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Effect of *E Mal*$^{(R)}$ (α, β-Arteether) on Hepatic Function in Albino Wistar Rats

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Abstract: *E MAL$^{(R)}$(α,β-arteether) is a drug of choice, across all age-groups, for the management of multi-drug resistant P. falciparum complicated and uncomplicated malaria. There is paucity of reports on the influence of the drug on liver function. This research, therefore, investigated the effect of EMAL$^{(R)}$, an artemisinin derivative, on hepatic function in albino Wistar rats. The animals, of both sexes, weighing 200±20g, were randomly assigned to five batches. Each batch was subdivided into five groups (n=5) for control, 1.5, 3.0, 5.0 and 10.0 mg E MAL$^{(R)}$/kg body weight, respectively. The test drug was administered intramuscularly, once daily for three consecutive days. At the end of the three-day administration, the relevant parameters for hepatic function were assessed. The results showed E MAL$^{(R)}$ given, respectively, at 1.5, 3.0, 5.0 and 10.0 (mg/kg body weight) significantly raised serum aspartate aminotransferase (AST), elevated serum alkaline phosphatase (ALP) and protein concentrations concentration compared to control group. The histological presentations of liver in the 1.5 mg and 3.0 mg/kg body weight doses of E MAL$^{(R)}$, showed no derangements compared to control group whereas the liver in the 5.0 mg/kg and 10.0 mg/kg body weight of drug showed dilated central veins and sinusoids, indicative of derangement compared to control group. It may be concluded that the drug at therapeutic dose (3.0 mg/kg body weight) may be well tolerated; but, higher doses might predispose to liver injury. Therefore caution is advised due to possible tendency for hepatocellular damage.*

Key Words: *E Mal$^{(R)}$ (α, β-Arteether), Hepatic Function, Malaria, Wistar rats.*

1. INTRODUCTION:

The hepatic system is a major pathway in the biotransformation of drugs at therapeutic, experimental, or toxic doses. Serum levels of liver enzymes are sensitive bio-indicators usually employed in the diagnosis and prognosis of some disease conditions (Ilic *et al.*, 2010). When the hepatocellular plasma membrane is damaged, the enzymes, normally, present in the cytosol, are released into the blood stream; the enzymes are quantified to assess the extent of liver injury. The liver is also very important in the biosynthesis of plasma proteins. Moreover, the cytoarchitecture of the hepatic parenchyma is built around central veins, sinusoids and hepatocytes. Over-the-counter drugs, like paracetamol, have been implicated in hepatocellular damage, the extent of which is demonstratable biochemically and histologically (Iyanda and Adeniyi, 2011).

2. LITERATURE REVIEW:

Alpha, beta-Arteether (α, β-Arteether) is an oil-soluble ethyl derivative of dihydroartemisinin, which is an efficient erthrocytic schizontocidal drug for the treatment of multi-resistant *P. falciparum* malaria. It is available as injection for intramuscular use only. Alpa,beta-arteether shows rapid schizontidal action and brings quick clinical improvement in falciparum malaria with low recrudescence rate. It has some gametocidal action too, which aids in cutting down the transmission of falciparium malaria.

In 2007, Nwanjo *et al.*, demonstrated that artemether, an artemisinin derivative, induces the transient and moderate elevations of liver transaminases as well as bradycardia. It has also been reported that artemether produces dose-dependent neurotoxic effects on the brainstem of the mouse (Nontrasert *et al.*, 2002). Increase in reactive oxygen species and extensive lipid peroxidation in cultured neurons has also been shown (Schmuck *et al.*, 2002).

The efficacy and safety of arteether have been established (Asthana *et al.*, 2001). The organ, liver, metabolizes artemisinin derivatives, including arteether. However, there is paucity of literature on the effect of arteether on the function of the hepatic system. The major aim of this research was to study the effect of *E Mal*$^{(R)}$ (α, β-Arteether) on the liver function in albino rats and the specific objectives were to determine the effect of *E Mal*$^{(R)}$ on liver enzymes and the histopathology of the liver.

3. MATERIALS AND METHODS:

3.1. Chemicals, Reagents and equipment:

Chemicals and reagents: Acetic acid, potassium iodide, sodium thiosulphate (0.1M) (May and Baker Dagenham, U.K.), starch solution (1%), chloroform, bromine, 95% alcohol, 0.1m NaOH, phenolphthalein (1&%), Sodium thiopentone 6mg/100g, body weight (Rotex Medica GMBH Germany), distilled water, NaOH (20g)(May and Baker, Dagenham England) , HNO_3 (2N) 20mL, Mercuric Nitrate (3g), Diphenyl carbazene (100g). The kits used for alanine amino transferase ALT and Aspartate amino transferase were obtained from Ranox Laboratories UK, alkaline phosphatise assay kit (Deutsche Gesbes Germany). Biuret reagent, Potassium sodium tartarate -, ($KnaC_4$ $H_4O_6 4H_2O$), serum albumin (1g).

