

**LIVER AND KIDNEY FUNCTION TESTS AND HISTOLOGICAL STUDY ON
MALARIA PARASITE INFECTED MICE ADMINISTERED WITH SEED EXTRACT
OF *PICRALIMA NITIDA***

ABSTRACT

The liver and kidney are very important organs of the body that play vital roles of detoxification of compounds and removal of waste products respectively. In this research, some liver and kidney function tests were carried out on albino mice infected with malaria parasite to know the effect of treatment with extract on them. Pulverised dried seeds of *Picralima nitida* were extracted using ethanol. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total bilirubin, total protein, urea and creatinine levels and histopathological studies on liver and kidney tissues were performed. From the results of the experiment, significant ($p < 0.05$) reductions were observed in serum levels of urea, creatinine of the treated groups compared to the positive control (group infected with malaria parasite and treated with 3 % tween 80) on days 3 and 5 post treatment. There was non-significant ($p > 0.05$) reduction in serum AST and ALT activities of the groups treated with 20, 40 and 80 mg/kg b.w. of the extract on days 3 and 5 post treatment compared to the positive control. Groups treated with 20 and 40 mg/kg b.w. of the extract showed significant ($p < 0.05$) reduction in bilirubin level on days 3 and 5 post treatment compared to the positive control. All the treated groups showed significant ($p < 0.05$) elevation in serum total protein on day 3 post treatment while the group treated with 80 mg/kg b.w. of the extract showed significant ($p < 0.05$) elevation in total protein on day 5 treatment compared to the positive control. Histopathological studies on the hepatic tissues of the treated mice showed lesser inflammatory cells around the portal areas than the untreated. No lesions were noticed in the renal tubules of both the treated and untreated mice. From the results of this experiment, it is now obvious that *Picralima nitida* seeds possess good hepato-protective properties.

Key words: Liver and kidney function tests, *Picralima nitida*, serum ALT and AST, serum total bilirubin, serum total protein, serum urea and creatinine.

INTRODUCTION

Plasmodium berghei is a unicellular protozoan parasite that infects mammals other than humans. It was first described by Vincke and Lips in 1948 in the Belgian Congo. It is found in the forests of Central Africa, where its natural cyclic hosts are the thick-knee rat and the mosquito (*Anopheles durenii*) (Vincke and Lips, 1948).

Like all malaria parasites of mammals, *P. berghei* infects the liver after being injected into the bloodstream by a bite of an infected female mosquito. After a short period of development and multiplication, these parasites leave the liver and invade the erythrocytes. *P. berghei* infections may also affect the brain and can cause cerebral complications in laboratory mice. The complete genome of *P. berghei* has been sequenced and it shows a high similarity both in structure and gene content with the genome of the human *Plasmodium falciparum* (Hall *et al.*, 2005; Kooij *et al.*, 2006).

The parasite can be genetically manipulated and so, is often used for the analysis of the function of malaria genes using the technology of genetic modification (Janse *et al.*, 2006; Janse *et al.*, 2011). A number of genetically modified *Plasmodium berghei* cell lines have been generated and these transgenic parasites are tools to study and visualize the parasites in the living host (Amino *et al.*, 2005; Franke-Fayard *et al.*, 2006).

Livers function tests (LFTs of LFs) are group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver (Lee, 2009). The parameters measured include prothrombin time (PT/INR), activated partial thromboplastin time (a PTT), albumin, bilirubin and others. Aspartate aminotransferase and alanine aminotransferase (AST and ALT) are useful biomarkers of liver injury in a patient with some degree of intact liver (Johnston, 1999). Some tests are associated with functionality (e.g. albumin), some with cellular integrity (e.g. transferases), and some with conditions linked to the biliary tract (e.g. gamma-glutamyl transferase and alkaline phosphate) (McClatchey, 2002). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver diseases, distinguish among different types of liver disorders, gauge the extent of known liver damage and follow the response to treatment.

Albumin is the main constituent of total protein made specifically by the liver and can easily be measured. Its levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephritic syndrome, where it is lost through the urine. The consequence of low albumin can be edema since the intravascular oncotic pressure becomes lower than the extravascular space (Menge and Schwiebert, 2005).

Alanine aminotransferase (ALT), also known as serum glutamate pyruvic transaminase (SGPT), is a member of transferase family. It catalyses the reversible reaction in which an α -amino group is transferred between alanine and glutamine (Gaze, 2007).

This enzyme is found mainly in liver and small amount in heart, muscle, and kidney. In healthy subjects, serum ALT levels are low. However, when cells are damaged, for instance, acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, this enzyme may leak into the blood stream and its levels are significantly elevated (Hayashi *et al.*, 2003; Berg *et al.*, 2006).

Aspartate aminotransferase (AST) is similar to ALT in that it is another enzyme associated with parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscles, so is not specific to the liver (www.notebook.co.uk, 2014). So, elevated AST levels are not specific for liver damage. Aspartate aminotransferase, as with all aminotransferases, operates via dual substrate recognition; that is, it is able to recognize and selectively bind two amino acids (Asp and Glu) with different side-chains (Hirotsu *et al.*, 2005). In either case, the aminotranferase reaction consists of two similar half-reactions that constitute what is referred to as a ping-pong mechanism. In the first half-reaction, amino acid 1 (e.g., L-Asp) reacts with the enzyme-PLP complex to generate ketoacid 1 (oxaloacetate) and the modified enzyme-PMP. In the second half-reaction, ketoacid 2 (α -ketoglutarate) reacts with enzyme-PMP to produce amino acid 2 (L-Glu), regenerating the original enzyme-PLP in the process.

