Nephrotoxicity of Therapeutic Dose of Dihydroartemisinin-Piperaquine Phosphate in Male and Female Animals

Fatai A. Kareem1, Mutiu A. Alabi2*, Fatai Akinwunmi3 and Ayodeji O. Obatoye4

1Department of Science Laboratory Technology, School of Science and Technology, Gateway Polytechnic, Saapade, Ogun State.
2Bioresources Development Centre, National Biotechnology Development Agency, P.M.B. 3524 Ogbomoso, Oyo State.
3Institute for Human Resources Development, Federal University of Agriculture, P.M.B 2240 Abeokuta, Ogun State.
4Department of Crop Processing and Utilization unit, Cocoa research Institute of Nigeria, Ibi Ayunre, Ibadan, Oyo state.
*Corresponding author: mutiualabi@gmail.com

Abstract
Dihydroartemisinin-piperaquine phosphate (DHAPP) is an artemisinin based combined therapy and is very effective in treating malaria in areas of high resistance to conventional malaria. The present study investigates the toxicological effects of the use therapeutic dose of DHAPP in male and female rats. Thirty adult Wister rats of both sexes weighing between 180 and 210 g were grouped into three consisting of 5 males and 5 females per group. The control group was orally administered with normal saline, the test and recovery groups were given body weight 15.4 mg/kg of DHAPP orally for three days after which the recovery group were allowed to recover from the drug’s effect for another three days. The blood samples were collected through cardiac puncture into heparinised tubes centrifuged at 5000 rpm for 10 minutes. The kidney were also removed, weighed, blotted dry and homogenised. The supernatant of each of the plasma and kidney was kept in clean bottles, stored at -4ºC and were used for kidney function and histological analysis. An increment was observed in the protein, creatinine and urea levels in the plasma while in the kidney, the levels decreased. In the plasma and kidney, all the biochemical parameters were observed to return to normal when the animals were left to recover. Sex related differences were noted in most of the groups in the plasma and kidney of the enzyme activities. Histological examination also revealed an intoxication of the kidney cell of the rats. It could therefore be inferred from these results that increases in protein, creatinine and urea levels in the blood plasma showed the possibility of abnormality in the renal system when the drug was administered. It is therefore recommended that the drug should be administered with caution.

Keyword: Dihydroartemisinin-piperaquine phosphate (DHAPP); Artemisinin; Antimalarial; therapeutic dose.
1. Introduction

Malaria is one of the most serious health challenges facing the world today. It is a mosquito-borne infectious disease of humans and other animals caused by *Plasmodia* and are also definitely the single most destructive and dangerous infectious agent in the developing countries of the world [1-3]. This disease results from the multiplication of *Plasmodium* parasites within red blood cells. Studies revealed that five species of *Plasmodium* can infect and be transmitted by humans [4].

There were an estimated 225 million cases of malaria worldwide in 2009 [5]. An estimated 655,000 people died from malaria in 2010, a decrease from the 781,000 who died in 2009, accounting for 2.23% of deaths worldwide [5]. However, a 2012 meta-study from the University of Washington and University of Queensland estimates that malaria deaths are significantly higher [6].

Severe malaria disease is largely caused by *Plasmodium falciparum* while that caused by *Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* is generally a milder that is rarely fatal [4]. *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques which can also infect humans [7, 8].

Currently, artemisinin-based combination therapy (ACT) is recommended for the treatment of *P. falciparum* malaria [5, 9]. Fast acting artemisinin-based compounds are combined with a drug from a different class. Such drugs may include lumefantrine, mefloquine, amodiaquine, sulfadoxine, piperaquine and chlorproguanil [9].

Malaria transmission can be reduced by preventing mosquito bites, use of mosquito nets and repellents, or by mosquito control measures such as spraying with insecticides and draining stagnating water in which they can breed [10, 11]. Despite a clear need, no vaccine offering a high level of protection currently exists, however, efforts to develop one are ongoing [12]. A number of medications (antimalarial drugs) are also available to prevent malaria (prophylaxis) while travelling to malaria endemic countries [13].

