCHE.

h. Identification and the estimation of the density of Plasmodium parasite in the subjects were carried out as follows: Plasmodium spp was determined in the blood of the control and the test subjects using Giemsa thick blood staining technique described by Cheesbrough [27]. Estimation of parasite number was carried out by multiplying average number of parasites per high power field (100x objective) by 500 within 10 [27,28].

2.3. Ethical Consideration

The proposal was reviewed and approved by the Research and Ethical Committee of Baptist Medical Centre, Saki-Oyo state- Nigeria before the commencement of the work. This is to protect the interest of patients to ensure that the patients and the community are not harmed in any form by the procedure. Only subjects that volunteered themselves for the study were recruited.

2.4. Data Analysis

The result obtained was subjected to statistical analysis to determine mean, standard deviation, correlation coefficient and 't' test at 0.05 level of significance using online: T-Test Calculator for 2 Dependent Means: www.socscistatistics.com/tests/ttestdependent/ A T-test calculator that compares 2 dependent population means for statistical significance.

3. Result

Table 1. Comparative analysis of Plasmodium Parasite density, plasma values of Albumin, Fibrinogen, Total Bile Acids and Lactate dehydrogenase before and after the administration of the extract

Mean \pm SE _M	Albumin (g/dl)	Fibrinogen (mg/dl)	Total Bile Acids (μ mol/L)	LDH (U/L)	Parasite density (μ L)
Values obtained before the treatment (Mean \pm SEM)	3.6 \pm 0.3	438 \pm 10.1	13 \pm 1.8	302 \pm 18	490 \pm 10.0
Values obtained after the treatment (Mean \pm SEM)	4.1 \pm 0.2	358 \pm 10.2	10.1 \pm 1.5	264 \pm 12	205 \pm 15
't' value	3.303	13.75	3.036	4.095	36.36
'p' value	0.0081	0.00001	0.0115	0.0032	0.00001
Level of significance at 0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Comment	Significant	Significant	Significant	Significant	Significant

Table 2. Comparative analysis of Plasmodium Parasite density, plasma values of Albumin, Fibrinogen, Total Bile Acids and Lactate obtained in the Control and in the test subjects before and after treatment

	Albumin (g/dl)	Fibrinogen (mg/dl)	Total Bile Acids (µml/L)	LDH (U/L)	Parasite density (per µL)
Values obtained from the Control subjects (n=50) (Mean ±SE _M)	3.9±0.2	264±10.6	7.4±0.6	253±13.1	0
Values obtained before the treatment (Mean ±SE _M)	3.6±0.3	438±10.1	13±1.8	302±18	490±10.0
't' values	0.832	12.30	2.68	2.93	97
'p' values	0.246	0.0033	0.058	0.0495	5.3 x 10 ⁻⁵
Level of significance at 0.05	>0.05	<0.05	>0.05	<0.05	<0.05
Comment	Not significant	Significant	Not significant	Significant	Significant
Values obtained after the treatment/Control values	4.1±0.2	358±10.2	10.1±1.5	264±12	205±15
't' value	0.707	7.06596	1.52	0.62	40
'p' value	0.28	0.0097	0.134	0.3	0.0003
Level of significance at 0.05	>0.05	<0.05	>0.05	>0.05	<0.05
Comment	Not Significant	Significant	Not Significant	Not Significant	Significant