Total bilirubin includes both unconjugated and conjugated bilirubin. Unconjugated bilirubin is a breakdown product of haem (Pirone *et al.*, 2009). It is very hydrophobic and is mainly transported bound to albumin circulating in the blood. High concentrations of hydrophobic drugs and high free fatty acids can cause elevated unconjugated bilirubin. The liver is responsible for clearing the blood of unconjugated bilirubin, and about 30% of it is taken up by a normal liver on each pass of the blood through the liver by the some mechanisms.

Bilirubin can be conjugated with a molecule of glucuronic acid which makes it soluble in water. Some of the double-bonds in bilirubin isomerizes when exposed to light. This is used in the phototherapy of jaundiced newborns: the E, Z-isomers of bilirubin formed upon light exposure

are more soluble than the unilluminated Z, Z-isomer, as the possibility of intramolecular hydrogen bonding is removed (McDonagh *et al.*, 1980). This allows the excretion of unconjugated bilirubin in bile.

Erythrocytes generated in the bone marrow are disposed of in the spleen when they get old or damaged. This releases haemoglobin, which is broken down to haem as the globin parts are turned into amino acids (Cheifetz, 2010). The haem is then converted into unconjugated bilirubin in the reticuloendothelial cells of the spleen. This water-insoluble unconjugated bilirubin is then bound to albumin and sent to the liver. In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase making it water-soluble and afterwards goes into the bile. Conjugated bilirubin is not absorbed in the small intestine but instead is passed into the colon (Cheifetz, 2010). Colonic bacteria deconjugate and metabolize the bilirubin into colourless urobilinogen which is further oxidized to urobilin and stercobilin which give stool its characteristic brown colour. A trace amount of urobilinogen is reabsorbed into the enterohepatic circulation to be re-excreted in the bile: some of this is instead processed by the kidneys colouring the urine (Kuntz, 2008).

Kidney or renal function tests are common laboratory tests used to evaluate how well the kidneys are working. Such tests include: blood urea nitrogen (BUN), serum creatinine test, urinalysis and glomerular filtration rate (GFR) test (Kidney Function Tests, 2010). Urea is produced as a waste product of protein digestion by the liver. Normal human adult blood should contain 6-20 mg/dL of blood. The main causes of an increase in BUN are: high protein diet, decrease in glomerular filtrate rate (GFR) and in blood volume, congestive heart failure, gastrointestinal haemorrhage, fever and increased catabolism (Kasper *et al.*, 2005). Decreases in BUN are caused by severe liver disease, anabolic state and syndrome of inappropriate antidiuretic hormone (ADH).

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (www.medicinenet.com, 2014). Serum creatinine is an important indicator of renal health because it is an easily measured by-product of muscle metabolism excreted unchanged by the kidneys. It is synthesized by the liver from the methylation of glycocyamine by S-adenosyl methionine. It is then transported through the blood to the other organs, muscles, and brain where it is phosphorylated to phosphocreatine. During this reaction, creatine and phosphocreatine are catalyzed by creatine kinase, and a spontaneous conversion to

creatinine may occur (Allen, 2012). Creatinine is removed from the blood by the kidneys through glomerular filtration and also by proximal tubular secretion (Shemesh, 1985). There is little or no tubular reabsorption of creatinine. When its filtration in the kidney is impaired, blood creatinine levels rise and so its levels in blood and urine may be used to calculate the creatinine clearance (Crcl). Creatinine levels in the blood and urine correlates with the glomerular filtration rate (GFR) while blood creatinine levels alone are used to calculate the estimated GFR (eGFR).

Extracts of *Picralima nitida* parts (root, seed and stembark) have been shown to exhibit a broad spectrum of activity against bacterial strains (Nkere and Iroegbu, 2005). Among the susceptible bacteria were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella kintambo*. The Minimum Inhibitory Concentration (MIC) values for the ethanol extracts range from 6.25 to 50 mg/ml, while the MIC values for the cold water seed extracts (CWs) was 50 mg/ml showing that the ethanol extracts were more potent than the cold water extracts at very low concentrations. The activities of these extracts were possible because they contained a good number of phytochemicals such as alkaloids, saponins, flavonoids, steroids, tannins and terpenoids. Ubulom *et al.* (2011) have investigated into the antifungal activity of both aqueous and ethanolic extracts of *Picralima nitida* seeds against *Aspergillus flavus*, *Candida albicans* and *Microsporum canis* and found them to have activity against these organisms. However, the ethanol extract was found to have better antifungal activity than the aqueous extract. Okonta and Aguwa (2007) compared the hypoglycemic effect of glycosides extract with that of alkaloids extract of *P. nitida* seed and were able to establish that the hypoglycemic effect of the seed lies in the glycosides extract. The alkaloids extract reduced given i.p caused increase in mean fasting blood glucose levels while the glycosides extract reduced the blood glucose levels in normoglycemic and hyperglycemic rats.