3.2. Equipment: The Glassware and equipment were 2.5ml flask, Measuring cylinder, Pipettes/burettes, conical flasks, Fume cupboard, Microscope and slides, heating mantle. Beakers, Stainless steel pot, Weighing balance, Calibrated Water bottles, Portex, Cannula (0.5mm), Flame photometer (410c), Spectrophotometer, Refrigerator, Centrifuge Oessophageal Cannula, Vernia Calipers, Organ bath, Kymograph/drum, Meter rule, Electric lamp, Intubating syringe, Gallenkamp shaker, Thermometers, pH meter, Whatman filter paper (No1) and Volumetric flask.

3.3. Drug procurement:

The test drug, *E Mal*$^{(R)}$, was purchased from ROTECS Pharmacy RC. 1027569, Ebis Mechanic Road, Amarata, Yenagoa, Bayelsa State. Each 2ml vial of *E Mal*$^{(R)}$ contained 150mg α, β-Arteether with NAFDAC REG. NO. 04-8383.

3.4. Drug formulation for the experiments:

The test drug was prepared as a 150 mg/10ml stock. The original *E Mal*$^{(R)}$ (150mg/2ml) was introduced into a sterile plain bottle; then, 8 ml of sterile water (water for injection) were added, in order to obtain the stock from which the appropriate dose (weight/volume) was taken for administration in the corresponding animal.

$$\text{Dose per rat} = \frac{\text{mg } E\ Mal^{(R)} \text{ required} \times 10\text{ml stock } E\ Mal^{(R)}}{2\text{mg } E\ Mal^{(R)}} = \text{ml of } E\ Mal^{(R)} \text{ stock}$$

Where,

mg *E Mal*$^{(R)}$ required = group designation (mg *E Mal*$^{(R)}$/ kg body weight) x body weight (kg).

3.5. Mode of administration of *E Mal*$^{(R)}$:

E Mal$^{(R)}$ is approved for intramuscular administration only. The appropriate dose (w/v) from the stock drug was given at the anterior aspect of the mid-thigh muscle, using alternate limb, 24-hour interval, for 72 hours. One-milliliter hypodermic (insulin giving type) syringe-and-needle was used per injection. Wistar rats were administered graded doses of the drug intramuscularly once a day for three consecutive days.

3.6. Animal groupings per drug strength:

The animals (both sexes), 220 ± 40 grams body weight, were randomly assigned to five batches. Each batch was subdivided into five groups of five rats each based on the strength of *E Mal*$^{(R)}$ administered.

Group I: Control (rat, chow, water *ad libitum*, No drug)
Group II: 1.5 mg *E Mal*$^{(R)}$/ kg body weight (rat chow, water *ad libitum*, drug)
Group III: 3.0 mg *E Mal*$^{(R)}$/kg body weight (rat chow, water *ad libitum*, drug)
Group IV: 5.0 mg *E Mal*$^{(R)}$/kg body weight (rat chow, water *ad libitum*, drug)
Group V: 10.0 mg *E Mal*$^{(R)}$/kg body weight (rat chow, water *ad libitum*, drug)

3.7. Experimental animals and maintenance:

Albino rats of the Wistar strain were used for this research. The rats weighed between 200 and 220g at start of experiments and were randomly chosen from both sexes. They were all kept in plastic cages with wire net covers. The ethics for the use of experimental animals were strictly adhered to. They were maintained in the animal facility of the Physiology Department, University of Calabar, at a temperature of 28 ± 2°C, 12 hours light and dark cycles. Each rat was kept in a separate cage. The cages were always kept neat. Food and water intake per rat was determined on daily basis and their individual body weight measured using an animal weighing balance.

3.8. Determination of food and water intake:

Water intake was measured using calibrated feeding bottle with stainless steel nozzles. The daily water intake was obtained by subtracting the volume of water remaining at the end of 24 hours of feeding from the initial amount in the feeding bottle at start of the day. The difference was the amount consumed for the day. The food intake was measured by weighing the amount of food left in the container after 24 hours and subtracting it from the initial amount of food at start of the day's feeding. The food containers were medium sized stainless steel plates to avoid spillage of food.

3.9. Liver function tests:

Blood was collected by cardiac puncture of the anaesthetized (sodium thiopentone, Rolex Medial GMBH Germany) animals. Blood samples from each rat were collected using syringes and needles and separated into sample bottles and allowed to stand for 30 minutes for clotting to take place. These were then centrifuged at 300g for 10 minutes. The serum extracted into fresh test tubes and stored in a refrigerator for analysis of alkaline phosphate (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

3.10. Measurement of alkaline phosphatase:

This is by optimized standard method recommended by the Deutsche Geseiischage fur Klinische Chemic GSCC (1972).

4. PRINCIPLE:

P-nitrophenyl phosphate is hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing the substrate p-nitrophenyl phosphate and brought to room temperature. The solution was mixed, initial absorbance read after 1minute. The reaction was allowed to stand for 3minutes and the absorbance read again at 405nm. Alkaline phosphates activity was calculated from

UI = $2760 \times \Delta$Anm/minute micro

Where UI = Unit of alkaline phosphatise affinity

$\Delta A =$ Change in absorbance

Details of reagent composition are presented in Appendix 4.

Measurement of Alanine and Aspartate Amino Transferases

The measurement of AST and ALT activities in the serum were done using endpoint colorimetric-diagnostic kit (Randox; Laboratories UK) based on Reitman and Frankel (1952) method.