Scientists do not yet totally understand the complex that promotes humans against malaria and so the search for a vaccine to eliminate the disease is considered to be one of the most important projects in public health [14]. With the drug resistance strains of *Plasmodium*, the use of anti-malaria drugs, singly has failed to curtail the prevalence of malaria, globally, particularly multi-drug resistance falciparum, so WHO has recommended that acute uncomplicated resistance falciparum malaria should be treated by combining one of the artemisinin compound with another effective erythrocytic schizontocide [15].

Dihydroartemisinin-piperaquine phosphate (DHAPP) is an artemisinin based combination therapy drug consisting of dihydroartemisinin (40mg) and piperaquine phosphate (320mg). Piperaquine is a bisquinoline, first synthesized in the sixties in China and France, which is as effective as chloroquine [3]. The tolerability, efficacy, pharmacokinetic profile and low cost of piperaquine make it a promising combination drug for an artemisinin combined based therapy (ACT). DHAPP, being an artemisinin based combined therapy is very effective in treating malaria in areas of high resistance to conventional anti-malaria drug. The drug is usually being prescribed as an alternative to other artemisinin combined therapy such as Coartem [5]. The artemisinin-derivatives (artemether, artesunate, and dihydroartemisinin) are currently the most potent anti-malarial medicines in the market [3]. They are widely available in the different pharmaceutical dosage forms including tablets, injections, suppositories and dry powders [16].

Dihydroartemisinin-piperaquine phosphate (DHAPP) is an affordable drug generally used by people in Nigeria for treatment of malaria [3]. Many people are still unaware of its efficacy as an antimalarial agent because it is not very popular. There is also paucity of information on the toxicological effects of the administration of the drug.

The objective of the research work is to investigate the safety of the use of therapeutic dose of dihydroartemisinin-piperaquine phosphate on biochemical parameters as well as to assess the histology of the kidney on the rats administered with dihydroartemisinin-piperaquine phosphate. This research work is of significance to malaria treatment as it assesses the toxicological effects of the use of therapeutic dose of dihydroartemisinin-piperaquine phosphate on adult male and female rats.

2. Materials and Methods

2.1 Chemicals and Reagents. The chemicals used include diethyl ether from May and Baker Ltd., Dagenham, England, phosphate buffer (Na₂HPO₄ and NaH₂PO₄) from Randox Laboratories Ltd. Crumlin, United Kingdom, sodium chloride (NaCl), ethylene diaminetetraacetic acid (EDTA) were purchased from BDH Chemical Limited, Poole, England. Distilled water was obtained from the laboratory of Biochemistry Department, Olabisi Onabanjo, University, Ikenne Remo Campus, Ikenne, Nigeria. All other chemicals used are of analytical grade.

2.2 Animal Ethics. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Use of Animals [17].
2.3 Drugs, Animals and Diet. The dihydroartemisinin-piperaquine phosphate (DHAPP) tablet was obtained from Bliss GVS Pharma Limited, India. Thirty adult Wistar strain rats of both sexes (15 male and 15 female), weighing between 180 and 210g were obtained from the Physiology Department, Olabisi Onabanjo University, Remo Campus, Ikenne, Ogun State.

The rats were fed with standard rat pellets (Top Feed Nigeria Ltd., Ibadan, Nigeria) and water ad libitum and were housed in separate improvised cages under photo-period controlled environment (12 hours dark, 12 hours light cycles; 24-25°C) at the animal house of Olabisi Onabanjo University, Biochemistry Department, Remo Campus, Ikenne, Ogun State.

2.4 Experimental Design. The rats were divided into three groups consisting of five male and five female each which were housed separately in an improvised animal cages. Group I (Control Group) consists of five male and five female Wister rats which were fed with rat pellets and water ad-libitum throughout the study. They were orally administered with normal saline; Group II (Test Group) consists of five male and five female Wister rats were fed with rat pellets and water ad-libitum. They were orally administered with 15.4 mg/kg body weight of DHAPP for three days; and Group III (Recovery Group) consists of five male and five female Wister rats were fed with rat pellets and water ad-libitum. They were orally administered with 15.4mg/kg body weight of DHAPP for three days and were allowed to recover from the drug’s effect for another three days.