In traditional medicine, numerous plants have been used for cognitive disorders, including memory loss and antiaging. In accordance, *Picralima nitida* fruits and seeds have been found to possess memory enhancing property (Mojisola *et al.*, 2012) and so could be applied in the treatment of cognitive disorders like Alzheimer's disease, amnesia, depression and schizophrenia. Soladoye *et al.* (2010) performed ethnobotanical survey of plants used in the treatment of haemorrhoids in South-Western Nigeria and found *Picralima nitida* fruit to be very effective. From the survey, a total of 143 plant species belonging to 58 families were found to be

useful for the treatment of haemorrhoids. The most prominent family among these plant families is the *Leguminosae*, and then others include *Euphorbiaceae*, *Apocynaceae*, *Meliaceae*, *Compositae* and *Poaceae*. The seed extract of *P. nitida* (Enomfon and Ime, 2004) showed inhibition of β -glucosidase activity and so the exploitation of this potential could be of immense value in the current search for new therapeutically effective drugs with inhibitory effects against β -glucosidase which is implicated in HIV infectivity of cells. The aim of this study however, is to ascertain the protective effect of the seed extract of *Picralima nitida* on hepatic and renal tissues so as to advice the users better on how to administer the drug.

MATERIALS AND METHODS

Materials

Collection of *Picralima nitida* seeds

The seeds of *Picralima nitida* were collected from Isuofia, Aguata Local Government Area of Anambra State, Nigeria and were authenticated by Mr. Alfred Ozioko of the Bioresource Development and Conservation Programme (BDGP) Research Centre, Nsukka, Nigeria.

Instruments/Equipment

The following instruments were used for the experiment: Adjustable micropipette (PERFECT, USA), Chemical balance (Gallenkamp, England), Digital photo colorimeter (E1,312 Model, Japan), Incubator (Gallenkamp, England), Microscope slides (UNESCOPE, USA), pH meter (Pye, Unicam 293, England), Refrigerator (Kelvinator, Germany) and Spectrophotometer (NOVASPEC LKB Biochrome, model 4049, Germany)

Chemicals/reagents

The commercial kits used for this study were products of Randox, QCA, USA and Biosystem Reagents and Instruments, Spain.

Methods

Extraction

Seeds of *Picralima nitida* plant were harvested and then dried at room temperature (29-35 °C) for three weeks, after which they were pulverized into powdered form with a Creston high speed milling machine. The powdered seeds (1 kg) were then macerated in 5 volume (w/v) absolute

ethanol at room temperature for 48 hours. Afterwards, the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was finely filtered using Whatman qualitative filter paper, concentrated and evaporated to dryness using rotary evaporated at an optimum temperature of between 40 and 45 °C (to avoid denaturation of the active ingredients). The concentrated extract was stored in the refrigerator for subsequent studies.

Experimental design

A total of 180 albino mice of either sex weighing 20-34 g were housed in separate cages, acclimatized for one week and then divided into six groups of thirty mice each as follows:

Group 1: Normal control (Normal mice treated with the vehicle, 3% tween 80)

Group 2: Positive control (Mice inoculated with malaria parasite and treated with 3% tween 80)

Group 3: Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract

Group 4: Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract

Group 5: Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract

Group 6: Standard control (Mice inoculated with malaria parasite and treated with the standard drug, artesunate)

The route of administration (treatment) was via oral route with the aid of an oral intubation tube.

Procurement of parasitaemia

Malaria parasitaemia (*Plasmodium berghei*) was obtained from malaria-infected mice at Veterinary Medicine Department of University of Nigeria, Nsukka. Ten drops of the parasitized blood obtained with the aid of a capillary tube through the ocular region of the mice, was diluted with 1 ml of normal saline. Thereafter, 0.2 ml of the diluted parasitized blood was used to passage three mice that served as the host from where subsequent ones were passaged.

Assay of aspartate aminotransferase (AST) activity

The activity of aspartate aminotransferase (AST) was assayed by the method of Reitman and Frankel (1957) as outline in Randox kit.

Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazone with 2, 4-dinitrophenylhydrazine.

The AST substrate phosphate buffer solutions (0.5 ml each) were pipetted into both the reagent blank and sample test tubes. Serum sample of 0.1 ml was added to the sample test tube only and mixed thoroughly. Then, 0.1 ml of distilled water was added to the reagent blank. Afterwards, the entire reaction medium was well mixed and incubated in a water bath at 37⁰ C for 30 minutes.

A volume, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the reagent blank and the sample test tubes immediately after incubation. Also, 0.1 ml of the sample was added to blank only. The medium was mixed and allowed to stand for exactly 20 minutes at 25⁰ C. Finally, 5.0 ml of sodium hydroxide (NaOH) solution was added to both the blank and sample test tubes and mixed thoroughly. After 5 minutes, the absorbance of sample was read at 550 nm against the sample blank after 5 minutes. The activity of AST in mice serum was obtained from the already calibrated table.

Assay of alanine aminotransferase (ALT) activity

The activity of alanine aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit.

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Aliquots, 0.5 ml of the ALT substrate phosphate buffer solutions were pipetted into two sets of test tubes labeled B (sample blank) and T (sample test). The serum sample (0.1 ml) was added to the sample test (T) test tubes only and mixed thoroughly and then, incubated in a water bath at temperature of 37⁰ C for 30 minutes.

A volume, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to each of the test tubes labelled T (sample test) and B (sample blank) immediately after the incubation. Also, 0.1 ml of serum sample was added to sample blank (B) only. The entire medium was mixed thoroughly and allowed to stand at 25⁰ C for exactly 20 minutes. After this, 5.0 ml of sodium hydroxide (NaOH) solution was added to each test tube and mixed thoroughly. The absorbance of sample against the sample blank was read at a wavelength of 550 nm against the sample blank after 5 minutes. The activity of ALT in the serum was obtained from a pre-calibrated table.

Determination of total bilirubin concentration

Total bilirubin concentration was determined using the method of Jendrassik and Grof (1938) as outlined in the Randox kit.