Principle

The pyruvate produced by transamination reaction between L-alanine and ketoglutarate with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone is used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine is used to measure aspartate aminotransfarase (AST). Both AST and ALT were read at 540nm wavelength.

1) X - oxoglutamate + L-alanine $\xrightarrow{ALT}$ L. Glutamate + pyruvate.
2) X - oxoglutamate + L-aspartate $\xrightarrow{AST}$ L-glutamate + oxaloacetate

5. Histology of the liver:

Permanent preparations using routine biopsy method (Osim *et al.*, 1993) was employed. Tissue sections were treated with traditional haematoxlin and eosin stains (Appendix 7). Other special stains used included silver, reticulum, trichrome and PAS (Periodic Acid Schiff).

5.1. Precautions:

Tracheal cannulation was done immediately after the animal was made to sleep to avoid suffocation.

The animal was dissected along the *linea alba* to avoid excessive bleeding.

A lamp was placed near the animal to avoid hypothermia.

The right doses of the chemicals were always used.

The intubating syringe was carefully inserted into the oesophagus to avoid entrance of dye into the lungs.

6. STATISTICAL ANALYSIS :

All results are presented as mean $\pm$ standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the least significant difference (LSD) procedure for significant F values. $P<0.05$ was considered significant. Computer Software (SPSS and Excel Analyzer) was used for the analysis.

7. DISCUSSION:

The effect of *E MAL*$^{(R)}$ (α, β-arteether) on hepatic function in the albino Wistar rat was studied. The serum levels of hepatic enzymes, e.g, AST (aspartate transferase), ALT (alanine transferase) and ALP (alkaline phosphatase) are sensitive biomarkers in the evaluation of liver function. In this study, *E MAL*$^{(R)}$ is associated with raised serum levels of these enzymes compared with control. Moreover, at 5.0 and 10.0 mg/kg, *E MAL*$^{(R)}$ treated rats showed dilated central veins and sinusoids in the liver tissues. The acute level of liver enzymes may not correlate well with the degree of hepatocellular damage (Daniel and Marshal, 2005). In 2013, Okunola *et al* showed that the histological and biochemical effect of arteether on the liver were within normal range in the Wistar rat. However, Davidson (1994) had earlier reported arteether-related cyto-toxicity in the liver, central nervous system, kidney, bone marrow, heart and reproductive organs. Recently, Cheeraratana *et al.,* (2008), demonstrated that C-^{14} labeled arteether was distributed quickly and localized in the cytoplasmic cortex of the kidney and homogeneously in the liver, four hours post-injection in mice. They showed that the terminal half-life of the labeled arteether in the blood was 1.8 hours with a blood: kidney: liver ratio of 1:5:2. Ilic *et al* (2010) have revealed that elevation in activity of AST and ALT can occur as early as twenty-five minutes post-paracetamol administration. Such an increase was found to be an indication of the hepatotoxic effect of paracetamol (Sallie *et al*, 1991). This may explain the acute drug-induced-liver-injury tendency of *E MAL*$^{(R)}$ in our findings.

7.1. Serum total protein, albumin and globulin:

Serum levels of total protein, albumin and globulin, are also ancillary biomarkers in evaluating liver function, especially in chronic liver disease. Total protein consists mainly of albumin and globulin. Albumin is synthesized by the hepatocytes. Only minimal changes in serum albumin are seen in drug-related hepatotoxicity (Daniel and Marshal, 2005). Okunola *et al (*2013) reported reduced levels of total protein and albumin in rats treated with arteether. In our study, however, *E MAL*$^{(R)}$ was shown to be associated with raised total protein (1.5, 3.0 and 10.0 mg/kg body weight), albumin (5.0 mg/kg) and globulin (1.5, 3.0 and 10.0 mg/kg body weight). This suggests that the extent of tissue injury, in our findings, was not enough to compromise the biosynthesis of albumin by the hepatocytes. It takes several weeks of impaired liver function before serum albumin level drops, eg, liver cirrhosis. Albumin has along plasma half-life (15-20 days) with approximately 4% degraded per day. Because of this slow turnover, the serum albumin level is not a good indicator of acute or mild hepatic dysfunction.

8. RESULTS:

Effect of *E Mal*$^{(R)}$ on water intake:

The mean daily water intake in the control group was 16.03+1.88ml, while that for 1.5mg/kg, 3.0mg/kg, 5.0mg/kg and 10.0mg/kg body weight were 21.50+0.90, 21.25+2.08, 16.65+1.76, and 21.95+0.47ml respectively (Figure 1). There was no significant difference between control group and *E Mal*$^{(R)}$ treated 1.5, 3.0 or 5.0mg/kg groups. However, the change in water intake was significantly higher ($p<0.05$) in 10.0mg/kg body weight when compared with control.

