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Dihydroartemisinin Photoirradiation: Reduced Antiplasmodic Activity and Toxicological Implications

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ABSTRACT

Many antimalarial drugs are photoreactive, inducing varied phototoxic reactions. Dihydroartemisinin, the active metabolite of all artemisinins possesses sesquiterpene ring with an endoperoxide bridge which is essential for the antiplasmodial activity, and is a strong site for photochemical reactions. This accelerated photochemical stability study evaluated the physicochemical and biological implications of photo irradiation of dihydroartemisinin. Dihydroartemisinin (0.04% w/v) in aqueous methanol solution (50% v/v) was irradiated for 1 hour using ICH Photostability testing guidelines at 365nm. Photoirradiated samples were analysed using thin layer chromatography (TLC), ultraviolet spectrophotometry (UV) and high performance liquid chromatography (HPLC). Antiplasmodial activity, packed cell volume (PCV), liver enzyme assay and histopathology were also determined. Photo irradiation of the dihydroartemisinin solution gave golden brown colour with formation of needle like crystals and two additional TLC spots. The UV spectra showed a broad band at 229 – 302nm and the presence of four new photodegradation products in the HPLC chromatogram. Significant reduction in body weights ($p=0.0001$), PCV ($p=0.0002$) and antiplasmodial properties were observed with the irradiated dihydroartemisinin samples. Aspartate aminotransferase and alanine aminotransferase levels of the mice were significantly increased. Photo irradiated samples showed various stages of hepatotoxicity; massive hepatic degeneration and necrosis of the liver with hyperplasia and hemosiderin laden kupffer cells as well as multifocal lymphocytic infiltration. This study confirms that dihydroartemisinin has the potential of undergoing photodegradation with reduction in antiplasmodial activity and possible hepatotoxicity.

Keywords: Dihydroartemisinin, Photodegradation, Antiplasmodial activity, Hepatotoxicity

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INTRODUCTION

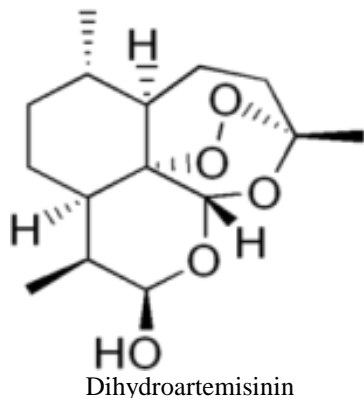
Most pharmaceutical products being complex organic molecules may react photochemically when exposed to sunlight or artificial ultraviolet (UV) light and get degraded in the process. Much care is therefore often required in ensuring the stability of light sensitive pharmaceuticals, which are liable to react photochemically (Greenhil and McLelland, 1990). The implication of photochemical degradation of drug compounds is diverse and may include reduction or loss of activity which will result in their not being effective at the required dose or formation of toxic products (Miranda *et al*, 1999). Drug compounds that have been reported to be photoreactive include various types of antibiotics especially quinolone antibiotics (ofloxacin, ciprofloxacin)(Matsumoto *et al*, 1992;Stahlmann and

Lode, 1999) and non-steroidal anti-inflammatory (diclofenac, ketoprofen, caprofen) sometimes with phototoxic effects(Quintero and Miranda, 2000; Vargas *et al* 1996; Tonnesen, 2004).

Almost all drugs previously used in the treatment of malaria have been reported to be photoreactive, examples of such antimalarials with photosensitization reactions are chloroquine, amodiaquine, mefloquine (Tonnesen, 2004; Oke, 1999; Motton *et al*, 1999). Currently, malaria drugs are plagued with varied problems of which multi drug resistance plays a key role. This has led to the introduction of artemisinin and its derivatives obtained from Chinese herb *Artemisia annua* (Meschnick *et al*, 1996). Some of the derivatives that have been reported to be more effective in the treatment of falciparum malaria, which is the most lethal form of the disease are artemether, arteether, artesunate, dihydroartemisinin (DHA) (Jansen, 2010; Davis *et al*,

2005). The World Health Organisation has recommended that artemisinin combination therapies (ACT) i.e. their combination with other antimalarial drugs should be first-line therapy for treatment of *Plasmodium falciparum* malaria (Olliaro and Taylor, 2003).

Artemisinin and its derivatives lack the nitrogen containing ring which is common in other class of antimalarial drugs, while possessing a basic sesquiterpene ring with an endoperoxide bridge which is essential for their antiparasmodial activity (Olaniyi *et al*, 2000).



Dihydroartemisinin, an epoxy pyrano benzodioxepinol compound is the active metabolite of all artemisinin compounds which is also available as a semi synthetic derivative of artemisinin. It is widely used as intermediate in the preparation of the other artemisinin derived anti-malaria drugs as well as a drug formulation itself. Oral dihydroartemisinin combination preparation has been shown to be effective in the treatment of multidrug-resistant uncomplicated *Plasmodium falciparum* (Tjitra *et al*, 2012).

Photochemical degradation potential of drug compounds have been related to some functional groups such as peroxides, nitro aromatic group, N-oxide function which generates highly reactive singlet oxygen (Olaniyi *et al*, 2000). The endoperoxide bridge of artemisinin derivatives is a possible susceptible point for photo chemical reaction.

