

Original Research Article

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. Liver and kidney function tests are routine clinical tests to ascertain the level of integrity and functionality of these organs. 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. The biochemical parameters; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, serum total bilirubin, total protein, urea and creatinine concentrations and histopathological studies on liver and kidney tissues were performed. Treatment lasted for 5 days during which analyses were done on days 3 and 5 post treatment. Group 1 (normal mice treated with 3 % tween 80) and group 2 (malaria-infected mice treated with 3 % tween 80) served as normal and positive controls respectively. Groups 3, 4 and 5 were treated with 20, 40 and 80 mg/kg b.w. of the extract, respectively. Group 6 that received 5 mg/kg b.w. of artesunate served as the standard control. From the results of the experiment, significant ($p < 0.05$) decreases were observed in serum concentrations of urea, creatinine of the treated groups compared to the positive controls on days 3 and 5 post treatment. There was non-significant ($p > 0.05$) decrease in serum AST and ALT activities of the treated groups on days 3 and 5 post treatment compared to the positive control. Groups 3 and 4 showed significant ($p < 0.05$) decrease in bilirubin concentration on days 3 and 5 post treatment compared to the positive control. All the treated groups showed significant ($p < 0.05$) increase in serum total protein on day 3 post treatment while group 5 showed significant ($p < 0.05$) increase in total protein on day 5 treatment compared to the positive control. Histopathological studies on the liver section of the treated mice showed lesser inflammatory cells around the portal areas than the untreated. No lesions were noticed in the tubules of the kidney sections 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.

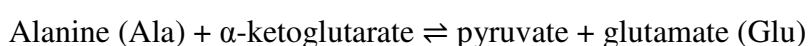
INTRODUCTION

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).

66 In either case, the aminotranferase reaction consists of two similar half-reactions that constitute
 67 what is referred to as a ping-pong mechanism. In the first half-reaction, amino acid 1 (e.g., L-
 68 Asp) reacts with the enzyme-PLP complex to generate ketoacid 1 (oxaloacetate) and the
 69 modified enzyme-PMP. In the second half-reaction, ketoacid 2 (α -ketoglutarate) reacts with
 70 enzyme-PMP to produce amino acid 2 (L-Glu), regenerating the original enzyme-PLP in the
 71 process.

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

78 Bilirubin can be conjugated with a molecule of glucuronic acid which makes it soluble in water.
 79 Some of the double-bonds in bilirubin isomerizes when exposed to light. This is used in the
 80 phototherapy of jaundiced newborns: the E, Z-isomers of bilirubin formed upon light exposure
 81 are more soluble than the unilluminated Z, Z-isomer, as the possibility of intramolecular
 82 hydrogen bonding is removed (McDonagh *et al.*, 1980). This allows the excretion of
 83 unconjugated bilirubin in bile.

84 Erythrocytes generated in the bone marrow are disposed of in the spleen when they get old or
 85 damaged. This releases haemoglobin, which is broken down to haem as the globin parts are
 86 turned into amino acids (Cheifetz, 2010). The haem is then converted into unconjugated bilirubin
 87 in the reticuloendothelial cells of the spleen. This water-insoluble unconjugated bilirubin is then
 88 bound to albumin and sent to the liver. In the liver, bilirubin is conjugated with glucuronic acid
 89 by the enzyme glucuronyltransferase making it water-soluble and afterwards goes into the bile.
 90 Conjugated bilirubin is not absorbed in the small intestine but instead is passed into the colon
 91 (Cheifetz, 2010). Colonic bacteria deconjugate and metabolize the bilirubin into colourless
 92 urobilinogen which is further oxidized to urobilin and stercobilin which give stool its
 93 characteristic brown colour. A trace amount of urobilinogen is reabsorbed into the enterohepatic
 94 circulation to be re-excreted in the bile: some of this is instead processed by the kidneys
 95 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).

MATERIALS AND METHODS

Materials

Animals

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

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 (BDCP) 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.

Procedure

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 (B) and sample test (T) test tubes. Serum sample of 0.1 ml was added to the sample test (T) tubes only and mixed thoroughly. Then, 0.1 ml of distilled water was added to the reagent blank (B). Afterwards, the entire reaction medium was well mixed and incubated in a water bath at 37⁰ C for 30 minutes.

Immediately after incubation, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the reagent blank (B) and the sample test tubes. It was mixed thoroughly and allowed to stand for exactly 20 minutes at 25⁰ C. Finally, 5.0 ml of sodium hydroxide solution was added to both the blank and the reagent test tubes respectively and mixed thoroughly. The absorbance of sample was read at the wavelength of 550 nm against the reagent blank after 5 minutes.