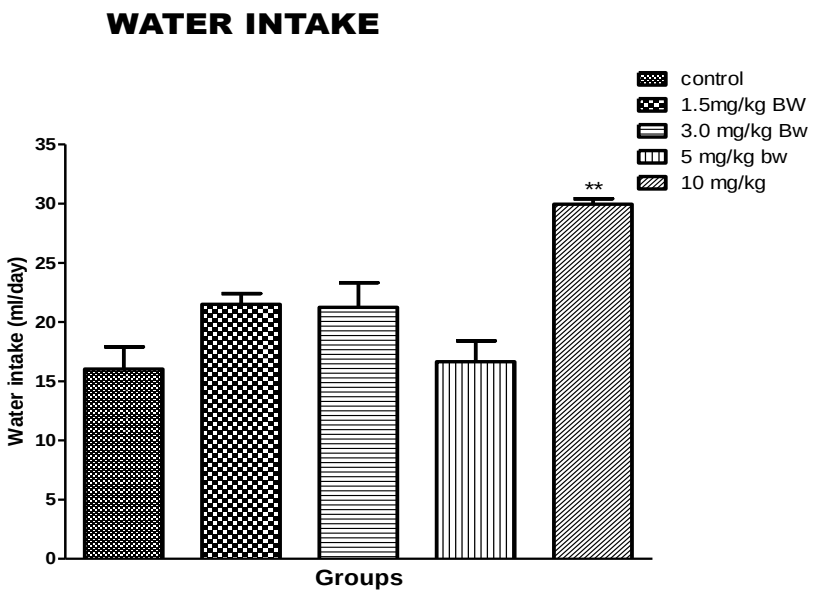

FIGURE 1: Mean Water intake in the different experimental groups. Values are expressed as Mean ± SEM, n=5
**p <0.05 versus Control

Effect of *E Mal*$^{(R)}$ on liver enzymes:

The alanine aminotransferase (ALT) levels in 5.0mg/kg group was significantly higher ($p<0.05$) than that in other doses and in controls, respectively (Figure 2) The aspartate aminotransferase (AST) levels in control, 1.5, 3.0, 5.0 and 10.0mg/kg groups were 77.67+1.53, 118.50+3.84, 92.50+2.78, 126.75+0.63 and 95.50+2.38 IU/L respectively. There is significant increase in AST levels with E MAL compared with control. However 3.0mg/kg elicited lower levels than 1.5mg/kg and 5.0mg/kg. There was significant higher levels of AST with 5.0mg/kg than with all other test groups ($p<0.05$, Figure 3) The alkaline phosphatase (ALP) level in the serum of rats in control, 1.5, 3.0, 5.0 and 10.0mg/kg groups were 125±5.01, 177.00+1.29, 117.00+1.29, 202.50+0.96, and 155.00+0.26 IU/L respectively. The differences were statistically significant among all the experimental groups at 95% confidence level with the lowest level at 3.0mg/kg and highest level with 5.0mg/kg of *E Mal*$^{(R)}$ (Figure 4).