A preliminary study on artesunate, another artemisinin derivative reported photochemical degradation with loss of antiparasmodial activity after ultraviolet irradiation (Badmus, 2011). Hence, there is need to evaluate the photodegradation potential of dihydroartemisinin, which is the active metabolite of this class of drugs and is currently within first line treatment of malaria by World Health Organization (WHO). This study aims to evaluate the biological implications of photochemical irradiation of dihydroartemisinin.

MATERIALS AND METHODS

Identification and assay of dihydroartemisinin powder (DHA) (I.P., 2015)

The purity and chemical content of the pure dihydroartemisinin was assessed by determining the melting point (Stuart melting point apparatus), chemical identification test, thin layer chromatography (TLC) and chemical assay.

Photochemical irradiation of dihydroartemisinin

- Dihydroartemisinin (DHA) solution (0.04% w/v) in aqueous methanol solution (50% v/v) was irradiated with near UV fluorescent lamp for 1 hour (ICH Photostability testing guidelines, 1996). The irradiated solution was concentrated to 30ml using rotary evaporator at 40°C after which it was evaporated to dryness under nitrogen gas. The obtained dry irradiated sample was labelled as direct irradiated DHA (DPD).
- The irradiation was repeated with another DHA solution (0.04% w/v) in aqueous methanol solution (50% v/v). The irradiated solution was extracted with diethyl ether (3 x 100ml), which was concentrated to 30ml using rotary evaporator at 40°C after which it was evaporated to dryness under nitrogen gas. The obtained dry irradiated sample was labelled as ether extract of DPD (EPD).

Physicochemical analysis of irradiated DHA solutions:

All the photochemical irradiation reactions were monitored for degradation by observing colour change, ultraviolet-visible spectrophotometry (Helios Ganama), while analytical thin layer chromatography (TLC) was on silica gel GF₂₅₄ using ethylacetate: methanol: ammonia (6.9 : 1.73: 1.3) (M₁), acetone: ethylacetate: toluene: glacial acetic acid (0.9: 4.48:4.48: 0.02) (M₂), toluene: ethylacetate (6:4) (M₃) as mobile phases and methanol-sulphuric acid solution followed by activation on hot plate for visualisation. The samples were analysed using isocratic high pressure liquid chromatography (HPLC) on reversed phase RP C₁₈ at a flow rate of 1.0ml/minute, acetonitrile: water (6:4) as mobile phase and UV detection at 216nm.

Biological evaluation

Animals and parasite: Thirty five healthy male Wistar albino mice weighing 22.14 ± 2.7 g (20 - 24g) obtained from the animal house of Institute of Advanced Medical Research and Advanced training (IAMRAT), University of Ibadan, Ibadan were used for the study. The mice kept at ambient temperature and humidity with 12 h light and

dark cycles, were allowed to acclimatize for one week. The animal study was done in accordance with the National Institute of Health Guidelines for Care of Laboratory animals of 1985. They were fed with pellets (Ladokun feeds) and water *ad libitum*. The mice were randomly distributed into seven groups (n=5) according to the research design. *Plasmodium berghei*, chloroquine sensitive ANKA strain (NK-65 strain) used for the study was a kind donation from Prof. O. G. Ademowo of the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan and maintained by passage in mice.

Research design

The following research groups were used for the study;

- PDD - parasitized animals administered unirradiated DHA
- EPD - parasitized animals administered ether extract of photolysed DHA
- DPD - parasitized animals administered dried aqueous photolysed DHA
- DW - parasitized animals administered water
- DMSO - parasitized animals administered dimethyl sulfoxide
- DWH – healthy animals administered water only
- DMSOH - healthy animals administered dimethyl sulfoxide only

In vivo plasmodial studies (Rane's test) (Ryley and Peters, 1970)

Inoculum preparation and parasitaemia load determination: Standard inoculum was prepared from a donor mouse with Chloroquine-sensitive NK-65 strain of *P. berghei* parasitized erythrocytes. Infected blood from the donor mouse was obtained by cardiac puncture after anaesthesia with chloroform. Microscopic examination of the thin blood film was used to establish parasitaemia.

Induction of parasitaemia: The experimental parasitized mice were inoculated intraperitoneal with 0.2ml of infected blood containing a standard inoculum of 10^6 parasitized erythrocyte suspensions in normal saline (0.2ml) from the donor mouse. They were left for 72 hours after which the parasitaemia level were determined by collecting blood from the caudal vein and a smear made on a clean slide, fixed in methanol, allowed to dry and then stained with Giemsa stain and the parasitaemia level determined.

Drug administration: Solutions of the pure unirradiated DHA (PDD) and photoirradiated DHA samples (EPD and DPD) used for this experiment were prepared in

aqueous dimethylsulfoxide (DMSO) and administered orally using oral canula at a dose of 1.2mg/kg on day 0 and 0.6mg/kg for the next 4 days.

Evaluation of the antiplasmodial activity: Tail snips was used to prepare thin blood films on days 0 (after establishment of parasitaemia, before drug administration), 1, 2, 3, 4 and 5 after drug administration. The parasitaemia level was determined from thin blood films fixed in methanol, and stained with Giemsa stain. Percentage reduction in parasitaemia was calculated using the equation below;

$$\% \text{ Reduction in Parasitaemia} = \frac{\text{Initial Load} - \text{Load at Time } T}{\text{Initial Load}} \times \frac{100}{1}$$

Determination of body weight

The body weights of the experimental animals were determined on days 0, 2, 4, and 10 of treatment.