The AST substrate phosphate buffer solutions (0.5 ml each) were pipetted into both the reagent blank (B) and sample test (T) test tubes. The serum sample of volume, 0.1 ml was added to the sample test (T) test tubes only and mixed thoroughly. Then, 0.1 ml of distilled water was added to the reagent blank (B). Thereafter, the entire reaction medium was well mixed and incubated at 37° C for 30 minutes in a water bath.

A volume, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the reagent blank (B) and the sample test tubes immediately after incubation. Also, 0.1 ml of the sample was added to blank (B) 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 (B) and sample test (T) 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.

Procedure

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.

Procedure

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 (1951)

Procedure

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.

Procedure

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

Determination of serum creatinine concentration

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

Procedure

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

Histopathology of Organs

Procedure

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

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

RESULTS

In table 1, the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and the total protein concentration are presented. In normal mice, the AST activity of found to be 28.60 ± 1.17 IU/L; the ALT activity was 44.60 ± 2.78 IU/L and the total protein

276 concentration was 6.74 ± 0.19 g/dl. In the positive control group, AST and ALT activities
 277 increased non-significantly ($p > 0.05$) compared to the normal control. The total protein
 278 concentration in the positive control decreased significantly ($p < 0.05$) compared to the normal
 279 control. In group 3, the AST activity was assayed to be 30.20 ± 3.58 IU/L on day 3 post
 280 treatment. This activity decreased non-significantly to 29.40 ± 3.44 IU/L on day 5 post treatment.
 281 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
 282 treatment which decreased non-significantly to 29.00 ± 3.02 and 28.80 ± 2.85 IU/L on day 5 post
 283 treatment respectively. The protein concentration was observed to be 5.78 ± 0.10 g/dl in group 3,
 284 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
 285 concentrations increased non-significantly to 5.86 ± 0.10 , 6.00 ± 0.11 and 6.34 ± 0.05 IU/L
 286 respectively on day 5 post treatment. Group 6 (standard control) showed AST activity of $30.80 \pm$
 287 2.60 IU/L on day 3 of post treatment which decreased to 29.40 ± 1.57 IU/L on day 5 post
 288 treatment. The standard control showed ALT activity of 45.40 ± 1.47 IU/L on day 3 post
 289 treatment which decreased further to 44.40 ± 1.75 IU/L on day 5 post treatment. The standard
 290 control produced 5.74 ± 0.12 g/dl on day 3 post treatment and 6.08 ± 0.09 g/dl on day 5 post
 291 treatment. The effect of treatment with ethanol seed extract of *Picralima nitida* on AST activity
 292 and total protein concentration of the treated mice was dose-dependent while its effect on ALT
 293 activity of treated mice was non dose-dependent.

294 In table 2 are presented the mean values of serum bilirubin (0.34 ± 0.05 mg/dl), urea ($20.00 \pm$
 295 0.32 mg/100 ml) and creatinine (0.72 ± 0.01 mg/100 ml) of normal mice. There is significant ($p <$
 296 0.05) increases in serum urea and creatinine and non-significant ($p > 0.05$) increase in serum
 297 bilirubin concentrations in the positive control compared to the normal control. In the treated
 298 groups (groups 3, 4 and 5), the mean values for bilirubin on day 3 post treatment were $1.46 \pm$
 299 0.10 , 1.28 ± 0.11 and 0.88 ± 0.10 mg/dl respectively. On day 5 post treatment, the concentrations
 300 further decreased non-significantly to 1.40 ± 0.11 , 1.22 ± 0.11 and 0.78 ± 0.10 mg/dl
 301 respectively. The standard control showed bilirubin concentrations of 1.12 ± 0.12 mg/dl on day
 302 3 post treatment and 1.04 ± 0.13 mg/dl on day 5 post treatment. Compared to the positive
 303 control, groups 3 and 4 showed non-significant ($p > 0.05$) decreases in serum bilirubin
 304 concentrations on days 3 and 5 post treatment. The treatment with ethanol seed extract of
 305 *Picralima nitida* showed a dose-dependent effect on serum bilirubin concentration in the treated
 306 mice. In normal mice, the serum urea concentration 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 concentration 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 concentration of the treated mice followed a non dose-dependent fashion. The standard control showed serum urea contrations of 26.40 ± 0.51 mg/100 ml on day 3 post treatment with a decrease 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 ($p < 0.05$) decreases in serum urea concentration compared to the positive control. The serum creatinine concentration 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 concentrations 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 ($p < 0.05$) decreases in serum creatinine concentration on days 3 and 5 post treatment. The effect was non dose-dependent. There was a significant ($p < 0.05$) increase in serum creatinine concentration in the positive control compared to the normal control. The standard control showed serum creatinine concentration 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 (portal hepatitis) (white arrow) in groups 2,5 and 6 while group 1 shows normal portal area(PA) and hepatocytes(black arrow). The severity of cellular infiltration (inflammation) 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)