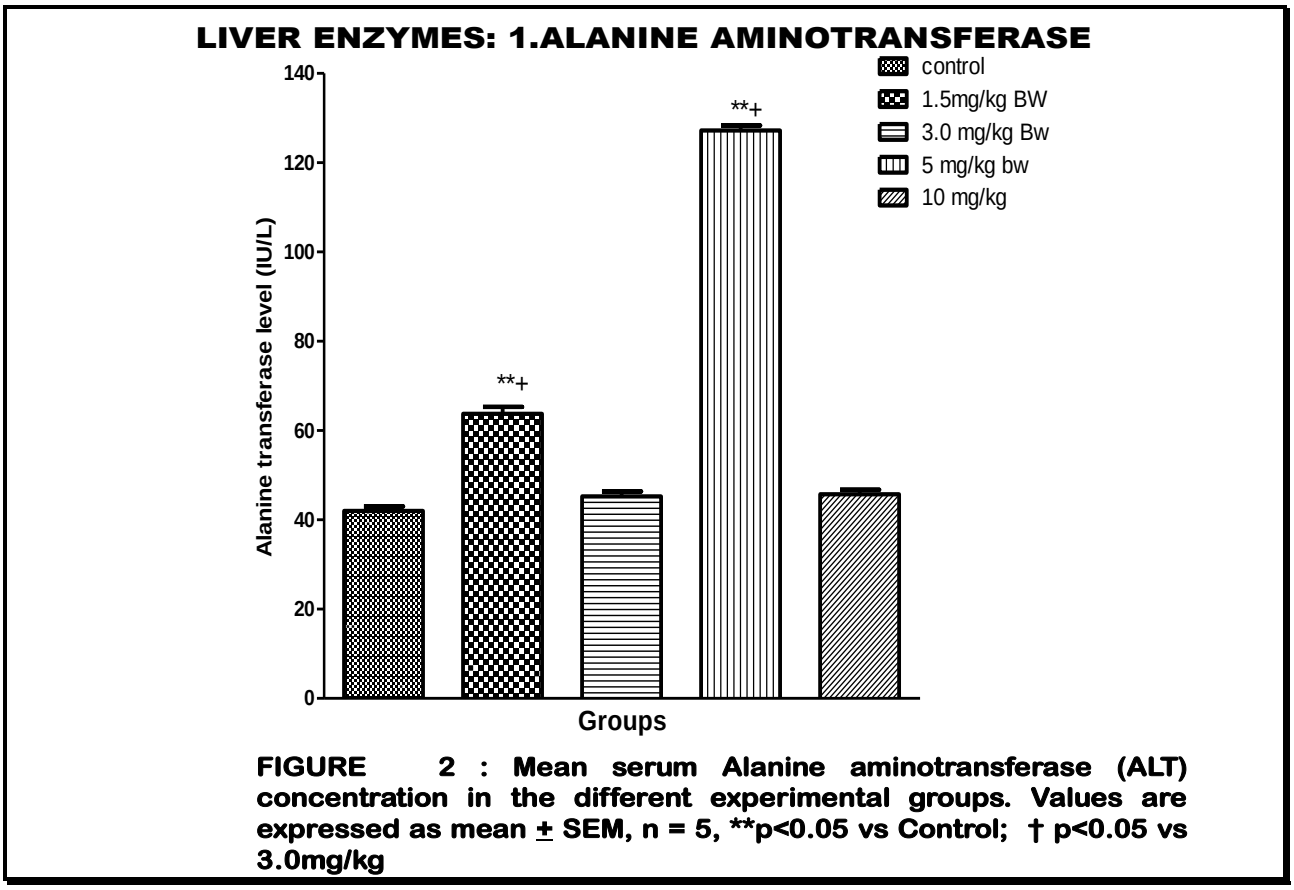

FIGURE 2 : Mean serum Alanine aminotransferase (ALT) concentration in the different experimental groups. Values are expressed as mean ± SEM, n = 5, **p<0.05 vs Control; † p<0.05 vs 3.0mg/kg

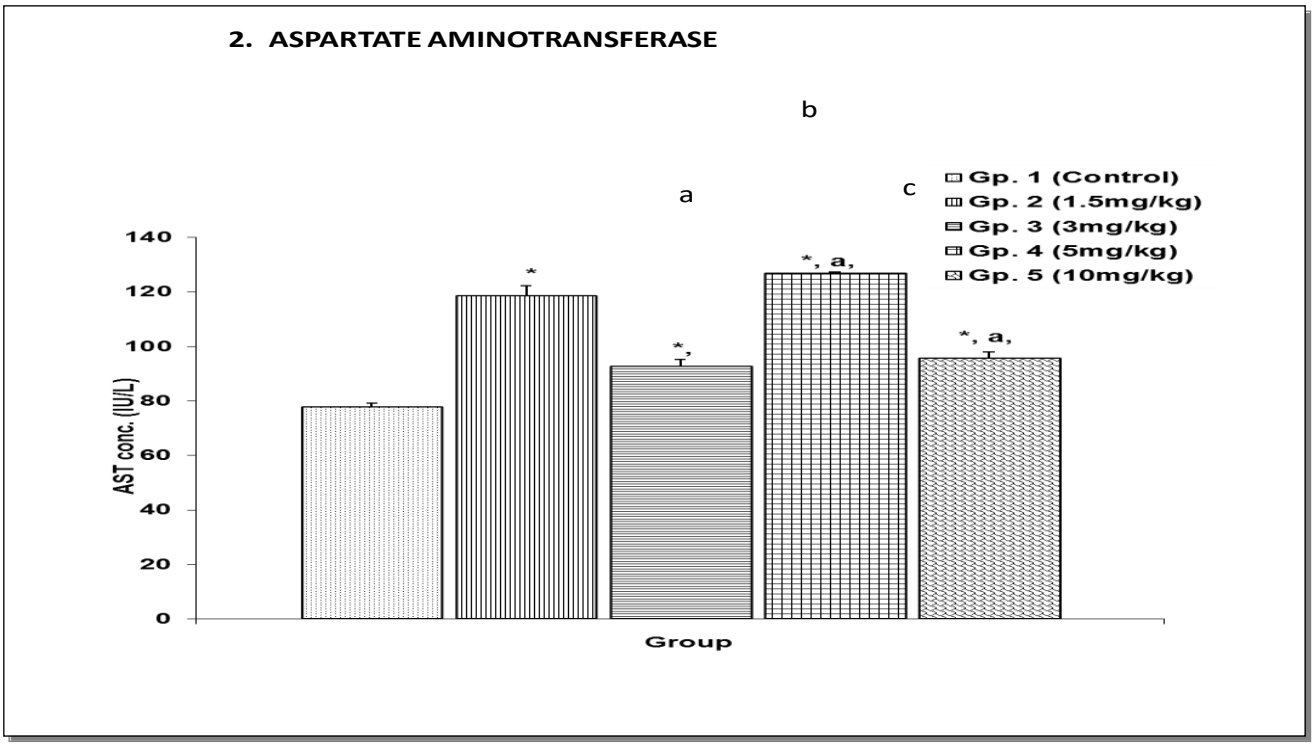

Figure 3: Mean Aspartate Aminotransferase Concentration in the Various Experimental Group. Values are expressed as Mean ± SEM, n= 5, p< 0.005