Packed cell volume (PCV) determination

Packed cell volume (PCV) (%) of the mice were determined on days 0, 5 and 10 of drug administration using blood collected from the caudal vein into heparinised micropipettes and centrifuged at 2500 r.p.m for fifteen minutes using microhaematocrit centrifuge.

Liver Enzyme Assessment

The sera obtained from blood (3 mL) collected into labelled non-heparinised bottles from ocular vein of the mice on day 10 were analysed for two liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using an auto analyser (Reflotron-plus machine, Roche Germany).

Histopathological evaluation

The animals were sacrificed on day 10 and kidney, liver and heart were harvested. Histopathological examination using conventional methods was carried out at Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan.

Statistical analysis

Data were expressed as mean \pm S.E.M. and subjected to statistical analysis using Student t-test and ANOVA with the Duncan's Multiple Range test used for the post test where appropriate. Differences were considered significant at $p < 0.05$

RESULTS

The pure DHA used for the study conformed to the official specification for identification and chemical

content of 98.3% w/w. Physicochemical analysis of photoirradiated DHA confirmed photodegradation with golden brown colour and formation of needle like crystals respectively for EPD and DPD after concentration. The UV spectra of the photoirradiated DHA showed loss of the main λ_{max} at 192nm observed in the unirradiated DHA, while varied λ_{max} were

observed between 220 and 400nm which appeared as a very broad band (Fig. 1). HPLC chromatogram obtained for the photoirradiated samples showed the presence of four new photodegradation products at retention times 1.58, 2.37, 3.13 and 3.34minutes observed, compared to the retention time of 3.52 and 5.2 obtained with pure DHA (Fig. 2).

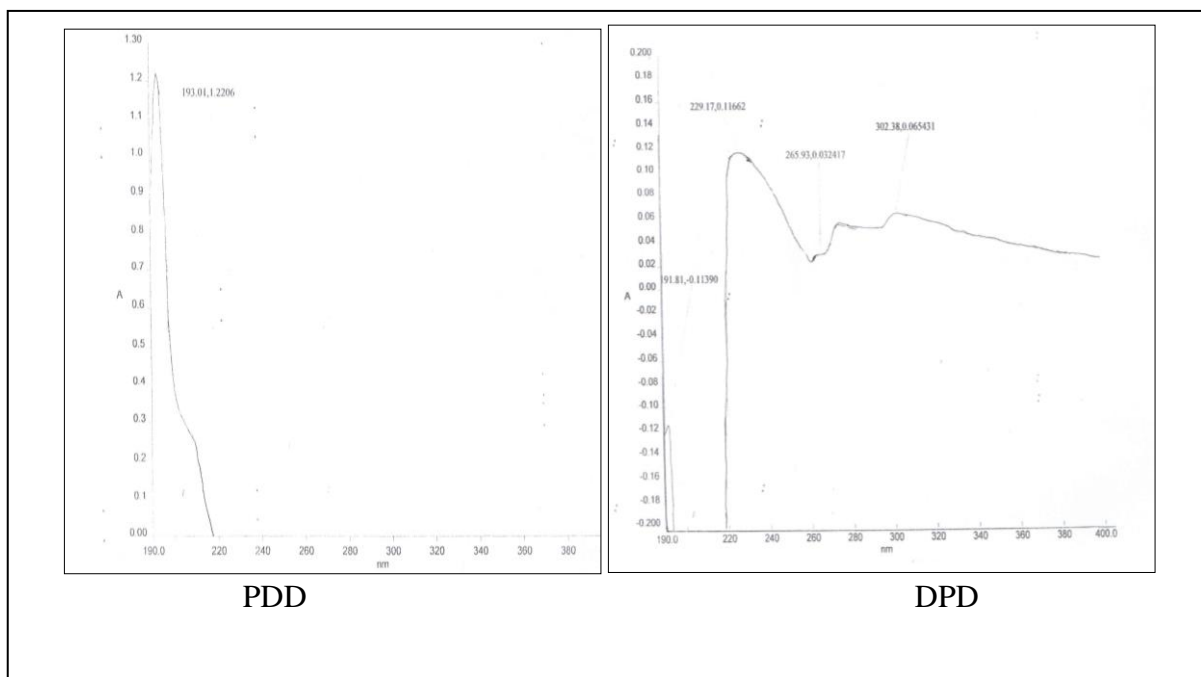


Figure 1: UV spectra of the unirradiated (PDD) and irradiated Dihydroartemisinin (DPD) showing the effect of photodegradation.

