

Alterations in Antioxidant Status and Biochemical Indices Following Administration of Dihydroartemisinin-Piperaquine Phosphate (P-ALAXIN[®])

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Abstract: Dihydroartemisinin-piperaquine phosphate (P-ALAXIN[®]) is a fixed-dose artemisinin-based combination therapy (ACT). Despite the efficacy and tolerability of this drug, there are adverse effects associated with it. This study was designed to investigate potential toxicity of two different doses of the drug in rats. Thirty rats (Wistar strain) weighing 200±20g were completely randomised into three treatment groups; Group A (control), Group B (Therapeutic dose) and Group C (double therapeutic dose of P-ALAXIN[®]). Creatinine, Urea and Bilirubin levels were significantly ($p<0.05$) elevated in the plasma of the rats that received the two doses of P-ALAXIN[®] by 67 and 117%; 33 and 67%; 85 and 119% respectively when compared to the control. Activities of plasma ALP, ALT, AST and GGT were also significantly ($p<0.05$) higher in the two treated group by 22 and 32%; 26 and 36%; 18 and 34%; 43 and 91% respectively. Moreover, plasma Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in the two treated groups were significantly increase ($p<0.05$) by 24 and 38%; 38 and 75%; 37 and 88% and 28 and 64% respectively. These were accompanied by a significant ($p<0.05$) increase in the MDA level by 33 and 45% respectively in the two treated groups. Furthermore, the two doses of P-ALAXIN[®] significantly ($p<0.05$) reduced hepatic ascorbic acid and GSH by 44 and 58% and 44 and 75% respectively, with a concomitant reduction in activities of hepatic GST. Similarly, there was a significant reduction in the activities of hepatic Catalase, and SOD. These data indicate that therapeutic and double therapeutic doses of P-ALAXIN[®] has adverse effects on both enzymic and non-enzymic antioxidant status and induces marked renal and liver damages and oxidative stress in rats.

Keywords: Dihydroartemisinin-piperaquine phosphate, Therapeutic and Double therapeutic doses, Oxidative stress Renal and Liver damage.

I. Introduction

Malaria is a vector-borne infectious disease caused by different strains of the protozoan parasites of the genus *Plasmodium*. The incidence of malaria worldwide is estimated to be 300-500 million clinical cases each year with about 90% of these occurring in Africa, south of the Sahara mostly caused by *P. falciparum* [1]. Effective treatment of malaria has been a great challenge due to the development of resistance of parasite especially *P. falciparum* to most antimalarial agents. [2] Emphasis on malaria treatment has therefore shifted from the inexpensive, effective and orally administered chloroquine to artemisinin and its derivatives [3].

The artemisinins are highly efficacious against multi-drug resistant *P. falciparum* and capable of producing up to 10,000 fold reductions in parasite biomass per asexual cycle [4, 5]. However, it has been reported that due to treatment failures associated with monotherapy and artemisinins, combined therapy of artemisinin with other antimalarial agents known as the artemisinin-based combination treatments (ACTs) have been recommended [6, 7, 8].

Artemisinin has a very different mode of action from conventional antimalarial and this makes it particularly useful in the treatment of resistant infections [9]. The combinations of Artemisinin derivatives having a short half-life with another drug with a longer duration of action provides a synergistic activity leading to elimination of remaining parasites [10].

The most widely used artemisinin derivatives are artesunate, artemether and dihydroartemisinin (DHA). Based on available safety and efficacy data, different therapeutic options of artemisinin-based combination treatments (ACTs) are now available which include: Artemether-lumefantrine; Artesunate-amodiaquine; Artesunate-Sulfadoxine/Pyrimethamine; Artesunate-Mefloquine; Dihydroartemisinin-piperaquine phosphate etc. [11].

Dihydroartemisinin and piperaquine phosphate combination under the trade name P-ALAXIN[®] is a film coated tablet containing 40mg of Dihydroartemisinin (DHA) and 320mg of piperaquine phosphate (PQP). The drug combination is known to be effective against *P. vivax*, *P. malariae* and the multi-resistant *P. falciparum* malaria parasites. DHA is the active metabolite of artemisinin and its derivatives (Fig. 1a). These derivatives have more potent blood schizonticidal activity than the parent compound. DHA is the most potent antimalarial of this group of compounds but it is also the least stable. Oral dihydroartemisinin is rapidly absorbed and has a short

elimination half-life, although little is known of its metabolism [12]. DHA mainly interferes with protein and nucleic acid synthesis and with the membrane structures of the food vacuole, nucleus, mitochondria, and endoplasmic reticulum leading to the dissociation of ribosomes from ER and the autophagosis of parasite [13].