A volume, 0.2 ml of sulphanilic acid was pipetted into the sample blank tube and sample tube. This was immediately followed by the addition of 0.05 ml of sodium nitrite to the sample tube. Caffeine (10 ml) and 0.2 ml of sample were also pipetted into each of the sample blank tube and sample tube. These mixtures were mixed and incubated for 10 minutes at 20-25⁰ C. Finally, 1.0 ml of tartrate was pipetted into the sample blank tube and sample tube. These mixtures were once again mixed, incubated at 20-25⁰ C for 30 minutes and their absorbances read at 578 nm against the sample blank.

Determination of Total Protein

Total protein concentration was determined using the method of Lowry *et al.* (1951)

Eleven sets of test tubes were set up in a rack and filled with different concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µl) of Bovine Serum Albumin (BSA). A known volume, 2 ml of solution D (48 ml of 2 % Na₂CO₃ in 0.1 N NaOH; 1 ml of 1 % NaK Tartarate in H₂O and 1 ml of 0.5 % CuSO₄.5H₂O in H₂O). The mixture was incubated for 10 minutes at room temperature. An aliquot, 0.2 ml of dilute Folin-phenol as added to each test tube and vortex immediately. Afterwards, the contents were incubated for 30 minutes and the absorbance readings taken at 600 nm.

Determination of serum urea concentration

The concentration of serum urea was determined using the method of Tietz (1994) as outlined in Randox kits, UK.

A known volume, 10 µl of the sample was pipetted into the sample tube; 10 µl of the standard was also pipetted into the standard tube while 10 µl of distilled water was added to the blank tube. A volume, 10 µl sodium nitroprusside and 10 µl urease were added to each of the three tubes. The tubes were mixed and incubated at 37⁰ C for 15 minutes. Then, 2.50 ml of phenol was added to each of the three tubes followed by addition of 2.50 ml of sodium hypochlorite. After

274 mixing, they were incubated at 37⁰ C for 15 minutes and the absorbance of the sample was read
275 against the reagent blank at 546 nm.

276 **Determination of serum creatinine concentration**

278 The concentration of serum creatinine was determined using the method of Tietz (1994) as
279 outlined in Randox kits, UK.

280 A volume, 100 µl, of distilled water was pipetted into the blank tube. Also 100 µl of the
281 standard was added to the standard tube while 100 µl of the sample was pipetted into the sample
282 tube. Then, 100 µl of the working reagent was pipetted into each of the three tubes and mixed.
283 The absorbance of the sample was read against the blank at 492 nm.

284 **Histopathological Study**

286 The tissue specimens (liver and kidney) were excised from the sacrificed rats and fixed in 10%
287 formaldehyde buffered saline (formal saline). After some days, the processing started with the
288 dehydration in graded levels of alcohol (about 70-100%) in ascending order to remove the water
289 content.

290 After the dehydration, the tissues were cleared in xylene impregnated with paraffin wax and
291 sectioned at 5 microns thickness using rotary microtone. The sections were floated on a water
292 bath maintained at a temperature of 2-3°C below melting point of the paraffin wax after which
293 the sections were dried on a hot plate maintained at a temperature of 2-3°C above the melting
294 point of the paraffin wax used. After proper drying, the sections were stained and mounted using
295 haematoxylin and eosin in such a way as to avoid air bubbles.

296 **Experimental Animals**

297 The animals used for this study were white albino mice of either sex weighing 20-34 g. The mice
298 were between 3-4 months old and were obtained from the Animal Unit of Faculty of Veterinary
299 Medicine, University of Nigeria, Nsukka.

300 Clearance and approval for the humane use and handling of laboratory animals were given by the
301 ethical committee of Biochemistry Department, University of Nigeria Nsukka.

RESULTS

In table 1, the serum AST and ALT activities and the total protein level are presented. In normal mice, the AST activity was found to be 28.60 ± 1.17 IU/L; the ALT activity was 44.60 ± 2.78 IU/L and the total protein level was 6.74 ± 0.19 g/dl. In the positive control group, AST and ALT activities were non-significantly ($p > 0.05$) elevated compared to the normal control. The total protein level in the positive control was significantly ($p < 0.05$) reduced compared to the normal control. In group 3, the AST activity was shown to be 30.20 ± 3.58 IU/L on day 3 post treatment. This activity was non-significantly reduced to 29.40 ± 3.44 IU/L on day 5 post treatment. In groups 4 and 5, the activity of AST was 29.60 ± 3.01 and 29.00 ± 3.02 IU/L on day 3 post treatment which reduced non-significantly to 29.00 ± 3.02 and 28.80 ± 2.85 IU/L on day 5 post treatment respectively. The total protein level was observed to be 5.78 ± 0.10 g/dl in group 3, 5.96 ± 0.10 g/dl in group 4 and 6.04 ± 0.08 g/dl in group 5 on day 3 post treatment. These concentrations were non-significantly elevated to 5.86 ± 0.10 , 6.00 ± 0.11 and 6.34 ± 0.05 IU/L respectively on day 5 post treatment. Group 6 (standard control) showed AST activity of 30.80 ± 2.60 IU/L on day 3 of post treatment which reduced to 29.40 ± 1.57 IU/L on day 5 post treatment. The standard control showed ALT activity of 45.40 ± 1.47 IU/L on day 3 post treatment which reduced further to 44.40 ± 1.75 IU/L on day 5 post treatment. The standard control produced 5.74 ± 0.12 g/dl on day 3 post treatment and 6.08 ± 0.09 g/dl on day 5 post treatment. The effect of treatment with ethanol seed extract of *Picralima nitida* on AST activity and total protein level of the treated mice was dose-dependent while its effect on ALT activity of treated mice was non dose-dependent.