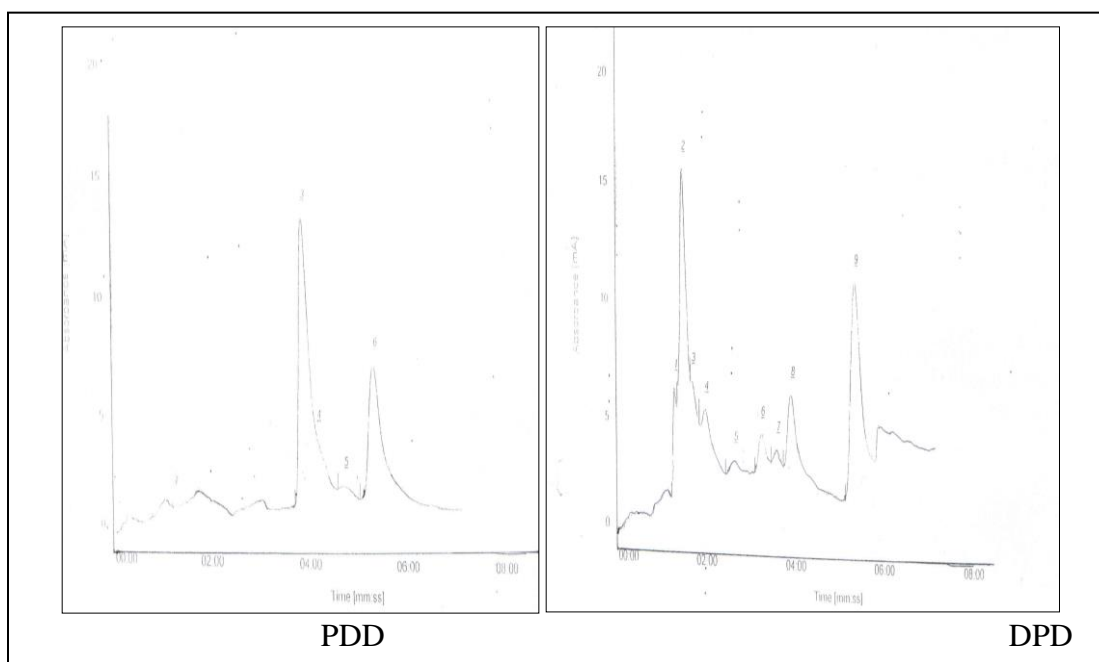


Figure 2: HPLC chromatogram of the unirradiated (PDD) and irradiated Dihydroartemisinin (DPD) showing the effect of photodegradation

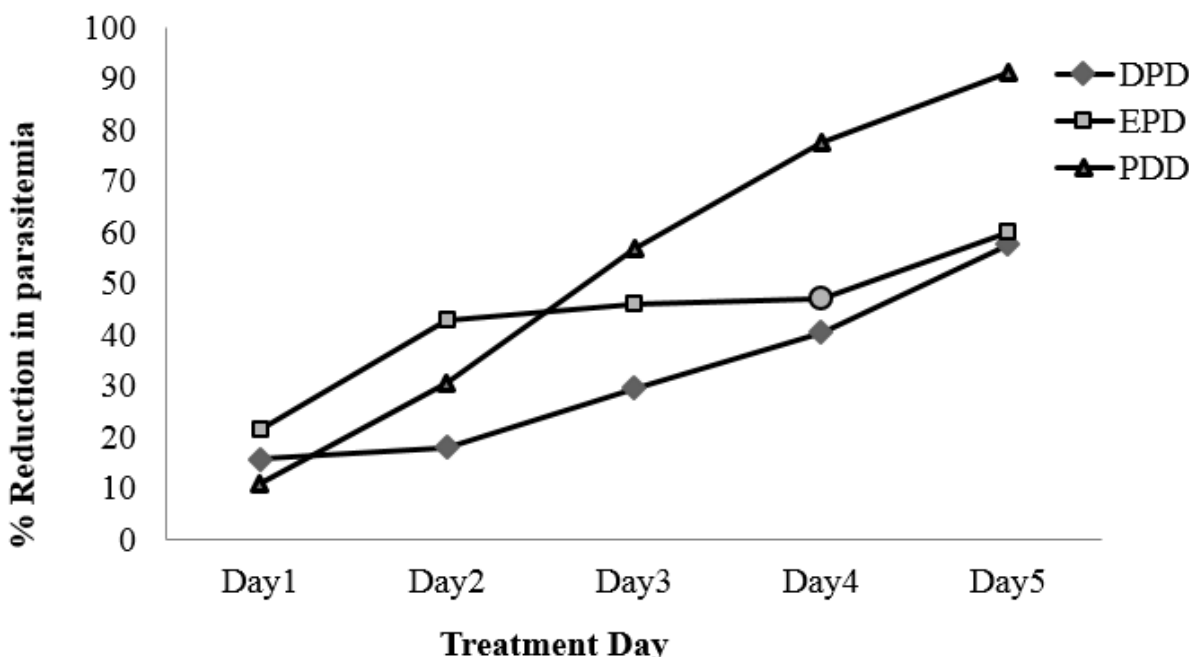


Figure 3: Percentage (%) reduction in parasitaemia of parasitized mice at different days after treatment with the irradiated and unirradiated aqueous solutions of dihydroartemisinin
 [Code: DPD: Photolysed DHA; EPD: Ether extract of photolysed DHA; PDD: unirradiated dihydroartemisinin]

Table 1: R_f values of the photo irradiated DHA samples in the three mobile phases

Group	DPD	EPD	PDD
M ₁	0.59	0.59	0.59
	0.66	0.67	
	0.72	0.73	
M ₂	0.36	0.36	0.36
	0.40	-	
	0.48	-	
	0.63	-	
	0.73	0.73	
M ₃	0.78	0.78	0.72
	0.72	0.72	
	-	-	
	0.84	0.84	

The TLC of EPD and DPD samples were similar with two prominent new spots in all the mobile phases used (Table 1).

The biological evaluation of the photoirradiated samples revealed a significant reduction in antiplasmodial properties for all the irradiated DHA samples ($p = 0.0002$) against *Plasmodium berghei* by day 5 of the drug administration, when compared with the unirradiated DHA (PDD), however, there was no significant difference in the reduced antiplasmodial

activities of the two irradiated samples (DPD and EPD) (Fig. 3).