Piperaquine phosphate (Fig. 1b), a bisquinoline, structurally related to chloroquine has been shown to exhibit cardiovascular toxicity in overdose with significant electrophysiological effects on the ear [14]. PQP is widely distributed in the liver, kidneys, lungs and spleen. The high lipophilicity of piperaquine results in an extremely high distribution into parasite lipid membranes [15]. PQP interferes with the food vacuole membrane of the trophozoites leading to autophagosis of the parasite. Elimination of PQP is very slow with a half-life of about 9 days. The structural determinant of the activity of artemisinins is the endoperoxide bridge which is a specific feature of this type of compounds. It has been suggested that the parasiticidal activity starts with the reaction of artemisinins with haem iron, leading to the generation of activated oxygen species, such as oxygen radicals, or of a C-centred radical of artemisinin itself [16].

Nosten and White [5], had reported that if there is any toxicity observed in artemisinin combination treatments, it may be due to the non-artemisinin component as artemisinin derivatives alone may have relatively low toxicological effects. However, studies with animal had shown that artemisinin and its analogues cause acute hepatotoxicity in guinea-pigs [17] and have adverse effects on biochemical parameters in rabbits [18]. These result in the production of oxygen free radicals and peroxide formation which will lead to the deleterious effects of these agents [19, 20]. Despite decades of clinical use, there are limited published non-clinical *in vivo* and *in vitro* information of Dihydroartemisinin-Piperaquine Phosphate addressing pharmacokinetics, metabolism or toxicity [21]. Therefore, the present study was designed to investigate the effect of administration of different doses of Dihydroartemisinin-Piperaquine Phosphate (P-ALAXIN[®]) on the antioxidant status and some biochemical indices in rats.

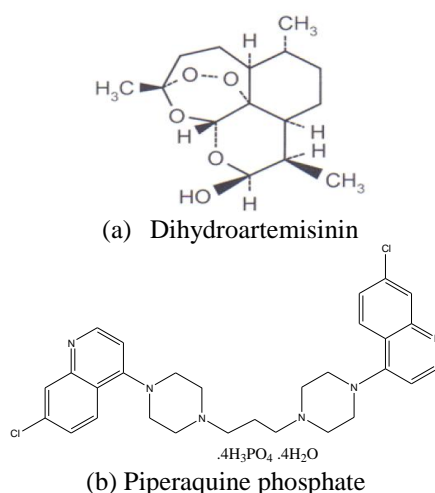


Fig 1: Structures of Dihydroartemisinin (DHA) and Piperaquine phosphate (PQP)

II. Materials And Methods

2.1 Chemicals and Reagents

P-ALAXIN[®]-Dihydroartemisinin plus Piperaquine Phosphate (360mg), was a product of BLISS GVS Pharmaceutical limited, India. Glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), epinephrine and hydrogen peroxide were purchased from Sigma chemical company (London, UK). Alanine transaminase (ALT), Aspartate transferase (AST), gamma glutamyl transferase (GGT), urea, creatinine, bilirubin, total cholesterol, HDL – cholesterol, LDL – cholesterol and triglycerides assay kits were products of Randox Laboratories Ltd, (Antrim UK). All other chemicals and reagents were of analytical grade and of highest purity.

2.2 Animals and Treatments

2.2.1 Animals

In bred 5-6week-old male rats (Wistar strain) weighing 200±20g were used in this study. The rats were bred and housed in the animal house of the department of Chemical sciences, Ajayi Crowther University, Oyo, Nigeria. They were kept in wire meshed cages at room temp (28-30°C) and under controlled light cycle (12-hr light: dark). They were fed with commercial rat chow (Ladokun feeds, Ibadan, Nigeria) and supplied water *ad libitum*. All experiments were conducted without anaesthesia and protocol conforms to the guidelines of the National Institute of Health [22] for laboratory animal care and use.

2.2.2 Treatments

Thirty healthy male albino rats (wistar strain) were randomly divided into three groups of 10 rats each. Group I (Control) received physiological saline, while groups II and III were administered the therapeutic and double therapeutic dose of dihydroartemisinin plus piperazine phosphate (P-ALAXIN®) respectively as presented in Table 1. The treatments were administered in divided doses, twice daily for three days. The rats were sacrificed 24hrs after the last treatment.

Table 1: Experimental design

| EXPERIMENTAL GROUP | DAY 1 | DAY 2 | DAY 3 |
|----------------------------|----------------------|----------------------|----------------------|
| 1. CONTROL | Physiological saline | Physiological saline | Physiological saline |
| 2. THERAPEUTIC DOSE | 18 mg/ Kg b. w. | 18 mg/ Kg b. w. | 12g/ Kg b. w. |
| 3. DOUBLE THERAPEUTIC DOSE | 36 mg/ Kg b. w. | 36 mg/ Kg b. w. | 24 mg/ Kg b. w. |

b.w.= body weight

2.3 Collection of blood samples for plasma preparation

Blood was collected from the inferior *vena cava* of heart of the animals into heparinized tubes, and the rats were sacrificed by cervical dislocation. Plasma was prepared by centrifuging blood samples for ten minutes at 3000 x g in an Eppendorf (UK) bench centrifuge. The clear supernatant was used for the estimation of plasma lipid profiles and enzymes.