2.5 Animal Sacrificing and Sample Collection. Twenty four (24) hours after the last administration for groups A and B and fourth day after recovery for group C, the animals were anaesthetized using diethyl ether. Using sterile forceps, the animals were dissected and blood collected through cardiac puncture using Norject syringe (10ml). The blood collected was immediately transferred to heparinised bottle to prevent clotting. The blood samples collected were centrifuged at 3000 rpm for 10 minutes to separate plasma and the packed cells. The plasma was kept in a clean specimen bottles placed in ice bucket and stored frozen until they were analyzed for the kidney function tests.

The kidney were surgically removed, rinsed in ice cold 0.85% NaCl (normal saline), blotted dry and weighed. One gram of the liver were homogenized and centrifuged. The supernatant were kept in clean bottles and stored frozen at 4°C for assay.

2.6 Biochemical and Histological Analyses. The protein concentration was determined by Biuret method [18], creatinine concentration was determined by the method of Jaffé [19] and urea concentration was determined by Berthelot’s reaction [19]. The histology of the kidney was studied as described by the method of Drury et al. [20].

2.7 Statistical Analysis. The data were analysed using SPSS version 17.0 and the values were expressed as mean ± SEM (Standard Error of Mean). The means of the groups were compared using one way ANOVA (Analysis of Variance) while the sex differences were compared using Primer of Biostatistics version 3.01 and level of significance was determined using Duncan Multiple Range Test (DMRT) at p<0.05.

3. Results and Discussion

3.1 Effect of Dihydroartemisinin-Piperaquine Phosphate (DHAPP) on Protein, Creatinine and Urea in the Plasma of Experimental and Control Rats. There was a significant increase in plasma protein (at p<0.05) in both sexes of the rats when the drug was administered but the level was reduced during recovery (Table 1). During the recovery process, the values were brought towards normal. Sex related difference was observed only in control group (Table 1) Also, there is a significant increase in the creatinine level in the plasma of both sexes when the drug was administered but was reduced during recovery (Table 1). Moreover, sex differences were observed in the male and female DHAPP treated group. The differences were higher in the male animals than in the females (Table 1). The result showed that there is significant increase in the urea level in the plasma of both male and female animals when the drug was administered which was reduced during recovery. There was also sex related differences in both treated and recovery groups (Table 1).

The result (Table 2) of the treatment on the homogenate from the kidney shows a reduction in the kidney protein, creatinine and urea level of male and female animals. A significant difference was observed in protein level between the males and females animals in the control, when DHAPP was administered as well as when allowed to recover (Table 2). Sex related differences were also noted in the creatinine level of control animals and recovery group which was higher in male than in female. The result also shows that there was sex related differences in the control, DHAPP treated and recovery group in the kidney protein, creatinine and urea level of the animals.
Table 1. Effect of dihydroartemisinin-piperaquinephosphate (DHAPP) on protein, creatinine and urea in the plasma of experimental and control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (g/100ml)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Control</td>
<td>3.66±0.11&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>4.54±0.17&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>0.12±0.01&lt;sup&gt;ax&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHAPP treated</td>
<td>5.34±0.07&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>5.24±0.07&lt;sup&gt;hx&lt;/sup&gt;</td>
<td>0.34±0.01&lt;sup&gt;by&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recovery</td>
<td>4.58±0.09&lt;sup&gt;hx&lt;/sup&gt;</td>
<td>4.74±0.23&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.14±0.02&lt;sup&gt;bx&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean of 5 replicates ± SEM (Standard Error of Mean) and data were subjected to one way analysis of Variance (ANOVA) and level of significance were done using Duncan Multiple Range Test (DMRT) at P < 0.05 of SPSS version 17.0 software. Different superscripts along a column indicate significant difference (a<b<c). Sex differences were determined using Primer of Biostatistics version 3.01. Different superscripts across the row indicate significant difference (x<y).