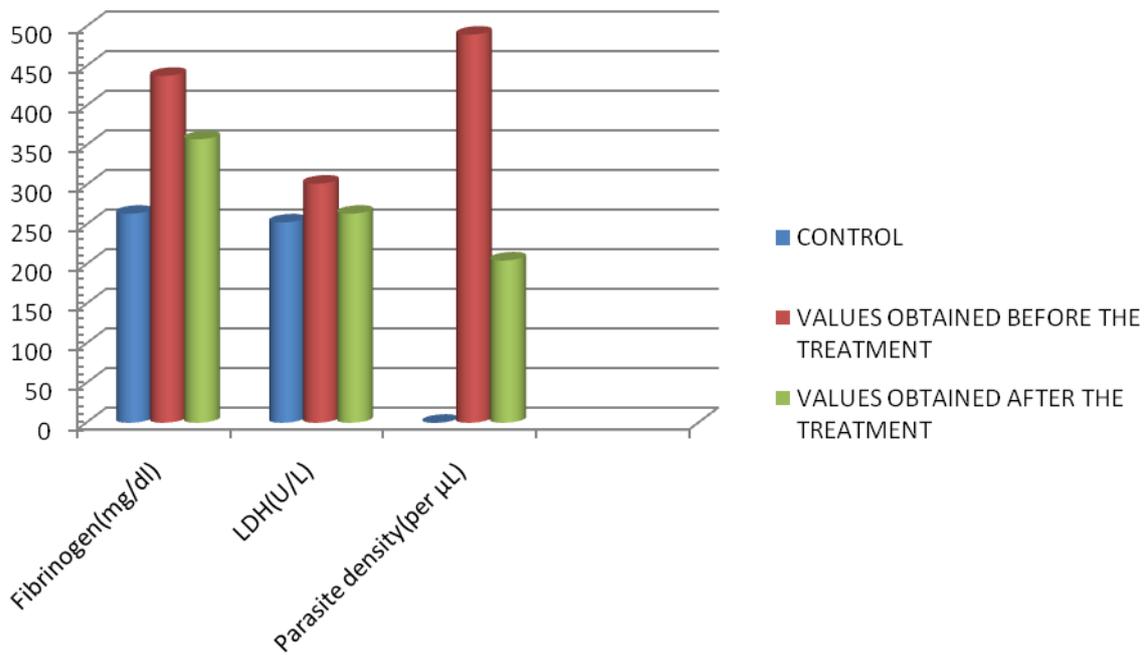


Figure 1.

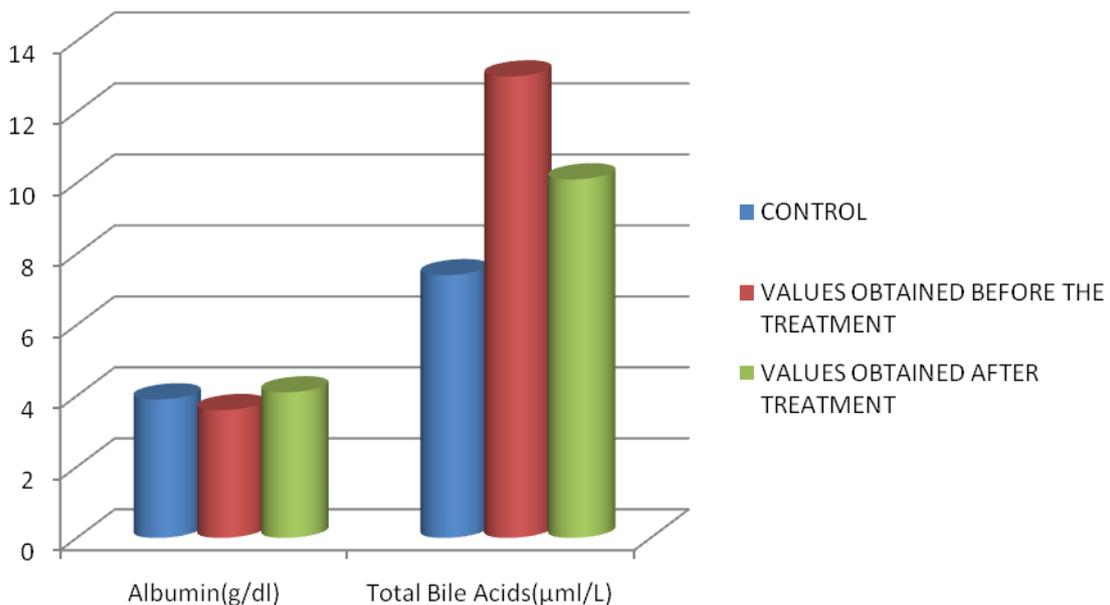


Figure 2.