2.4 Preparation of cytosolic fractions

The liver, excised from rat, blotted of blood stains, rinsed in 1.15% KCl was homogenized in 4 volumes of ice-cold 0.01 M potassium phosphate buffer, (pH 7.4). The homogenates were centrifuged at 12,500 g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

2.5 Renal and liver functions test

Plasma creatinine, urea and bilirubin determination was done using Sigma diagnostic kits. Methods for creatinine assays are based on colorimetric alkaline picrate methods [23] with Creatinine-picrate complex measured at 492nm. The urea determination method was based on the fearon reaction [24], with the Diazine chromogen formed absorbing strongly at 540nm. The dimethyl sulphoxide method by [24] was used for bilirubin determination. The dimethyl sulphoxide form a coloured compound with maximum absorption at 550nm.

2.6 Determination of plasma AST, ALT, ALP and GGT activities

Plasma AST, ALT, ALP and GGT activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reltman and Frankel[25]. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546nm and ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. ALP was determined in accordance with the principles of Tietz [26]. The p-nitrophenol formed by the hydrolysis of p-Nitrophenyl phosphate confers yellowish colour on the reaction mixture and its intensity can be monitored at 405nm to give a measure of enzyme activity. GGT activity was measured based on a modification of the method described by Theodorsen *et.al.* [27] using Abbott diagnostic kit.

2.7 Determination of plasma lipid profiles

The plasma total cholesterol, HDL- cholesterol, LDL- cholesterol and triglycerides were determined using Randox diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder [28].

2.8 Assay of non-enzymatic antioxidants and lipid peroxidation

Hepatic vitamin C was determined chemically according to the method of Erel *et al.* [29] using dinitro phenyl hydrazine (DNPH), while hepatic glutathione was determined according to the method of Jollow *et al.* [30]. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was read in a spectrophotometer. Reduced GSH is proportional to the absorbance at 412 nm. The extent of lipid peroxidation (LPO) was

estimated by the method of Vashney and Kale [31], the method involved the reaction between malondialdehyde (MDA; product of LPO) and thiobarbituric acid to yield a stable pink chromophore with maximum absorption at 532 nm.

2.9 Determination of antioxidant enzymes

The procedure of Misra and Fridovich [32] as described by Magwere *et al.* [33] was used for the determination of hepatic superoxide dismutase (SOD) activity by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 and 30°C. Hepatic catalase activity was determined according to the method of Asru [34] by measuring the reduction of dichromate in acetic acid to chromic acetate at 570 nm. Hepatic Glutathione S-transferase (GST) activity was determined by the method described by Habig *et al.* [35] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

2.10 Protein determination

Protein determination of plasma and all fractions was estimated by the method of Lowry *et al.* [36] using bovine serum albumin as standard.

2.11 Histopathological studies

The method of Baker and Silverton [37] was employed for the processing of liver for histopathological studies.

2.12 Statistical analysis

Results were expressed as mean of 10 replicates \pm SD. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and complemented with Student's t-test using StatPac[®] Statistical Software. Statement of statistical significance was based on $p < 0.05$.

III. Results

3.1 Effect of dihydroartemisinin- piperazine phosphate on the levels of plasma Creatinine, Urea and Bilirubin in rats

There were significant increases ($P < 0.05$) in the plasma Creatinine, Urea and Bilirubin levels of animals given the two doses of dihydroartemisinin- piperazine phosphate (P-ALAXIN[®]) when compared to the control (table 2). Specifically, the therapeutic and double therapeutic doses of P-ALAXIN[®] increased the plasma creatinine level by 67% and 117%; plasma urea by 33% and 67%; plasma Bilirubin by 85% and 119% respectively.

3.2 Effect of dihydroartemisinin- piperazine phosphate on the levels of plasma enzymes in rats

Administration of dihydroartemisinin- piperazine phosphate (P-ALAXIN[®]) significantly increased ($P < 0.05$) plasma ALP, AST, ALT and GGT activities of the animals when compared to the control (table 3). Precisely, ALP activity was increased by 22% and 32% and ALT activity increased by 26% and 36% in both the therapeutic and double therapeutic dose treated groups respectively. Furthermore, AST activity was increased by 18% and 34% and GGT activity increased by 43% and 91% in the treated groups respectively.