Similarly, a significant decrease in body weights ($p = 0.0001$) and PCV ($p = 0.0002$) was observed in all the treatment groups including the unirradiated DHA ($p = 0.0002$) (Fig. 4 and Fig. 5).

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of the mice were significantly increased with the irradiated samples (DPD and EPD), when compared with PDD group (unirradiated DHA) (Table 2).

Table 2: Effect of photo irradiated DHA on the liver enzymes (AST and ALT) of *Plasmodium berghei* infected mice

Group	AST Activities (U/L ± SEM)	ALT Activities (U/L ± SEM)
DPD	336.7±37.95	45.0±11.56
EPD	358.0±34.0	49.0±28.0
PDD	155.0±50.5	27.5±9.50
DMSO	389.5±25.0	28.0±15.0
DW	372.0±0.00	27.0±0.00
DMSOH	164.5± 5.50	37.5± 5.00
DWH	150.3±14.71	36.0± 2.65

Histopathological evaluation of the heart and kidneys showed normal morphology for irradiated and PDD unirradiated samples. However, the liver of the irradiated samples showed various stages of hepatotoxicity; DPD group showed massive

hepatic degeneration and necrosis with hyperplastic hemosiderin laden kupffer cells (Fig.6), while the EPD group had multifocal lymphocytic infiltration of liver with parasites not cleared (Fig.7).

DISCUSSION

Pharmaceutical products are capable of undergoing photodegradation because they are complex organic

compounds, and this could result in reduction or loss of therapeutic activity or formation of toxic compounds. Many antimalarials have been reported to be photoreactive with photo-induced dermatological problems and compromise of therapeutic activity including amodiaquine, chloroquine, mefloquine, primaquine, sulfadoxine, pyrimethamine (Tonessen, 2004; Motton *et al*, 1999; Meshnick *et al*, 1996; Jansen, 2010).

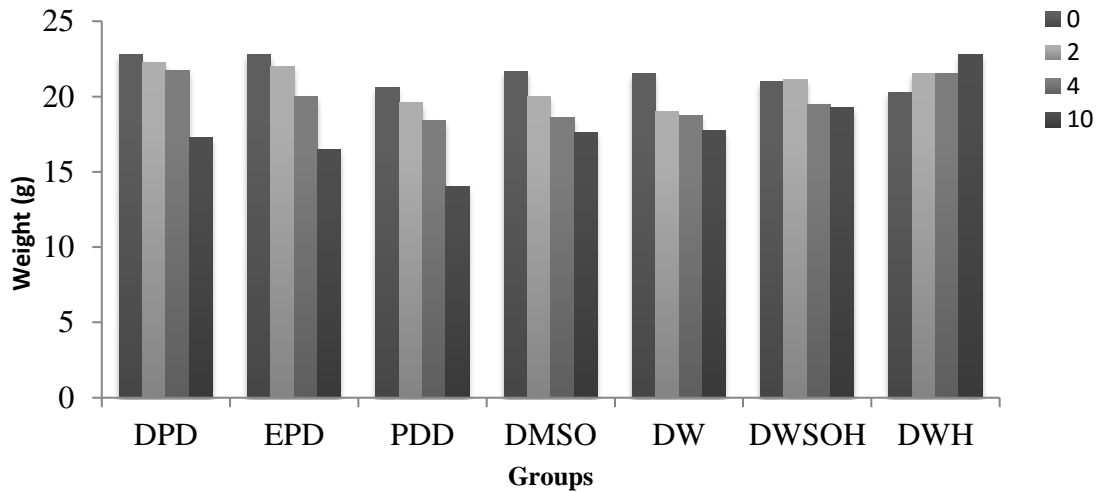


Figure 4: Weights (g) of the mice at different days after treatment with the irradiated and unirradiated aqueous solutions of dihydroartemisinin [Code: DPD: Photolysed DHA; EPD: Ether extract of photolysed DHA; PDD: Unirradiated DHA; DMSO: Dimethylsulfoxide on parasitized mice; DWP: distilled water on parasitized mice; DMSOH: Dimethylsulfoxide on healthy mice; DWH: distilled water on healthy mice]