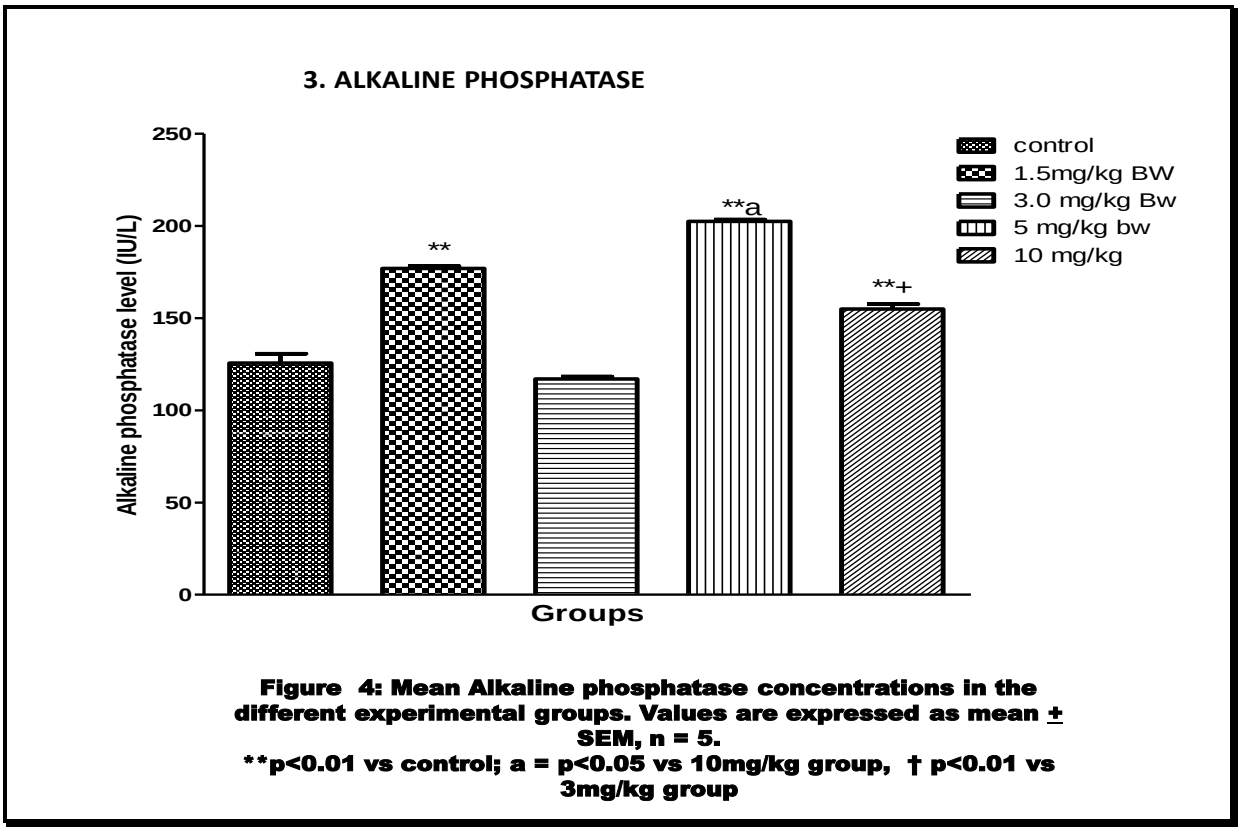

Figure 4: Mean Alkaline phosphatase concentrations in the different experimental groups. Values are expressed as mean ± SEM, n = 5.
****p<0.01 vs control; a = p<0.05 vs 10mg/kg group, † p<0.01 vs 3mg/kg group**

- **Effect of *E Mal*$^{(R)}$ on serum total proteins**

The total protein concentration in control, 1.5, 3.0, 5.0 and 10.0mg/kg body weight were 63.33+2.08, 87.00+0.91, 89.50+1.04, 66.00+0.82 and 74.75+0.99 g/L, respectively. These levels were significantly higher between control group and test group except for the 5.0mg/kg dose of *E Mal*$^{(R)}$ ($p<0.05$, Figure 5).