3.3 Effect of dihydroartemisinin- piperazine phosphate on the levels of plasma lipid profiles in rats

There were significant increases ($P < 0.05$) in the plasma lipid profile levels of rats following the administration of two doses of dihydroartemisinin- piperazine phosphate (P-ALAXIN[®]) when compared to the control (table 4). Exactly, plasma total cholesterol was increased by 24% and 38% ; plasma HDL-cholesterol by 38% and 75%; plasma LDL- cholesterol by 37% and 88% and plasma triglycerides by 28% and 64% in the therapeutic and double therapeutic dose groups respectively.

3.4 Effect of dihydroartemisinin- piperazine phosphate on the levels of hepatic antioxidant enzymes in rats

The hepatic antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) activities were significantly ($p < 0.05$) reduced following treatment with two doses of dihydroartemisinin- piperazine phosphate (P-ALAXIN[®]) when compared to the control (table 5). CAT and SOD activities were reduced in the treated groups by 26% and 71% and 18% and 94% respectively. Hepatic glutathione-S-transferase (GST) activity was also significantly reduced following treatment with therapeutic and double therapeutic doses of P-ALAXIN[®] by 25% and 76% respectively (fig. 4).

3.5 Effect of dihydroartemisinin- piperazine phosphate on the levels of hepatic non-enzymatic antioxidant and lipid peroxidation in rats

The hepatic level of non-enzymic antioxidants: ascorbic acid (Vit. C) and glutathione (GSH) were significantly ($p < 0.05$) depleted following treatment with therapeutic and double therapeutic doses of dihydroartemisinin- piperazine phosphate (P-ALAXIN[®]) when compared to the control: Vit C level was decreased by 45% and 131% (fig. 2) and GSH by 27% and 44% (fig.3) in the treated group respectively. MDA level was significantly elevated ($p < 0.05$) by 50% and 72% in the therapeutic and double therapeutic dose groups respectively compared to the control (fig. 5).

3.6 Effect of dihydroartemisinin- piperazine phosphate on the histopathology of kidney and liver of rats

Plates 1 and 2 are photomicrographs showing the effect of the therapeutic and double therapeutic doses of dihydroartemisinin plus piperazine phosphate combination antimalarial on the liver and kidney respectively. The therapeutic dose of the drug resulted in hepatic portal congestion and cellular infiltration by mononuclear cells (Plate 1B). A severe hepatic portal congestion and cellular infiltration by mononuclear cells was observed in the double therapeutic dose group (Plate 1C). The therapeutic dose of the drug did not produce any visible lesion on kidney cells (Plate 2B), however, a severe renal cortical congestion was observed in the double therapeutic dose group (Plate 2C).

TABLE 2: Effect of dihydroartemisinin plus piperazine phosphate (P-ALAXIN)[®] on plasma urea, creatinine, and bilirubin levels in rats.

| TREATMENT | CREATININE (mg/ dl) | UREA (mg/ dl) | BILIRUBIN (mg/ dl) |
|-------------------------|---------------------|------------------|--------------------|
| Control | 0.13± 0.01 | 24.8± 2.8 | 0.36± 0.02 |
| Therapeutic dose | 0.22± 0.01 (67%)* | 33.2± 1.9 (33%)* | 0.67± 0.02 (85%)* |
| Double therapeutic dose | 0.29± 0.01 (117%)* | 41.6± 1.7 (67%)* | 0.80± 0.04 (119%)* |

The values are the Means ± SD (range) for ten rats in each group. *Significantly different from the control ($p < 0.05$). Values in parenthesis represent (%) increase.

TABLE 3: Effect of dihydroartemisinin piperazine phosphate (P-ALAXIN)[®] treatments on plasma Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GGT).

| TREATMENT | ALP (U/L) | ALT (U/L) | AST (U/L) | GGT(U/L) |
|-------------------------|----------------|--------------|-------------------|------------------|
| Control | 101.4±3 | 61±1 | 71 ±1.2 | 7.4 ±1.1 |
| Therapeutic dose | 123.6± 3(22%)* | 77±2(26%)* | 83.8 ± 2.6 (18%)* | 10.6± 0.9 (43%)* |
| Double therapeutic dose | 134±4(32%)* | 83±1.6(36%)* | 95.2 ±2.6 (34%)* | 14.2±1.3(91%)* |

The values are the Means ± SD (range) for ten rats in each group. *Significantly different from the control, ($p < 0.05$). Values in parenthesis represent (%) increase.