In table 2 are presented the mean values of serum bilirubin (0.34 ± 0.05 mg/dl), urea (20.00 ± 0.32 mg/100 ml) and creatinine (0.72 ± 0.01 mg/100 ml) of normal mice. There was significant ($p < 0.05$) elevations in serum urea and creatinine and non-significant ($p > 0.05$) elevation in serum bilirubin levels in the positive control compared to the normal control. In all experimental groups of mice, the mean values for bilirubin on day 3 post treatment were 1.46 ± 0.10 , 1.28 ± 0.11 and 0.88 ± 0.10 mg/dl respectively. On day 5 post treatment, the levels further decreased non-significantly to 1.40 ± 0.11 , 1.22 ± 0.11 and 0.78 ± 0.10 mg/dl respectively. The standard control showed bilirubin levels of 1.12 ± 0.12 mg/dl on day 3 post treatment and 1.04 ± 0.13 mg/dl on day 5 post treatment. Compared to the positive control, groups 3 and 4 showed non-

significant ($p > 0.05$) reductions in serum bilirubin level on days 3 and 5 post treatment. The treatment with ethanol seed extract of *Picralima nitida* showed a dose-dependent effect on serum bilirubin level in the treated mice. In normal mice, the serum urea level was found to be 20.00 ± 0.32 mg/100 ml while that of the positive control was 34.80 ± 0.37 mg/100 ml. On day 3 post treatment, groups 3, 4 and 5 produced decreases in serum urea level as 32.60 ± 0.40 , 29.20 ± 0.37 and 31.00 ± 0.71 mg/100 ml respectively which decreased further to 30.80 ± 0.37 , 28.40 ± 0.51 and 24.40 ± 0.68 mg/100 ml respectively on day 5 post treatment. The effect exerted by the treated groups on serum urea level of the treated mice followed a non dose-dependent fashion. The standard control showed serum urea level of 26.40 ± 0.51 mg/100 ml on day 3 post treatment with a reduction of 1.00 mg/100 ml serum urea on day 5 post treatment. On days 3 and 5 post treatment, all the extract treated groups showed significant ($p < 0.05$) reductions in serum urea level compared to the positive control. The serum creatinine level of normal mice was found to be 0.72 ± 0.01 mg/100 ml and that of positive control was 1.33 ± 0.01 mg/100 ml. On day 3 post treatment, groups 3, 4 and 5 showed creatinine levels of 1.30 ± 0.01 , 1.14 ± 0.01 and 1.14 ± 0.02 which decreased to 1.22 ± 0.01 , 1.07 ± 0.01 and 0.99 ± 0.02 mg/100 ml respectively on day 5 post treatment. When compared with the positive control, groups 3, 4 and 5 showed significant ($p < 0.05$) reductions in serum creatinine concentration on days 3 and 5 post treatment. The effect was non dose-dependent. There was a significant ($p < 0.05$) elevation in serum creatinine level in the positive control compared to the normal control. The standard control showed the serum creatinine level of 1.07 ± 0.02 mg/100 ml on day 3 post treatment which decreased to 1.03 ± 0.02 mg/100 ml on day 5 post treatment.

The histomorphological changes in the liver sections of the different groups of mice (positive/untreated control, negative/normal control, standard control and treated groups) are shown in plates 1. There is infiltration of mononuclear cells (inflammatory cells) around the portal area (PA) (portal hepatitis) (white arrow) in groups 2,5 and 6 while group 1 shows normal PA and hepatocytes(black arrow). The severity of inflammatory cells infiltration is more in 2 than 5 and 6.

The changes in the kidney sections of the different groups of mice (positive/untreated control, negative/normal control, standard control and treated groups) are presented in plates 2. There is

no infiltration of mononuclear cells around the glomeruli (G) and renal tubules (arrow) in the kidneys of all the experimental groups.

Table 1: Table showing the values serum AST and ALT activities and total protein concentration

Groups	Day 3 of post treatment	Day 5 of post treatment	Parameters
AST (IU/L)			
1	28.60 ± 1.17*	N.D.	“
2	32.20 ± 3.20	N.D.	“
3	30.20 ± 3.58	29.40 ± 3.44	“
4	29.60 ± 3.01	29.00 ± 3.02	“
5	29.40 ± 3.01	28.80 ± 2.85	“
6	30.80 ± 2.60	29.40 ± 1.57	“
ALT (IU/L)			
1	44.60 ± 2.78	N.D.	“
2	49.40 ± 3.03	N.D.	“
3	48.80 ± 2.85	48.00 ± 2.88	“
4	49.20 ± 2.62	46.60 ± 2.54	“
5	46.20 ± 2.50	45.20 ± 2.87	“
6	45.40 ± 1.47	44.40 ± 1.75	“
Total Protein (g/dl)			
1	6.74 ± 0.19*	N.D.	“
2	5.76 ± 0.10	N.D.	“
3	5.78 ± 0.10*	5.86 ± 0.10	“
4	5.96 ± 0.10*	6.00 ± 0.11	“
5	6.04 ± 0.08*	6.34 ± 0.05	“
6	5.74 ± 0.12	6.08 ± 0.09	“

* = p < 0.05 compared to the positive control (n=5)

The results are expressed as mean ± standard error of mean (S.E.M)