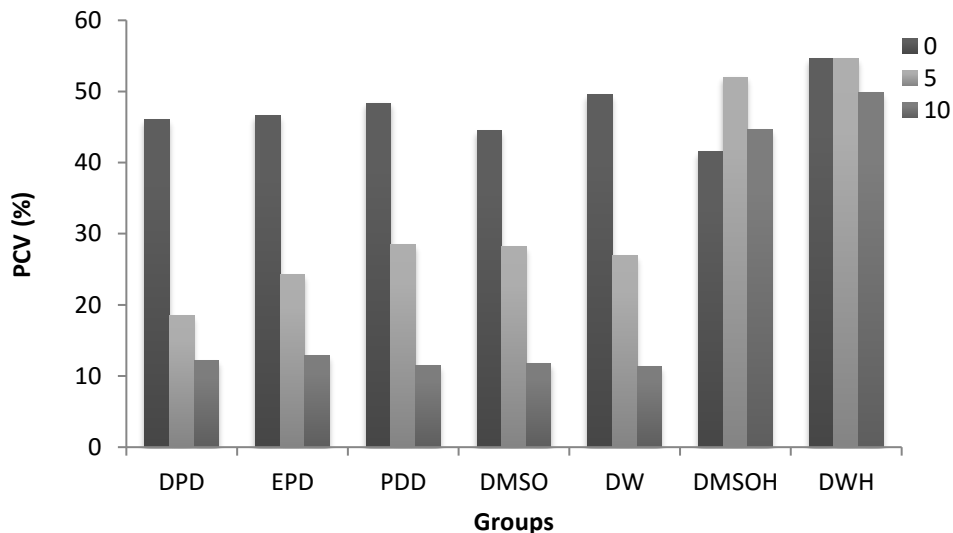


Figure 5: Packed cell volume (PCV) (%) of mice at different days after treatment with the irradiated and unirradiated aqueous solutions of dihydroartemisinin [Code: DPD: Photolysed DHA; EPD: Ether extract of photolysed DHA; PDD: Unirradiated DHA; DMSOP: Dimethylsulfoxide on parasitized mice; DWP: distilled water on parasitized mice; DMSOH: Dimethylsulfoxide on healthy mice; DWH: distilled water on healthy mice]

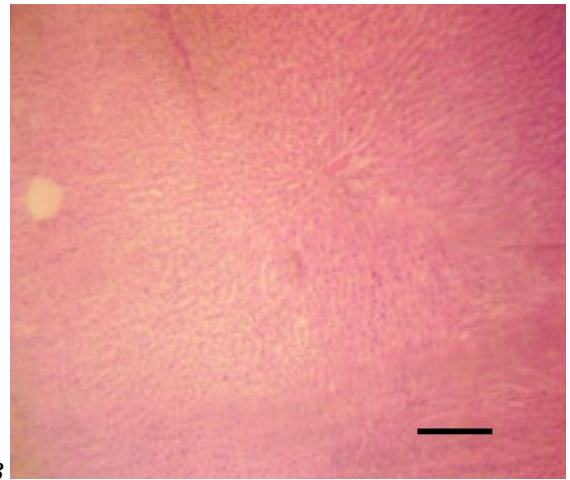
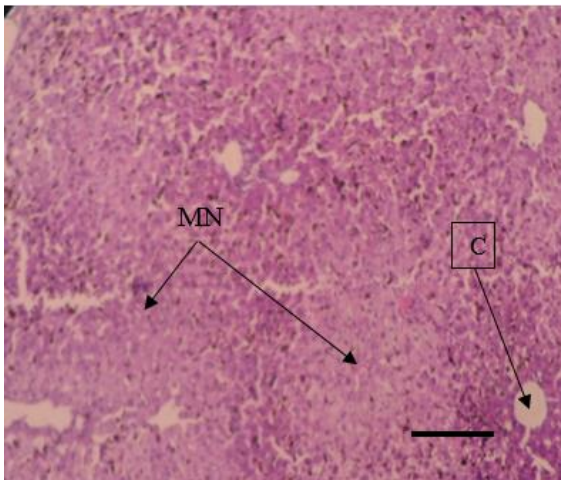


Plate 1:

Photomicrograph section of (a) liver from parasitized groups treated with DPD showing generalised multifocal necrosis (MN) and hemosiderin laden Kupffer cells (with arrows) and (b) Negative Control. H&E (Scale bar: 75 μ m) (MN: Multifocal Necrosis, C: Central vein)

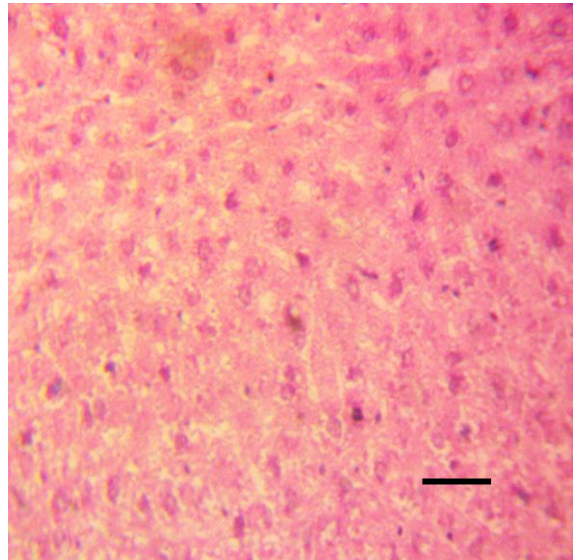
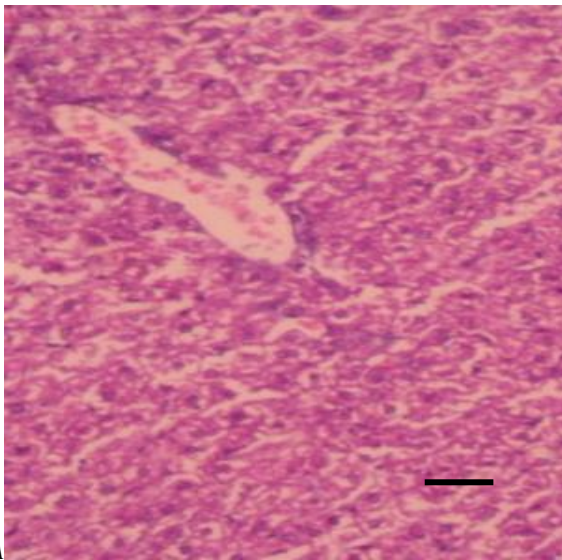


Plate 2:

Photomicrograph section of (a) liver treated with EPD showing massive hepatic degeneration and necrosis (rectangle) and (b) Negative Control. H&E (Scale bar: 1cm=50 μ m)

Dihydroartemisinin, the active metabolite of artemisinin derivatives has an endoperoxide bridge which is responsible for its therapeutic activity and a point for possible photodegradation. In view of the increasing number of reports on the phototoxic effects induced by new pharmaceuticals and the photosensitization potential of antimalarials such as chloroquine, amodiaquine, this study was carried out to evaluate the possible clinical implications of photo irradiation of DHA; its antiplasmodial activity and toxicity using animal model. The quality of the pure dihydroartemisinin was ascertained by its compliance with the official specifications.