TABLE 4: Effect of dihydroartemisinin plus piperazine phosphate (P-ALAXIN)[®] on plasma lipid profile in rats

| TREATMENT | TOTAL CHOLESTEROL (mg/ dl) | HDL- CHOLESTEROL (mg/ dl) | LDL- CHOLESTEROL (mg/ dl) | TRIGLYCERIDES (mg/ dl) |
|-------------------------|----------------------------|---------------------------|---------------------------|------------------------|
| Control | 46.8± 1.9 | 23.6±2.1 | 19.4±1.1 | 28.2±2.3 |
| Therapeutic dose | 58±0.7 (24%)* | 32.6±2.8(38%)* | 26.6±1.1 (37%)* | 36±1.2 (28%)* |
| Double therapeutic dose | 64±1.7(38%)* | 41.4±1.7 (75%)* | 36.4±2.2 (88%)* | 46.2± 1.9(64%)* |

The values are the Means ± SD (range) for ten rats in each group. *Significantly different from the control, $p < 0.05$. Values in parenthesis represent (%) increase.

TABLE 5: Effect of dihydroartemisinin piperazine phosphate (P-ALAXIN)[®] treatments on Hepatic Catalase Activities and Superoxide Dismutase (SOD) Activity in Rats.

| TREATMENT | CAT (µmol H ₂ O ₂ consumed/min) | SOD (Units) |
|-------------------------|---|----------------|
| Control | 0.33±0.02 | 6.6±0.55 |
| Therapeutic dose | 0.24±0.01 (26%)* | 5.4±0.55(18%)* |
| Double therapeutic dose | 0.19±0.01 (71%)* | 3.4±0.55(94%)* |

The values are the Means ± SD (range) for ten rats in each group. *Significantly different from the control, $p < 0.05$. Values in parenthesis represent (%) decrease

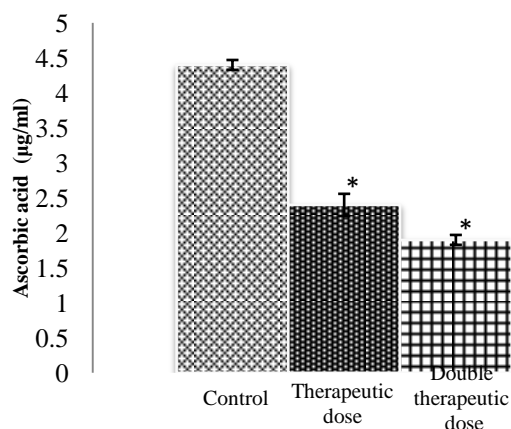


Figure 2: Effect of dihydroartemisinin piperazine phosphate (P-ALAXIN)[®] treatments on hepatic Vitamin C concentration in Rats. The values are the Means \pm SD (range) for ten rats in each group. *significantly different from the control, $p < 0.05$

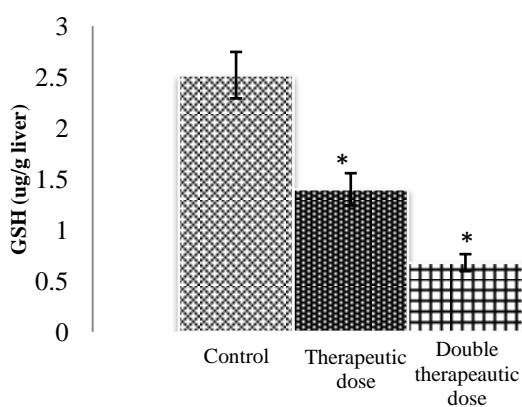


Figure 3: Effect of dihydroartemisinin piperazine phosphate (P-ALAXIN)[®] on Hepatic reduced Glutathione (GSH) level in Rats. The values are the Means \pm SD (range) for ten rats in each group. *significantly different from the control, $p < 0.05$.

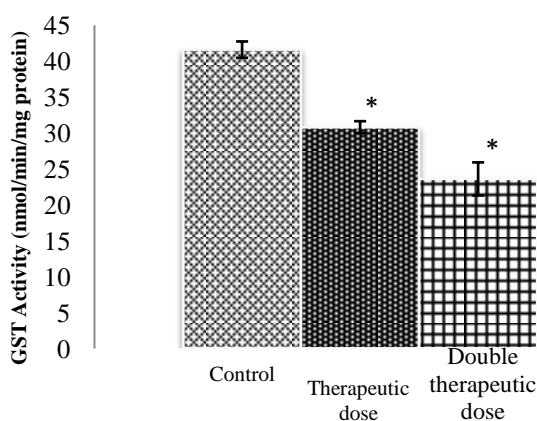


Figure 4: Effect of dihydroartemisinin plus piperazine phosphate (P-ALAXIN)[®] on Hepatic Glutathione-S-transferase (GST) activity in Rats. The values are the Means \pm SD (range) for ten rats in each group. *significantly different from the control, $p < 0.05$