N.D. = Not determined

Group 1: Normal control (Normal mice treated with the vehicle, 3% tween 80)

Group 2: Positive control (Mice inoculated with malaria parasite and treated with 3% tween 80)

Group 3: Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract
Group 4: Mice inoculated with malaria parasite and treated with 40 mg/kg b.w. of the extract
Group 5: Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract
Group 6: Standard control (Mice inoculated with malaria parasite and treated with the standard drug, artesunate)

Table 2: Table showing the serum total bilirubin, urea and creatinine concentrations

Groups	Day 3 of post treatment	Day 5 of post treatment	Parameters
			Bilirubin (mg/dl)
1	0.34 ± 0.05	N.D.	“
2	1.50 ± 0.11	N.D.	“
3	1.46 ± 0.10	1.40 ± 0.11	“
4	1.28 ± 0.11	1.22 ± 0.11	“
5	0.88 ± 0.10*	0.78 ± 0.10*	“
6	1.12 ± 0.12	1.04 ± 0.13	
			Urea (mg/100 ml)
1	20.00 ± 0.32*	N.D.	“
2	34.80 ± 0.37	N.D.	“
3	32.60 ± 0.40*	30.80 ± 0.37	“
4	29.20 ± 0.37*	28.40 ± 0.51	“
5	31.00 ± 0.71*	24.40 ± 0.68	“
6	26.40 ± 0.51	25.40 ± 0.51	“
			Creatinine (mg/100 ml)
1	0.72 ± 0.01*	N.D.	”
2	1.33 ± 0.01	N.D.	“
3	1.30 ± 0.01*	1.22 ± 0.01	“
4	1.14 ± 0.01*	1.07 ± 0.01	“
5	1.14 ± 0.02*	0.99 ± 0.02	“
6	1.07 ± 0.02	1.03 ± 0.02	“

* = p < 0.05 compared to the positive control (n=5)

The results are expressed as mean ± standard error of mean (S.E.M)

N.D. = Not determined

Group 1: Normal control (Normal mice treated with the vehicle, 3% tween 80)

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Group 3: Mice inoculated with malaria parasite and treated with 20 mg/kg b.w. of the extract
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Group 5: Mice inoculated with malaria parasite and treated with 80 mg/kg b.w. of the extract
Group 6: Standard control (Mice inoculated with malaria parasite and treated with the standard drug, artesunate)