The photoirradiated dihydroartemisinin samples changed colour from the clear transparent solution to pale yellow solution which turned brown with needle

like crystals. This indicates that the sample **was** not stable to the photo irradiation at 365nm. The UV analysis of the photoirradiated dihydroartemisinin were similar with the loss of the main λ_{max} at 192nm observed in the unirradiated DHA, while varied λ_{max} were observed between 220 and 400nm in form of a broad band (Fig. 1). The TLC evaluation of the irradiated DHA confirms the photo reactivity; all the mobile phases showed the presence of photodegradation products of which two were common to two irradiated products, while the unirradiated DHA gave only one spot. This was confirmed with the HPLC chromatogram obtained for the photo irradiated samples with the presence of four new photodegradation products, compared to the retention times of 3.52 and 5.2 obtained with pure DHA (Fig. 2). These confirms that DHA is photolabile with

the evidence of photodegradation products observed in the TLC and HPLC and the broad UV band between 220nm and 400nm where DHA does not normally have absorbance. Previous studies have reported such photodegradation reaction with many antimalarials including chloroquine, amodiaquine, halofantrine and even artesunate (Tonnesen, 2004; Motton et al, 1999).

However, there is no such report for DHA and other artemisinin derivatives. The unirradiated DHA gave two different retention times on HPLC which confirms the presence of α and β forms of DHA as earlier reported (Ilaria et al, 2010). The isomeric forms have been adduced to on-column epimerization of DHA, hence the reason why it was not evident on the TLC system used in this study.

Biological evaluation of the photoirradiated samples using animal model showed a significant reduction in the body weights of the parasitized mice for all treatments including the negative control group i.e. untreated groups (DW and DMSO) which is due to the infection (*Plasmodium berghei* parasite) ($p < 0.05$). This was confirmed with the non-significant effect on the weight observed in healthy mice administered two vehicles (DWH and DMSOH) (Fig. 2). The antiplasmodial activity of the irradiated samples showed a significant reduction; no significant difference was observed between the irradiated samples which were significantly lower than what was obtained with the unirradiated DHA ($p < 0.05$) (Fig. 1). Furthermore, a significant decrease in PCV was observed for all infected groups (Fig. 3), this is in agreement with previous antimalarial studies such as artesunate (Adegbolagun et al, 2014) where a reduction in PCV was observed in the presence of the malaria parasite.

Toxicological evaluation of the treated samples using the liver enzyme assay showed a significant increase in the AST and ALT levels in all the groups treated with the irradiated DHA when compared with the unirradiated DHA (Table 1). Similar increase in AST levels was observed with negative control groups (DW and DMSO), this can be attributed to the parasite (Table 1) as the non-parasitized groups were not so affected, however, the ALT levels of DW and DMSO groups were not so affected. Liver enzyme elevation has been associated with the plasmodium infections (Enemchukwu et al, 2014). This shows that the antiplasmodial activity of the unirradiated DHA protected against the elevation of the AST and ALT of the parasitized mice. The obtained result in this study further confirms earlier reports that DHA does not have any effect on the AST and ALT levels (Utoh-Nedosa et al, 2009). This result thus suggests a compromise of the antiplasmodial effect as well as the possible toxicity of

the irradiated drug samples. The elevated AST level with negative control groups is corroborated by an earlier report that since the liver is involved in the pathophysiology of malaria, it is usually accompanied by elevation of AST level (Maduka et al, 2008).

Histopathological evaluation of organs such as heart and kidney revealed that there was no significant effect on these organs as their morphologies were normal in the irradiated samples which were comparable to those of the unirradiated DHA. This indicates that DHA and its irradiated forms may not be cardiotoxic or nephrotoxic. However, the infected mice treated with the irradiated DHA (DPD and EPD) showed generalized multifocal necrosis of the liver with the presence of hyperplastic and hemosiderin laden kupffer cells (Fig. 6). Also, the EPD group showed multifocal lymphocytic infiltration of liver with non-clearance of the parasite (Fig. 7). These indicate the possibility of serious hepatotoxicity of the photo irradiated samples. Similarly, the unirradiated DHA was observed to induce massive hepatic degeneration and necrosis of the liver in the parasitized group, which is an indication of possible hepatotoxicity. This may corroborate an earlier report of the deleterious effect of DHA on the liver in the healthy state (Obianime and Aprioku, 2011).

This accelerated photochemical stability study showed that dihydroartemisinin (DHA) has the potential of undergoing photochemical degradation resulting in a reduction in antiplasmodial activity which is accompanied by phototoxic effects such as hepatotoxicity. This study shows the need for caution in the widespread use of dihydroartemisinin, especially in the tropics where there is abundant sunlight and high prevalence of malaria.