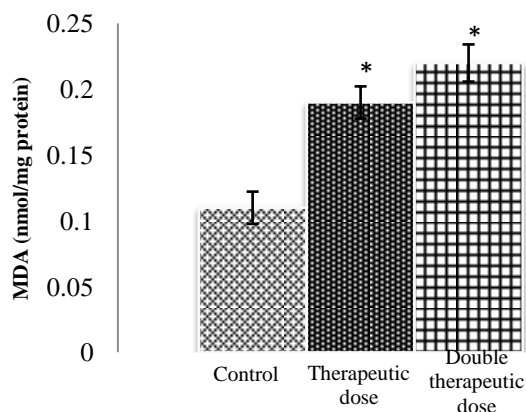


Figure 5: Effect of dihydroartemisinin plus piperazine phosphate, (P-ALAXIN[®]) on Hepatic Lipid peroxidation (MDA) level in Rats. The values are the Means \pm SD (range) for ten rats in each group. *significantly different from the control, $p < 0.05$.

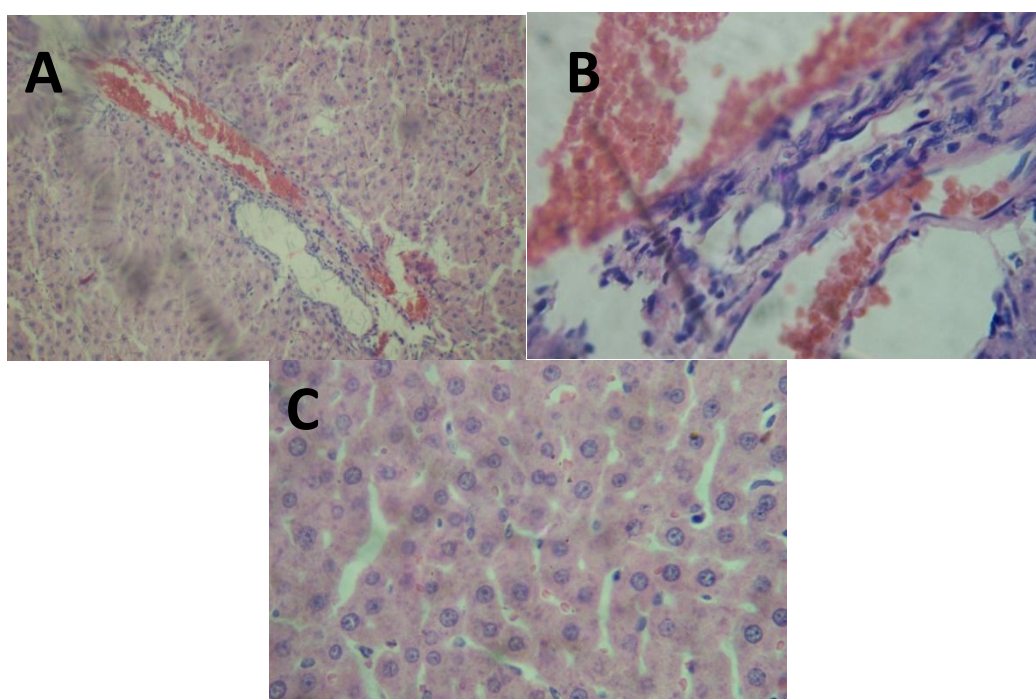
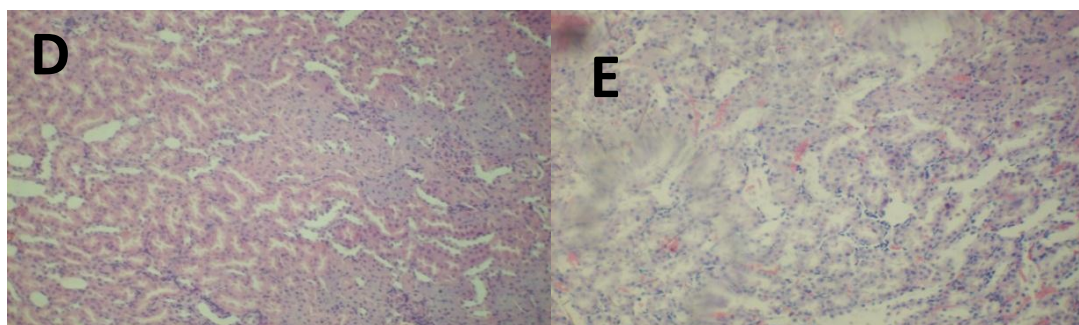


Plate 1: Photomicrographs of hepatocytes showing the effect of P-ALAXIN[®]:
A = Control: No visible lesions seen.
B = Therapeutic Dose: There is portal congestion and cellular infiltration by mononuclear cells
C = Double therapeutic dose: There is severe portal congestion and cellular infiltration by mononuclear cells