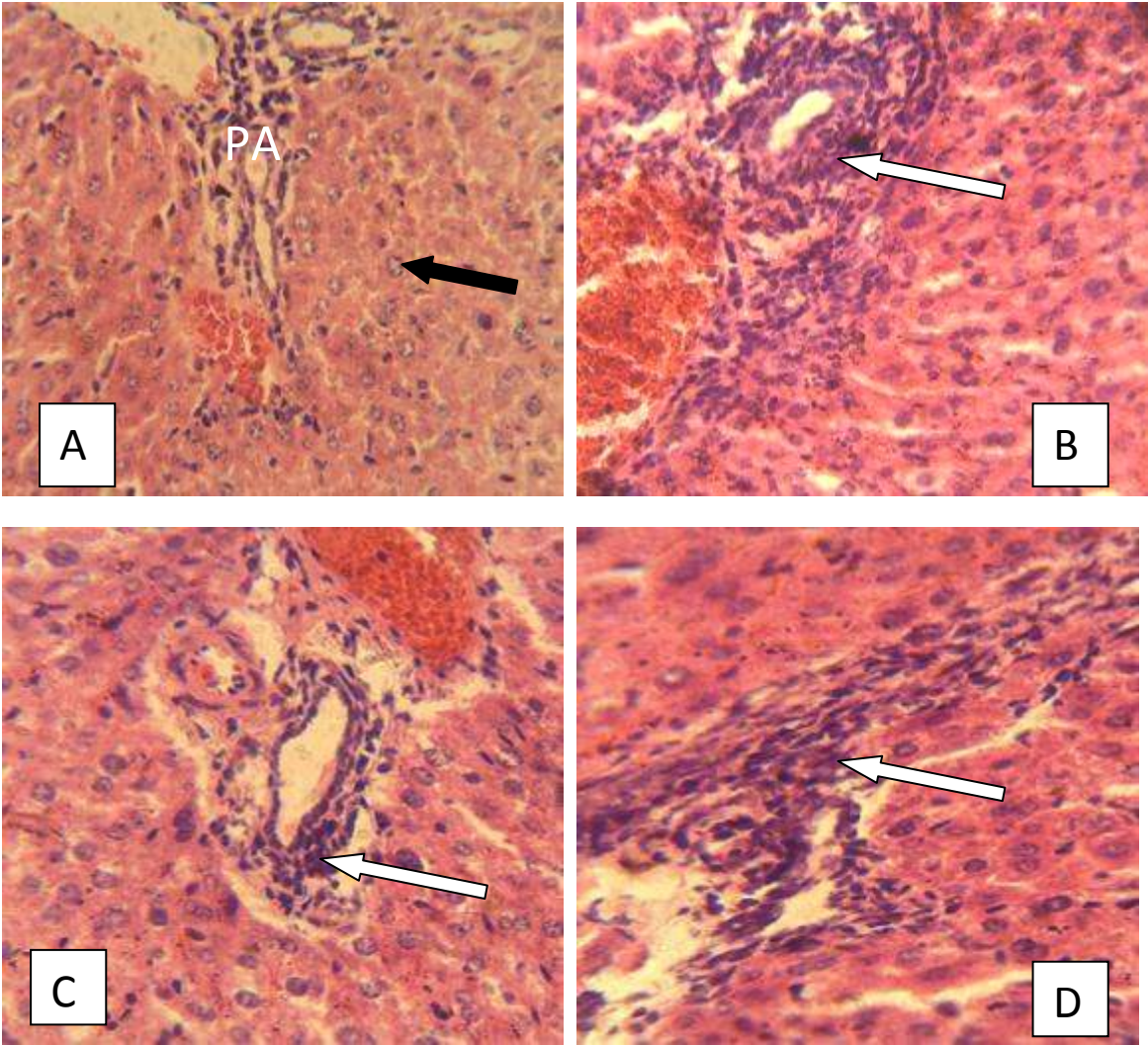


Plate 1: Photomicrograph of liver section of mice from experimental groups showing A-normal control, B-infected but untreated, C-infected and treated with artesunate, D-infected and treated with extract. The mononuclear infiltration of cells around the portal area-portal hepatitis (white arrow) in groups B,C and D while the group A shows normal portal area(PA) and hepatocytes(black arrow). Note that the severity of inflammatory cells infiltration is more in B than D and C. H and E x 400.

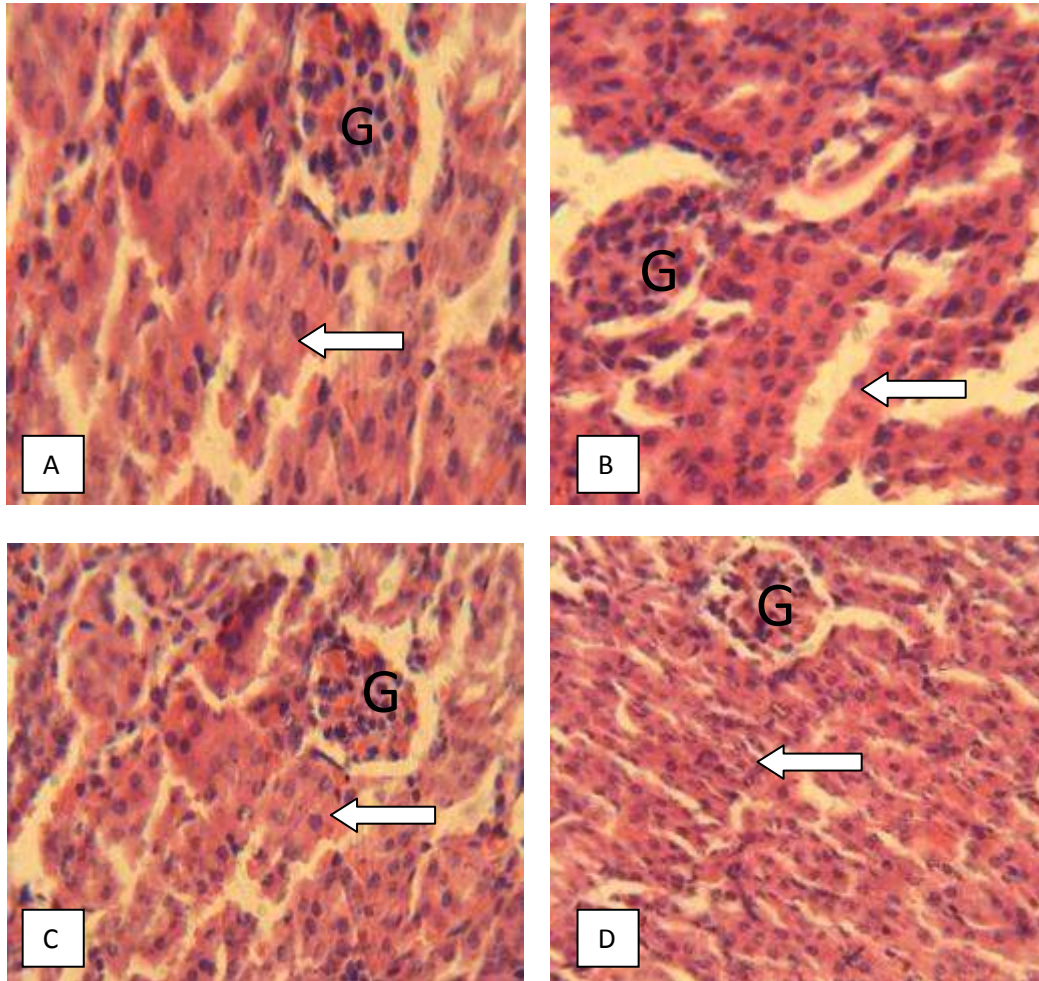


Plate 2: Photomicrograph of kidney section of mice from experimental groups showing A-normal control, B-infected but untreated, C-infected and treated with artesunate, D-infected and treated with extract. Note the apparently normal glomerulus(G) and renal tubules(arrow). H and E x 400.