348 **Table 2:** Table showing the serum total bilirubin, urea and creatinine concentrations
349

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	“

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

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

352 **N.D. = Not determined**

353

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

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

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

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

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

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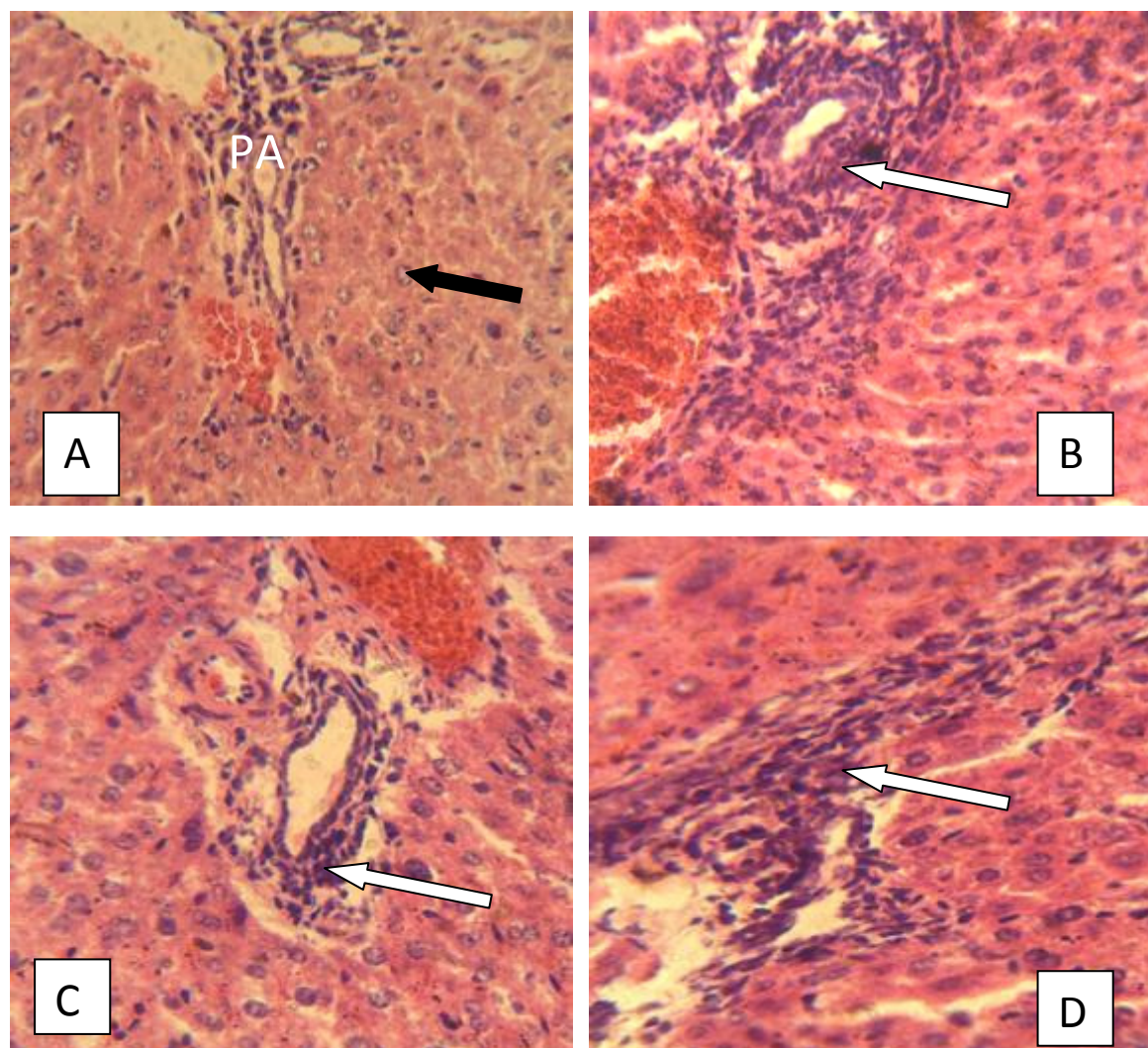