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REFERENCES

- Adegbolagun O. M., Emikpe B. O., Woranola I. O., Ogunremi Y. (2014). Synergistic effect of aqueous extract of *Telfaria occidentalis* on the biological activities of artesunate in *Plasmodium berghei* infected mice. African Health Sciences 14(1): 111-118.
- Badmus O. K, (2011): Photochemical degradation of artesunate and its chemical implication. Master of Science Project. Department of Pharmaceutical Chemistry, University of Ibadan, Nigeria.

- Davis T. M. E., Karunajeewa H. A., Illet K. F. (2005):** Artemisinin-based combination therapies for uncomplicated malaria. *Medical Journal of Australia* 182 (4): 181-185
- Enemchukwu B. N., Ibe C. C., Udedi S. C., Iroha A., Ubaoji K. I., Ogundapo S. S. (2014):** Liver assessment in malaria, typhoid and malaria-typhoid co-infection in Aba, Abia State, Nigeria. *Pakistan Journal of Biological Science* 17(6): 860-863.
- Greenhill J. W., McLelland M. A. (1990):** Photodecomposition of drugs In: *Progress in Medicinal Chemistry*. 27. Edited by P Ellis and GB West. Elsevier Science Publication, Amsterdam.
- Iaria D'Acquarica I., Gasparrini F., Kotoni D., Pierini M., Villani C., Cabri W., Di Mattia M., Giorgi F. (2010):** Stereodynamic investigation of labile stereogenic centres in dihydroartemisinin. *Molecules*. 15: 1309-1323.
- ICH Guidelines (1996):** International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Topic Q1B – Photostability testing of new active substances and medicinal products - CPMP/ICH/279/95.
- Jansen F. H.(2010):** The pharmaceutical death-ride of dihydroartemisinin. *Malaria Journal* 9: 212.
- Maduka I. C., Neboh E. E., Eyisi B. N. (2008):** Effect of Malaria Parasitaemia on liver Enzyme Tests. *International Journal of Tropical Medicine*. 3(3):49-52.
- Matsumoto M., Kojima K., Nagano H., Matsubara S., Yokota T. (1992):** Photostability and biological activity of fluoroquinolones substituted at the 8 Position after UV irradiation. *Antimicrobial Agents and Chemotherapy*. 36(8): 1715-1719.
- Meshnick S.R., Taylor T. E., Kamchonwongpaisan S. (1996):** Artemisinin and the antimalarial endoperoxide: From herbal remedy to target chemotherapy. *Microbiology Review*. 60: 301-315.
- Miranda M. A., Perez-Prieto J., Lahoz A., Morera I. M. (1999):** Photosensitization by drugs. *European Journal of Organic Chemistry*. 497-502.
- Motton A. G., Martinez L. J., Holt N., Sik R. H., Reszka K., Chignell C. F., Tønnesen H. H., Roberts J. (1999):** Photophysical studies of antimalarial drugs, *Photochemistry Photobiology*. 69: 282–287.
- Obianime A.W., Aprioku J. S. (2011):** Mechanism of action of artemisinins on biochemical, haematological and reproductive parameters in male guinea pigs. *International Journal of Pharmacology*. 7(1): 84 – 95.
- Oke J. M. (1999):** Comparative photostability study of Fancimef Tablets and its active ingredient. *African Journal of Biomedical Research*. 2(1): 35-39.
- Olaniyi A. A., Ayim J.S.K., Ogundanni A.O., Olugbade T.A. (2010):** Essential inorganic and organic pharmaceutical chemistry. Shaneson C.I. Ltd. Ibadan. pp 489-512.
- Olliaro P., Taylor W.R.J. (2003):** Antimalarial compounds: from bench to bedside. *The Journal of Experimental Biology*. 206: 3753-3759.
- Quintero B., Miranda M.A. (2000):** Mechanisms of photosensitization induced by drugs: A general survey. *Ars Pharmaceutica*. 41(1):27-46.
- Ryley J. F., Peters W. (1970):** The antimalarial activity of some quinoline esters. *American Journal of Tropical Parasitology* 84: 209–222.
- Stahlmann R., Lode H. (1999):** Toxicity of quinolones. *Drugs*. 58: 37-42
- The International Pharmacopoeia (2015):** World Health Organization Press, Geneva, Switzerland. 5th Edition.
- Tjitra E., Hasugian A.R., Siswantorol H., Prasetyorini B., Ekowatiningsih R., Yusnita E.A., Purnamasari T., Driyah S., Salwati E., Nurhayati Y.E., Januar E., Labora J., Wijayanto B., Amansyah F., Dedang T.A.D., Purnama A.T. (2012):** Efficacy and safety of artemisinin-naphthoquine versus dihydroartemisinin-piperazine in adult patients with uncomplicated malaria: a multi-centre study in Indonesia. *Malaria Journal*. 11:153
- Tønnesen H.H. (2004):** Photostability of Drugs and Drug Formulations. CRC Press, Washington DC, USA. 2nd Ed. Pp. 213 – 234.
- Utoh-Nedosa A.U., Akah P.A., Okoye T.C., Okoli C.O. (2009):** Evaluation of the Toxic Effects of Dihydroartemisinin on the Vital Organs of Wistar Albino Rats. *American Journal of Pharmacology & Toxicology*. 4(4): 169-173.
- Vargas F., Rivas C., Canudas N., Torrealba A., Sarabia Z. (1996):** Mechanism of phototoxicity induced by drugs. *Acta Cient. Venezuela*. 47; 223-230