Immunochemical Status of the Plasmodium Infected Patients

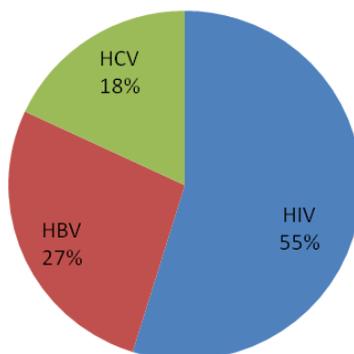


Figure 3.

The results obtained showed no significant difference when the values of plasma albumin, TBA, obtained in the Plasmodium non-infected control respectively (3.9 ± 0.2 g/dl; 7.4 ± 0.6 $\mu\text{mol/L}$) were compared with the mean values obtained from the test subjects before (3.6 ± 0.3 g/dl; 13 ± 1.8 $\mu\text{mol/L}$) and after (4.1 ± 0.2 g/dl; 10.1 ± 1.5 $\mu\text{mol/L}$) treatment with $p > 0.05$. There was also no significant difference in the mean value of LDH obtained in the control (253 ± 13.1 U/L) when the value was compared with the value obtained in the patients after treatment ($p > 0.05$). There was a significantly lower mean values of fibrinogen, LDH, Parasite density in the control (264 ± 10.6 mg/dl; 253 ± 13.1 U/L; 0 μL) compared with the values (438 ± 10.1 mg/dl; 302 ± 18 U/L; 490 ± 10.0 μL) obtained in the plasmodium infected subjects before treatment with $p < 0.05$. There was also a significantly lower mean values of fibrinogen and parasite density in the control (264 ± 10.6 mg/dl; 0 μL) than the values obtained in the Plasmodium infected patients after treatment with the extract (358 ± 10.2 mg/dl; 205 ± 15 μL) with $p < 0.05$. However there was a significantly higher mean values of Fibrinogen, TBA, LDH, and Parasite density of 438 ± 10.1 mg/dl, 13 ± 1.8 $\mu\text{mol/L}$, 302 ± 18 U/L and 490 ± 10 μL respectively in the Plasmodium infected subjects before treatment than the values of the these parameters obtained in the same subjects after the treatment (358 ± 10.2 mg/dl; 10.1 ± 1.5 $\mu\text{mol/L}$; 264 ± 12 U/L; 205 ± 15 μL) with $p < 0.05$. The results obtained also showed a lower significant difference in the plasma value of albumin in the Plasmodium infected subjects before treatment (3.6 ± 0.3 g/dl) than the value obtained in the same subjects after treatment (4.1 ± 0.2 g/dl) with $p < 0.05$.

The immunochemical status of the Plasmodium infected patients revealed: 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or anti-HCV which include: 9.7% (6) HIV, 4.8% (3) HBV and 3.2% (2) HCV while 8.1% (5) of them were detected to be alcoholics and 1.6% (1) was found to be under analgesic therapy using paracetamol.

4. Discussion

Significantly lower mean values of fibrinogen, LDH, Parasite density was obtained in the control compared

with the values obtained in the plasmodium infected subjects before treatment. This could be attributed to the fact that Fibrinogen is one of the positive acute phase proteins, and its plasma levels commonly increase in acute-phase conditions. It causes blood clotting to trap invading microbes in blood clots [20]. Raised plasma fibrinogen level in test subjects than the control subjects is consistent with the findings of Omoigberale *et al.*, [29] that found higher Fibrinogen levels of 3.40 ± 0.98 in children with malaria infection than 2.21 ± 0.81 in the controls. Fibrinogen is a protein produced by the liver. This protein helps stop bleeding by helping blood clots to form. A blood test can be done to tell how much fibrinogen you have in the blood. It may be elevated in any form of inflammation, as it is a positive acute-phase protein [38]. This explains the increase as a result of liver inflammation caused by the Plasmodium infection.