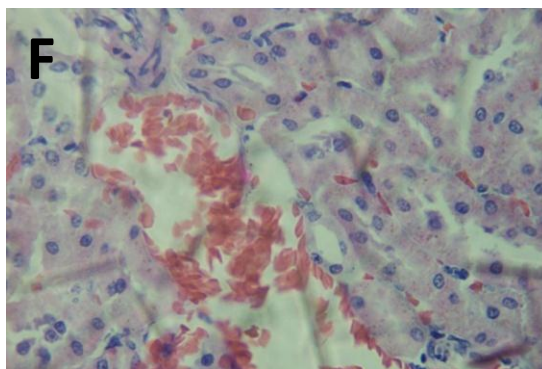


Plate 2: Photomicrographs of kidney cells showing the effect of P-ALAXIN[®]:

D = Control: No visible lesions seen.

E = Therapeutic Dose: No visible lesions seen.

F = Double therapeutic dose: There is severe renal cortical congestion.

IV. Discussion

In the present study, the potential hepatic and renal toxicity as well as effect on antioxidant status of the therapeutic and double therapeutic doses of dihydroartemisinin and piperazine phosphate (P-ALAXIN[®]) was investigated in rats. Dihydroartemisinin (DHA) is a reduced product of artemisinin and it is the primary metabolite of artemisinin and its derivatives [38]. DHA is water soluble, relatively unstable and very prone to oxidative reactions because of the lactol moiety in its structure. Piperazine is an antimalarial compound belonging to the 4-aminoquinolines. It is a highly lipophilic basic molecule [15]. The exact mechanism of action of piperazine is unknown, but it is reasonable to assume that the compound has similar targets as chloroquine considering the close structural resemblance.

It is known that many organs show separate and unique responses to factors that regulate antioxidant and some marker enzymes [39]. Marker enzymes and other biochemical indices used in this study are important and effective tools in the diagnosis of diseases and toxicity arising from drug administration. Our results indicate that administration of dihydroartemisinin plus piperazine phosphate induces marked renal and liver failure and affected both the enzymic and non-enzymic antioxidant system and lipid peroxidation.

Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys [40] and their serum/plasma concentrations are commonly used to screen for renal or cardiovascular diseases [41, 42]. Elevation of the plasma levels of creatinine and urea is an indication of abnormal renal function [43]. Plasma urea has been reported to increase in acute and chronic intrinsic renal disease and also when there is decreased effective circulating blood volume with decreased renal perfusion [44, 45]. Creatinine have been implicated in kidney diseases such as acute glomerulonephritis, nephrosclerosis and tubular necrosis [46, 47]. Elevated plasma bilirubin has been associated with hepatocellular damage, intra and extra-hepatic biliary tract obstruction, and neonatal jaundice [48, 49]. The results, therefore, indicate that renal and hepatic function may be impaired in animals treated with therapeutic and double therapeutic dose of P-ALAXIN[®].

The hepatocyte membrane distortion is associated with membranal leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum/plasma marker enzymes of acute hepatocellular damage namely ALT and AST, and ALP as a marker for hepatobiliary damage [50]. However, of these marker enzymes, ALT is the most reliable. AST is known to be present in abundance in the cardiac muscles, skeletal muscles, kidneys and testes, and ALP abundant in the growing bone. Thus, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes [51]. The elevated aminotransferases and phosphatases observed in the therapeutic and double therapeutic dose of P-ALAXIN[®] treated animals is highly indicative of hepatic toxicity [52, 53]. These findings are in line with the findings of other researchers [17, 54, 55, 56, 57]. Other antimalarial, such as chloroquine [58], amodiaquine [59], quinine [60] and halofantrine [61, 62] were also reported to elevate serum aminotransferases and phosphatases and may induce hepatic damage.

To prevent injury from reactive oxygen species (ROS), cells have developed defence systems. Besides scavenger molecules such as glutathione vitamin C, or α -tocopherol, specific enzymes, the antioxidants enzymes (AOE) fulfil this task. The expression of AOE can be regulated by oxidative stress itself [63, 64, 6, 65]. Our data indicates that the therapeutic and double therapeutic dose of P-ALAXIN[®] affects both enzymatic and non-enzymatic antioxidants. The antioxidant enzymes Catalase and SOD are part of the primary intracellular antioxidants defence mechanism against oxidative stress [28]. The decreased activities of SOD and CAT in the liver of the animals treated with the two doses of P ALAXIN[®] could be due to the organ's response to an

increased production of reactive oxygen species, as a result of exposure to the drugs and their metabolites. The finding of decreased SOD and CAT activities in the liver of these animals as a result of drugs administration is not unexpected as both SOD and CAT are co-regulated in tissues in response to toxic assaults [66]. This result is consistent with the findings of other researchers [53, 67, 68].