Plate 1: Photomicrograph of liver section of mice from experimental groups showing A-normal control,B-infected untreated,C-infected treated with artesunate,D-infected treated with extract.Note the mononuclear infiltration of cells around the portal area-portal hepatitis (white arrow) in groups B,C and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

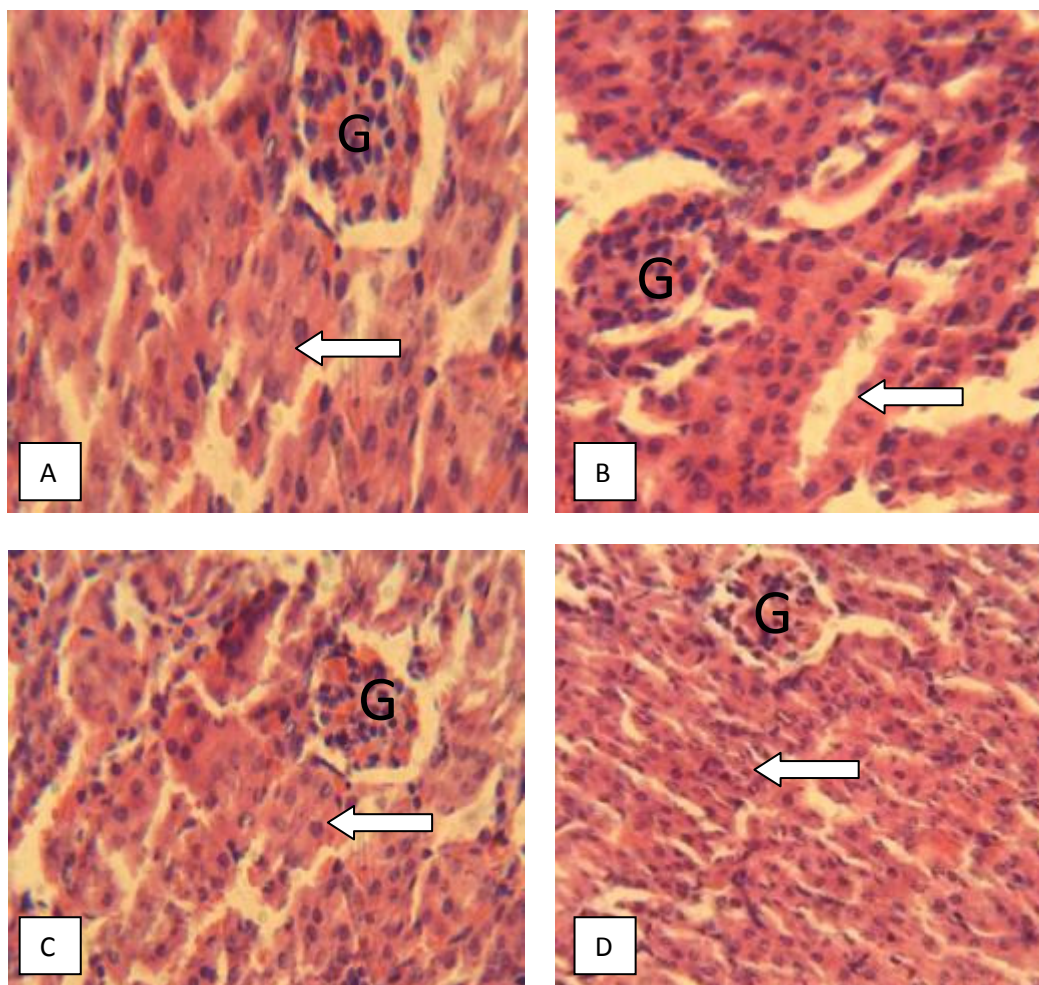


Plate 2: Photomicrograph of kidney section of mice from experimental groups showing A-normal control,B-infected untreated,C-infected treated with artesunate,D-infected treated with extract.Note the apparently normal glomerulus(G) and renal tubules(arrow).H and E x 400.

DISCUSSION

Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (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 the liver enzyme aspartate aminotransferase (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 alanine amino transferase (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 that 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 hepato-protective 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 indicatives that the extract has hepato-protective 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 erythrocytic stage and also indicates the apparent less toxic effect of the extract on body tissue.

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