Increased plasma Lactate Dehydrogenase has been associated with cell and tissue destructions. The pathophysiology of Plasmodia infection is also associated with tissue and cell destructions which is responsible for the higher concentration of this parameter in the patients than the control. This is consistent with the reports of Yeo *et al.*, [30] and Ladhani *et al.*, 2010 that as the infection progresses, haemolysis is indicated by elevated LDH, free haemoglobin and low haptoglobin. This also agrees with Garba and Ubom [31] that found higher mean serum LDH activity in plasmodium infected patients than the control and that the combination of acute hepatocellular injury and red cell haemolysis induced by the invading merozoites may account for the increase in serum LDH activity during this infection. Therefore serum LDH activity is a potentially valuable enzymatic marker of acute, uncomplicated *P. falciparum* malaria infection, especially in the absence of other complicating diseases known to be associated with normal serum LDH activities. There was also a significantly lower mean value of fibrinogen and parasite density in the control than the values obtained in the Plasmodium infected patients after treatment with the extract could be attributed to the presence of plasmodium in the test subjects even after treatment and the earlier explanation also holds for these findings. This could also be associated with the report of van Wolfswinkel *et al.*, [32] that the C-reactive protein, procalcitonin, fibrinogen, orosomucoid and cytokine

levels are raised in acute malaria and that moderate hyponatraemia can be seen, with normal plasma potassium level. However there was a significantly higher mean value of Fibrinogen, TBA, LDH, and Parasite density in the Plasmodium infected subjects before treatment than the values of the parameters obtained in the same subjects after the treatment. These findings could be attributed to the effectiveness of the extract of *Vernonia amygdalina* in the treatment of plasmodium infection as reported by previous authors [7,10,11]. The plasma Total Bile Acids could be used to determine liver dysfunction which could be found in plasmodium infection as the pathophysiology of malaria involves liver. Liver dysfunction was however found to be more in plasmodium infected subjects than the control and in the test subjects before treatment than after treatment considering the significant alterations in the value of plasma total bile acids [33,34]. Levels of TBA rise in many liver diseases, for example hepatitis and liver sclerosis [22]. Abnormal levels in fasting patients or immediately after a meal can be used to detect liver disease and damage, impaired liver function, intestinal dysfunction and perhaps a gall bladder blockage. Bile acid measurement may detect some forms of liver disease earlier than standard liver tests because bile acids levels correspond to liver function, rather than liver damage [23]. The concentration of LDH and Parasite density found to be higher in the plasmodium infected subjects before treatment than after treatment was due to the presence of plasmodium and red blood cells destruction by the parasite which decreases after the treatment with the extract of *Vernonia amygdalina*. The results obtained also showed a lower significant difference in the plasma value of albumin in the Plasmodium infected subjects before treatment than the value obtained in the same subjects after treatment. This is also due to the effectiveness of the extract of *Vernonia amygdalina* in the treatment of malaria infection as the plasma albumin is a negative acute phase protein whose level decreases upon infection/inflammation which was found to increase within the reference limit after treatment [7,10,11].

The viral immunochemical study of the patients revealed that 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or anti-HCV which is consistent with the report of Christina *et al.*, [35] that reviewed evidence of viral co-infection with malaria and Olaniyan and Babatunde [36] that reported 16.1% (114) (Female: 61 (53.5%); Male: 53 (46.5%)) of Plasmodium infected subjects co-infected with at least one of HIV, HBV or HCV.

Conclusion and Recommendation

There was a significant biochemical alterations in the concentration of plasma acute phase proteins (fibrinogen, albumin), Lactate dehydrogenase, Total Bile acids and parasite density in Plasmodium infected patients before the traditional treatment with extract of *Vernonia amygdalina* which was restored almost to normal after the treatment supporting the use of *Vernonia amygdalina* as an effective anti-plasmodia agent. The result obtained also revealed the immunochemical status of the Plasmodium infected patients as: 17.7% (11) out of the 62 Plasmodium infected patients were tested positive to HIV, HBsAg or

anti-HCV which include: 9.7% (6) HIV, 4.8% (3) HBV and 3.2% (2) HCV.

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