Results from this study further reveals that treatment with therapeutic and double therapeutic dose of P-ALAXIN[®] induced a significant decrease in hepatic glutathione-S-transferase (GST) activity, reduced glutathione (GSH) and vitamin C depletion. Glutathione-S-transferase is a family of multifunctional isozymes found in all eukaryotes, catalysing both glutathione dependent conjugation and reduction reactions [67]. One main function of GST is to catalyse the biotransformation of xenobiotics, including drugs detoxification in the mercapturic acid pathway, leading to the elimination of toxic compounds [69] and also acting as an antioxidant enzyme [70]. In this study, GST activities were inhibited in animals treated with the two doses of the drug. This observation, therefore, suggests that this drug may alter the expression and activities of antioxidant enzymes as a result of toxic metabolites generated during their biotransformation. The level of reduced GSH and vitamin C is a measure of non-enzymic antioxidant and cellular redox status of cells in higher animals [71]. Studies have shown that the redox state of intracellular vitamin C is controlled by the intracellular level of GSH [72]. Our results showed that administration of P-ALAXIN[®] decreased the overall redox status in the liver as indicated by a significant decrease in the level of GSH and Vitamin C. The observation on the decrease of GSH is in agreement with the findings of Bhattacharyya *et al.*[73], Ogunbayo *et al.* [74] and Adaramoye *et al.*[53] that observed significant decrease in GSH levels in the retina of rats, blood of rabbits and liver of rats respectively, following administration of chloroquine; artemether, coartem and halofantrine, while that of vitamin C is in consonance with the findings of Farombi *et al.*[75] that observed significant decrease in vitamin C in the blood of rats following treatment with chloroquine. The observed decrease in GSH levels and vitamin C further confirmed the formation of reactive oxygen species or toxic metabolites from the drug.

Our result also indicated an elevation in the levels of lipid profiles (plasma total cholesterol, HDL - cholesterol, LDL-cholesterol and triglyceride), with a concomitant increase in the concentration of malondialdehyde (MDA) in the P-ALAXIN[®] treated groups. It has been suggested that cholesterol is a general indicator of the level of lipid in the circulation [76] and cholesterol and polyunsaturated fatty acids (PUFA) are the main components of LDL. PUFA is the substrate required for MDA formation and the amount of peroxidized lipid formed may be related both to the amount of substrate and to the level of lipid peroxidation (LPO). Therefore, the more the lipid, the greater the amount of lipid peroxidation activity and the greater the amount of lipid peroxidation products such as MDA. Increase in the level of lipid peroxidation has been described as a biomarker of tissue damage [77]. The thiobarbituric acid (TBA) assay is a suitable means of determining the extent of lipid peroxidation in tissues by measuring the concentration of MDA. The high level of lipid peroxidation following the administration of P-ALAXIN[®] in the liver may be attributed to the redox imbalance caused by the drug. Depletion of the membrane antioxidant system is known to result in the oxidation of membrane lipids leading to accumulation of lipid peroxides [77]. The accumulation of LPO products observed in this study is supported by the findings of Magwere *et al.* [32]. The concomitant decrease in the GSH level and vitamin C (a nonenzymic antioxidant) and enzymic antioxidant indices (SOD, CAT and GST activities) of the animals imply an increase susceptibility of the tissues to radical species generated by the drugs.

The histopathological observations in the liver of both the therapeutic and double therapeutic doses of P-ALAXIN[®]-treated rats showed hepatic portal congestion and cellular infiltration by mononuclear cells and a severe hepatic portal congestion and cellular infiltration by mononuclear cells respectively. This could be due to the formation of highly reactive radicals because of oxidative threat caused by the drug. The accumulated hydroperoxides can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids. The therapeutic dose of the drug did not however, produce any visible lesion on kidney cells, but a severe renal cortical congestion was observed in the double therapeutic dose group. Based on the histology and other results in this study it can be seen that the administration of therapeutic and double therapeutic doses of P-ALAXIN[®] would have pronounced effect on the liver than the kidney. This may be due to the fact that the liver is the main site of drug metabolism, while the kidney is mainly involved in drug elimination.

V. Conclusion

In conclusion, our results suggests that the therapeutic as well as double therapeutic doses of P-ALAXIN[®] induced marked renal and hepatic failure and decreased the levels of antioxidant defence systems, with the effect more pronounced in the double therapeutic doses. As such caution should be taken in administering the drug beyond the therapeutic dose and the drug should be taken alongside with some antioxidants.

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