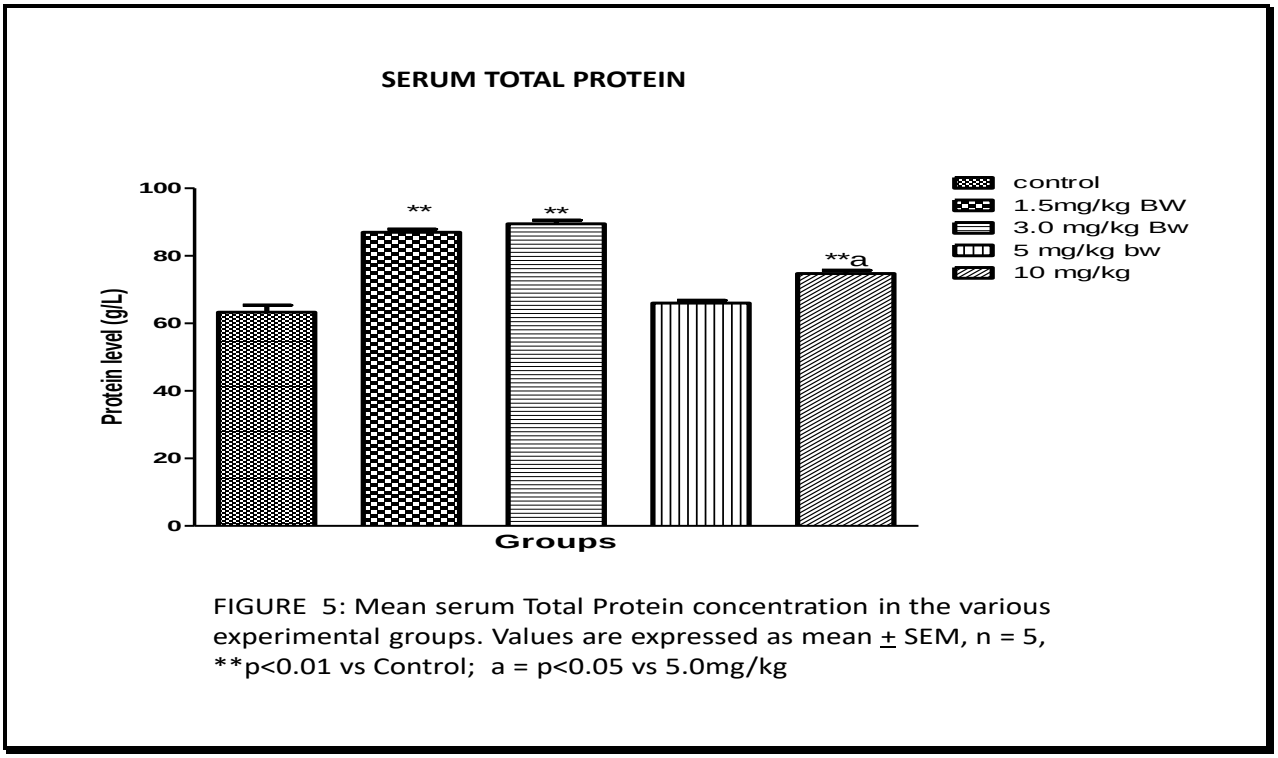

FIGURE 5: Mean serum Total Protein concentration in the various experimental groups. Values are expressed as mean ± SEM, n = 5, **p<0.01 vs Control; a = p<0.05 vs 5.0mg/kg

- **Effect of *E Mal*$^{(R)}$ on serum albumin**

Figure 6 shows that at 3.0mg/kg of drug, the serum albumin level was lower ($p<0.05$) than that of the control. With 5.0mg/kg dose, however, the level increased significantly.

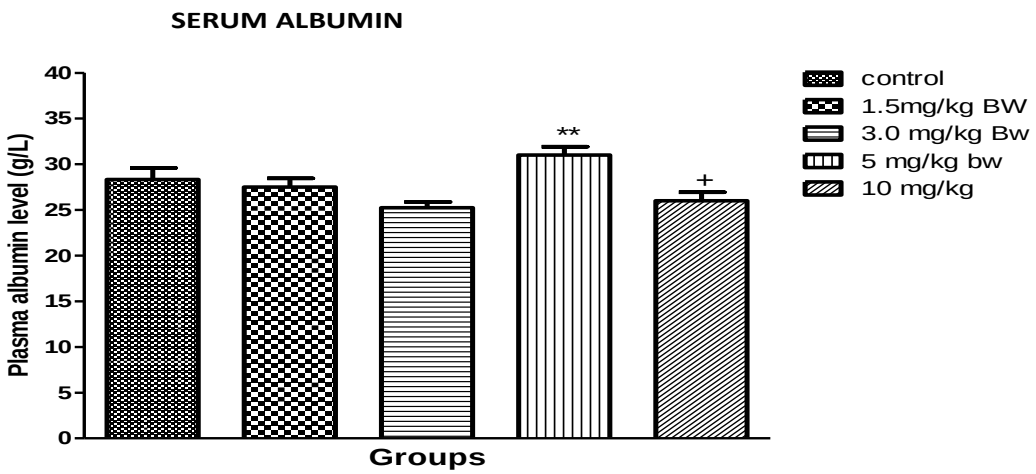

FIGURE 6: Mean serum Albumin concentration in the various experimental groups. Values are expressed as mean ± SEM, n = 5, **p<0.05 vs Control; † = p<0.05 vs 5.0mg/kg

- **Effect of *E Mal*$^{(R)}$ on serum globulin**

The serum level of globulin in ats of the control, 1.5, 3.0, 5.0 and 10.0 mg/kg groups were 35.00+ 0.87, 59.50 + 0.87, 64.25 + 1.55, 35.00 + 1.68 and 48.75 + 1.66 respectively. The differences were significantly among the between the control and test groups except for 5.0 mg/kg ($p<0.05$, Figure 7).