Table 2. Effect of dihydroartemisinin-piperaquinephosphate (DHAPP) on protein, creatinine and urea in the kidney of experimental and control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (g/100ml)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Control</td>
<td>5.19±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.78±0.08&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>3.70±0.15&lt;sup&gt;xy&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHAPP treated</td>
<td>4.51±0.06&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>4.15±0.05&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.49±0.01&lt;sup&gt;yx&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recovery</td>
<td>5.25±0.08&lt;sup&gt;by&lt;/sup&gt;</td>
<td>4.67±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.39±0.02&lt;sup&gt;by&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean of 5 replicates ± SEM (Standard Error of Mean) and data were subjected to one way analysis of Variance (ANOVA) and level of significance were done using Duncan Multiple Range Test (DMRT) at P < 0.05 of SPSS version 17.0 software. Different superscripts along a column indicate significant difference (a<b<c). Sex differences were determined using Primer of Biostatistics version 3.01. Different superscripts across the row indicate significant difference (x<y).

3.2 Photomicrograph of the Kidney of Rats Treated with Dihydroartemisinin-Piperaquine Phosphate (DHAPP). Histopathological examination revealed that DHAPP may cause progressive and degenerative changes in the kidney. The pathological observation showed degenerative changes in the DHAPP treated group while the control groups appear normal. The recovery groups were also observed to return to normal after the withdrawal of the drug.

Main function of the kidney is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Plate 1 showed the photomicrographs of the kidney of control, test and recovery groups. Histological study of the normal kidney revealed normal glomerulus surrounded by the Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes. The kidney of the tested rats group revealed renal tubular hypertrophy resulting in reduced luminal diameter especially in the male group. The recovery groups showed restoration to the normal renal structural arrangements with re-established tubular lumen.
The above changes in treatment rats were observed to be prominent in the female group when compared to the male.

**PLATE 1.** Showing photomicrographs of the kidney of control, test & recovery groups: a: Female Control, H&E, X100; b: Female Test, H&E, X100; c: Female Recovery, H&E, X100; d: Male Control, H&E, X100; e: Male Test, H&E, X100; f: Male Recovery, H&E, X100. Yellow arrow (renal tubule); Red arrow (Renal corpuscle)

A significant increase in the plasma protein, creatinine and urea level upon the administration of DHAPP was observed and this was reduced to normal when the animals were allowed to recover from the administration of the drug. It was revealed that the concentration of protein, creatinine and urea in the kidney significantly reduced when compared with their plasma concentration.
Creatinine and urea are excreted from the blood by the kidneys when there is abnormality in the body system. Increased level of creatinine in the treated rats as revealed in the present study may be due to post renal obstruction [21]. Damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes the accumulation in blood. Therefore, high level of plasma urea and creatinine will indicate kidney damage. The sex related differences observed could be attributed to physiological, hormonal and genetic differences between male and female animals [22].

The current investigations suggest intoxication of the kidney cells of the rats upon DHAPP administration as revealed by the biochemical and histological findings. The kidney cell damage may have been caused by highly reactive free radicals generated by DHAPP, which is also responsible for antimalarial actions as a result of the oxidative stress caused by DHAPP. The generation of ROS leads to accumulation of lipid peroxides leading to a change in permeability of the cells. The deleterious effects were considered to be caused by free radicals produced during peroxide formation. The findings in this study agree with the work of Olayinka and Ore [3] and Ngokere et al. [23] where DHAPP and artesunate were observed to cause degenerative changes in the kidney.

4. Conclusion

Dihydroartemisinin-Piperaquine Phosphate (DHAPP) as a form of artemisinin has been considered to have high safety margin, however, the results of this study confirmed that the administration of therapeutic dose of DHAPP may not be anaemic but may induce marked renal and hepatic damages.

It is recommended that care should be taken in administering Dihydroartemisinin-Piperaquine Phosphate (DHAPP). Lower concentration of the drug with an increase in the number of days required for the use of the drug should also be considered. It is also recommended that further research work should be done to study the haematological, hepatotoxicity and nephrotoxicity of the drug in malarial infected animal model and patients.

References