DISCUSSION

Elevated levels of AST and ALT (Kew, 2000) are indicative of damage to tissues or organs in the body such the parenchymal cells, red cells and other cells. However, ALT is a more specific enzyme marker for liver cells damage. The results of the effect of seed extract of *Picralima nitida* on AST activity of mice showed that group 3 had the highest mean AST activity, followed by group 4 while group 5 showed the least activity days 3 and 5 of post treatment. The extract treated groups produced better AST activity than the standard control on days 3 and 5 of post treatment. The highest ALT activity was shown by group 4 and the least activity was shown by group 5 on day 3 of post treatment. This effect of the extract on ALT of mice was non dose-dependent. However, group 3 showed the highest value; followed by group 4 and the least activity was shown by group 5 on day 5 of post treatment. The standard control showed better mean ALT activities than the extract treated groups on days 3 and 5 of post treatment. The evidence showing that the infected animals treated with the extract caused dose-dependent decreases in the activities of AST and ALT revealed that the extract protective effects on body liver and other tissues by lowering the activities of these enzymes. The time dependence of the effect of the treatment (table 1) showed greater effect on day 5 than day 3 of post treatment. This correlates with the findings of George *et al* (2011) who asserted that serum ALT and AST activities were significantly higher in positive control (group inoculated with malaria but not treated) when compared with group treated with graded doses of *Aframomum sceptrum*.

Urea (Kasper *et al.*, 2005) is produced as a waste product of protein digestion by the liver which is normally removed from the body through the process of ultrafiltration in the glomeruli of the kidneys. Creatinine, an important indicator of renal health, is a breakdown product of creatine phosphate in muscle produced at a fairly constant rate by the body (www.medicinenet.com, 2014). It is an easily measured by-product of muscle metabolism excreted unchanged by the kidneys. Group 3 showed the highest mean urea and creatinine concentrations followed by group 5 while group 4 showed the least concentrations on day 3 of post treatment. Group 3 showed the highest urea and creatinine concentrations followed by group 4 and group 5 showed the least serum urea and creatinine concentrations on day 5 of post treatment. Day 3 post treatment of standard control showed more appreciable decreases in urea and creatinine concentrations when compared with the extract treated groups but group 5 produced better effects than the standard

control on day 5 of post treatment. The evidence that the extracted treated groups caused non dose-dependent decreases in urea and creatinine concentrations on day 3 of post treatment and dose-dependent decreases on day 5 of post treatment revealed the ability of the extract to increase the kidney's functionality because it plays an active role in clearing urea and creatinine off the blood. The time dependence of the effects of the treatment (table 2) produced greater effects on day 5 than day 3 of post treatment. This correlates with the findings (Zailani *et al.*, 2009; Arise *et al.*, 2012) which stated that extracts of *Carica papaya*, *Alstonia boonei* and *Clerodendrum violaceum* Gürke have been observed to play vital roles in decreasing the serum urea and creatinine concentrations thus proving the efficacy of extracts in maintaining the integrity of the kidney function.

A rise in serum bilirubin concentration is an indication of liver damage, parenchyl or biliary (Collier and Bassendine, 2002). Bilirubin was found to be produced in highest concentrations by group 3 and in least amounts by group 5 on days 3 and 5 of post treatment. The standard control showed better bilirubin clearance effect on mice than the extract treated groups on days 3 and 5 of post treatment. Only group 5 showed a significant decrease in serum total bilirubin concentration among the extract treated groups when compared with the positive control indicating that this extract has bilirubin clearance effect at higher doses. The evidence showing that extract treatment caused a dose-dependent decrease in total bilirubin concentration revealed that this extract enhance the liver function of clearing the blood unconjugated hydrophobic bilirubin by combining it with glucuronic acid making it water-soluble conjugated bilirubin using the enzyme UDP-glucuronide transferase. The time dependence of the effect of the treatment (tables 1) showed greater effect on day 5 than day 3 of post treatment. This correlated with observation of Olorunnisola and Afolayan (2013) who showed that leaf extract of *Sphenocentrum jollyanum* administered to malaria-infected mice significantly decreased the total bilirubin concentration. In conformation to the present study too, crude aqueous extract of the fruiting body of medicinal mushroom, *Ganoderma lucidum* has been found to significantly reduce the total bilirubin level in malaria-infected mice (Oluba *et al.*, 2013).

Decreased levels of albumin, the main constituent of total protein are noticed in chronic liver diseases such as cirrhosis and nephritic syndrome where it is lost through the urine (Menge and Schwiebert, 2005). The highest protein concentrations were produced by group 3, followed by

group 4 and the least by group 3 on days 3 and 5 of post treatment. The extract treated groups produced better total protein than the standard control on days 3 and 5 of post treatment. The evidence showing that the extract treatment caused a dose-dependent increase in total protein revealed that the extract protected integrity of the liver which is involved in manufacturing of total proteins. The time dependence of the effect of the treatment (table 1) showed greater effect on day 5 than day 3 of post treatment.

The histomorphological changes in the liver sections of the different groups of mice (plates 1 – 4) showed inflammatory cells around the portal area (portal hepatitis) in groups 2, 5 and 6 while group 1 shows normal portal area (PA) and hepatocytes. The severity of cellular infiltration (inflammation) is more in 2 than 5 and 6; an indication that the extract produced hepatoprotective effect on the liver of the treated mice. The histomorphological changes in the Kidney sections of the different groups of mice (plates 5-8) showed normal glomeruli (G) and renal tubules in the kidneys of all the experimental groups.

The decreased activities of the liver function enzyme markers, serum bilirubin level and increase in total protein of the treated groups are indicative that the extract has hepatoprotective effect. The functionality of the kidney was enhanced by the administration of this extract thus serum amounts of urea and creatinine were significantly reduced in the treated mice. Nevertheless, prolonged treatment and higher doses with such extract can pose a serious danger and damage to these organs. The observed normal glomeruli and renal tubules in the untreated and treated groups shows that the kidney is not a target organ for the malaria parasite during its exo-erythrocytic stage and also indicates the apparent less toxic effect of the extract on body tissue.

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