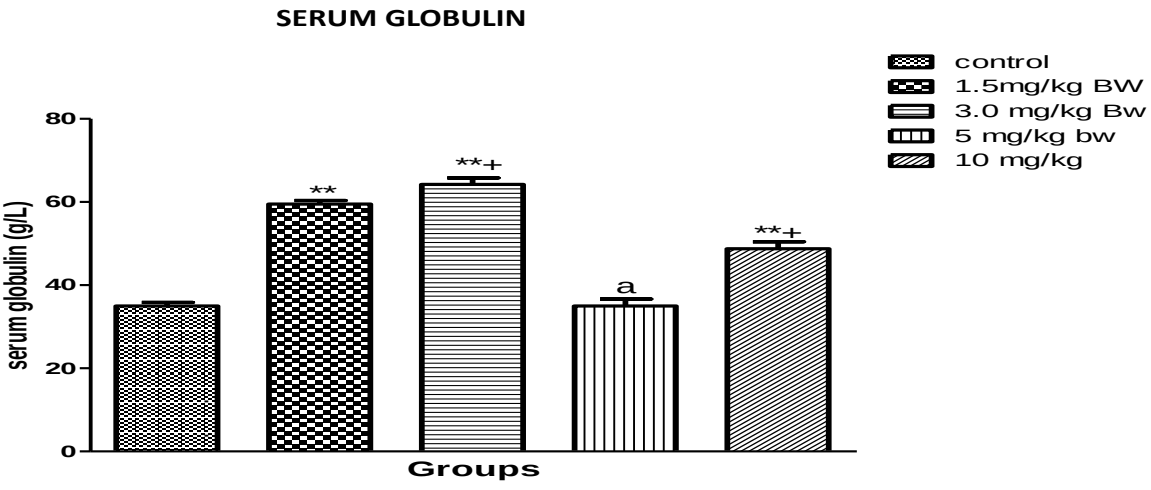

FIGURE 7: Mean serum Globulin concentration in the various experimental groups. Values are expressed as mean ± SEM, n = 5, **p<0.05 vs Control; † P<0.05 vs 1.5mg/kg; a = p<0.05 vs other *E Mal*$^{(R)}$ groups

- **Effect of *E Mal*$^{(R)}$ on liver histology**

Hepatocytes (H), central veins (CV) and sinusoids (S) were identified in the histological presentation of the liver tissues. It was normal study in the histology for the rats in control group and those administered *E MAL*$^{(R)}$ at 1.5 mg/kg body weight. Some degree of necrosis was shown in liver tissue at 3.0 mg/kg body weight of the drug. The central veins and sinusoids were seen to be dilated at *E MAL*$^{(R)}$ doses of 5.0 and 10.0 mg/kg body weight, respectively, suggestive of distorted hepatic cyto-architecture.

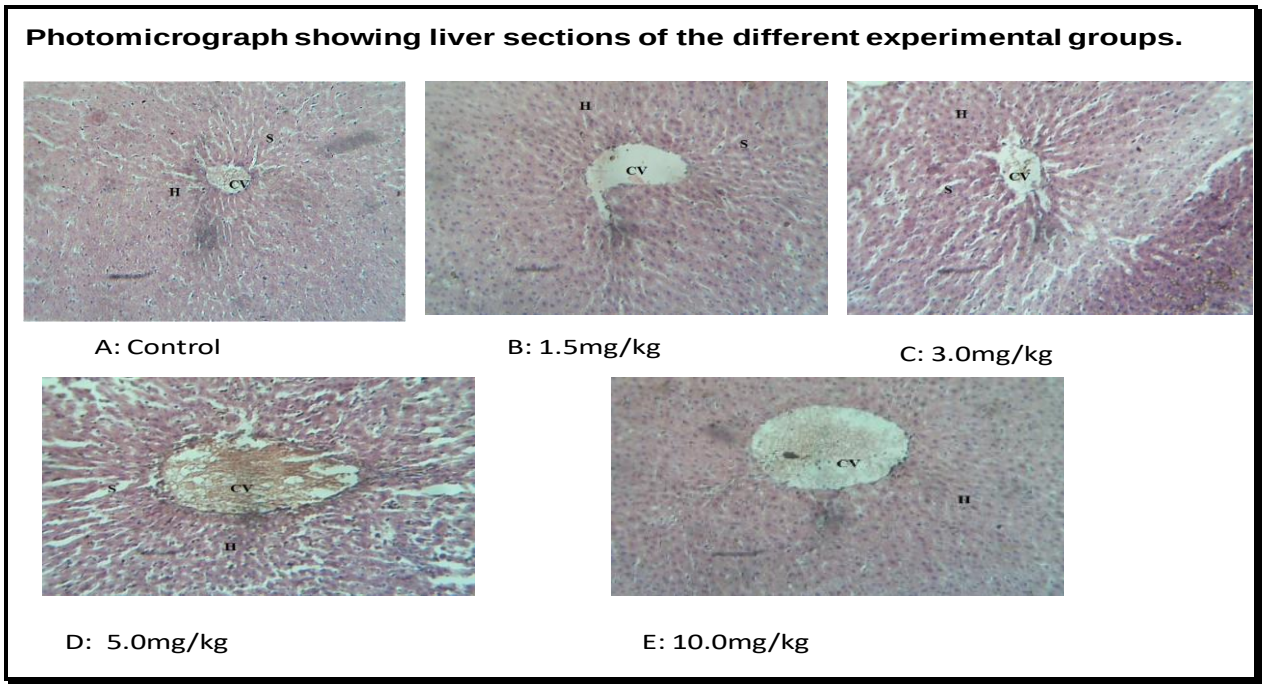

Figure 8: Photomicrograph showing sections of the different experimental Groups

9. CONCLUSION:

Results from our study showed that *E MAL*$^{(R)}$ (α, β-arteether) elevated serum alkaline phosphate (ALP), alkaline aminotransferase (ALT) enzymes, albumin and total protein concentrations in the albino wistar rat. The hepatic tissue in the 5.0 and 10.0 mg/kg body weight groups showed dilated central veins and sinusoids, suggestive of distorted liver cyto-architecture. Caution is advised in the use of the drug in patients with liver disease and subjects prone to hepatocellular damage. The current anti-malarial regimen of 150 mg (for adults) and 3 mg/kg body weight (for children), per day for three consecutive days may be well tolerated, However, higher doses may predispose to liver injury which leaves room for further studies.

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