

## Extra-Bone Marrow Sites of Haemopoiesis: Dihydroartemisinin Effects in Wistar Albino Rats

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**Abstract: Problem statement:** Iron is present in ferritin, the storage form of iron in the tissues; in the respiratory cytochrome enzymes; in hemoglobin of the blood and in the myoglobin of muscle. Dihydroartemisinin (DHA) has been shown to interact with heme groups *in vivo* and *in vitro*. This study investigated the effects of 5 day and 7 day oral dihydroartemisinin treatments on the blood and tissues of the lungs, the heart, the liver, the intestines, the spleen and the kidney of Wistar albino rats. The dosage regimens of dihydroartemisinin employed in the study were: A single dosage regimen of 1 mg kg<sup>-1</sup>; a repeated dosage regimen of 1; 2; 60 and 80 mg kg<sup>-1</sup>. **Approach:** The results of the study showed that dihydroartemisinin interacted with the hemoglobin of the blood and the myoglobin of muscle to stimulate new haemopoiesis in a concentration, repetition and time dependent manner in the tissues of the lungs, liver, spleen, intestine, heart and kidney of Wistar albino rats which was absent in the control rats. **Results:** Statistically significant increases were observed in the Packed Cell Volume (PCV) (P<0.01-0.03) and the total White blood cell count (P<0.01) of the DHA-treated rats but not in the control rats. It also stimulated hyperplasia of erythrocyte and leucocyte stem cells in the lungs, liver, intestine, spleen, heart and kidney of DHA-treated Wistar albino rats not seen in the controls. **Conclusion:** These haemopoetic effects of DHA were greater and of longer duration in 5 day DHA-treatment rats than in those of the 7 day DHA-treatment rats.

**Key words:** Dihydroartemisinin (DHA), bone marrow, wistar albino rats, haemopoetic effects, dosage regimen, blood cells, Packed Cell Volume (PCV), Blood Cell Count (WBC)

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### INTRODUCTION

The activity of artemisinin and synthetic endo-peroxides is related to their interaction with heme. And the potentiating of artemisinin *in vitro* and *in*

*in vivo* by natural and synthetic heme models has been reported.

Many studies have shown that artemisinin and dihydroartemisinin were active against many cell lines *in vitro*.

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Artemisinin-tagged transferrin was found to be highly selective and potent in killing cancer cells. Cancer cells including drug-resistant cancer cells are more susceptible to artemisinin drugs under conditions of high iron availability. Haem was demonstrated to cause a 0.6 V shift in the reduction potential of artemisinin. Artemisinin forms adducts with haem which retain the haem structure and lose the artemisinin structure. *In vitro* data suggest that artemisinin-haem adducts don't have much antimalarial activity.

Our study investigated the effect of dihydroartemisinin on the blood and six organs of Wistar albino rats.

### MATERIALS AND METHODS

Five rats which weighed 104-106 grams and 75-90 grams were evaluated for the effects of four oral dosage regimens and a repeated dosage regimen of Dihydroartemisinin (DHA). Four rats of equivalent weights as the test rats were given orally administered distilled water to serve as controls in each experiment.

The tested doses of DHA were 1 mg kg<sup>-1</sup> DHA; a repeated dosage regimen of 1; 2; 60 and 80 mg kg<sup>-1</sup> DHA.

The rats which weighed 75-90 grams received 1 mg kg<sup>-1</sup> rat weight of DHA for 5 or 7 days, rested for one week and received the same dosage regimen again for 5 days or 7 days. The rats which weighed 104-106 gms received the 1, 2, 60 or the 80 mg kg<sup>-1</sup> rat weight of DHA regimen for 5 or 7 days.

Blood samples from the test and control rats were collected from the subclavian artery 24 h after the administration of the last dose of each dosage regimen of DHA tested into blood collection bottles which contained EDTA anti-coagulant. The heart; liver; lungs; intestine (strip); spleen; and kidney of the sacrificed test and control rats were collected after gross anatomical observations of the organs *in situ*.

Photomicrographs of the tissues of these organs were prepared using conventional methods. The Packed Cell Volume (PCV) and the total white blood cell count of the blood of the test and control rats were determined using conventional haematological analytical methods.

### RESULTS

Gross anatomical examination of the dihydroartemisinin-treated and control rats showed that there was blood congestion (increased population of the blood cells) in the lungs, liver, heart, spleen and kidney of DHA-treated rats but not in the control rats (Fig. 1) which is an evidence of new haemopoiesis in the DHA-treated rats.

Comparison of the haematological data on the blood of the DHA-treated rats with those of the controls showed that dihydroartemisinin treatment produced statistically significant ( $P < 0.01-0.03$ ) increases in the packed cell volume (PCV) of the DHA-treated rats which was absent in the controls. Dihydroartemisinin treatment also produced large significant increases in the white Blood Cell Count (WBC) ( $P < 0.01$ ) of the DHA-treated rats.

The PCV of the blood of rats treated for 5 days with DHA ranged from 45-48% while those of rats treated for 7 days with DHA ranged from 30-39% against a normal of 42%. The red blood cells from 5 day DHA-treated rats were normochromic and normocytic; had polychromasia and contained many reticulocytes. Some red blood cells of the blood from 7 day DHA-treated rats were normocytic and normochromic while the rest were mildly hypochromic and normocytic.

The WBC counts of the 5-day DHA treatment rats ranged from 10500-13500 mm<sup>3</sup> while those of the 7-day treatments ranged from 3250-7050 mm<sup>3</sup> (against a WBC count of 4800-5200 mm<sup>3</sup> of the controls).



Fig. 1: Dark-red engorgement of the heart, lungs, liver, intestine, spleen and kidney of DHA-treated rat (a) absent in the control rat (b) under the influence of oral DHA illustrates these organs as haemopoietic sites in the body.

Dose, repetition and time dependent hyperplasia of dark-red stained clustered cells in muscle of DHA tested organs which were absent in the controls were interpreted as histological evidence of new haemopoetic activity in the lungs, liver, heart, intestine, spleen and the kidney (Fig. 2-7). The 2 mg kg<sup>-1</sup> dose of DHA produced the maximal response

for both the blood and tissue haemopoetic effects of DHA.

These evidences of new haemopoiesis seen in the tissue micrographs of the lungs, heart, intestine, liver, spleen and kidney of the DHA-treated rats but not in those of control rats were deduced as indications that these six organs are extra bone marrow sites of haemopoiesis.

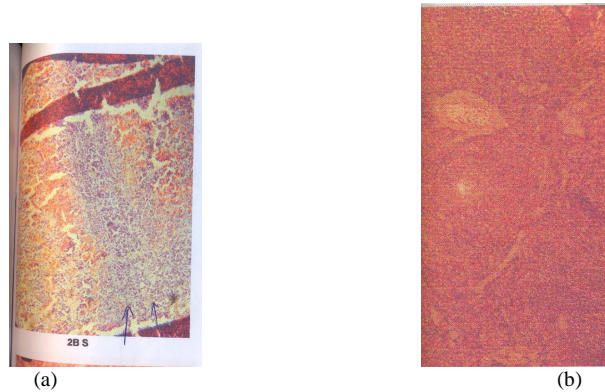


Fig. 2: The dark heavy staining of sites of new multiplication of cells in the photomicrograph of DHA-treated rat spleen (a) which are absent in that of controls (b) indicate new haemopoiesis in the DHA- treated rats

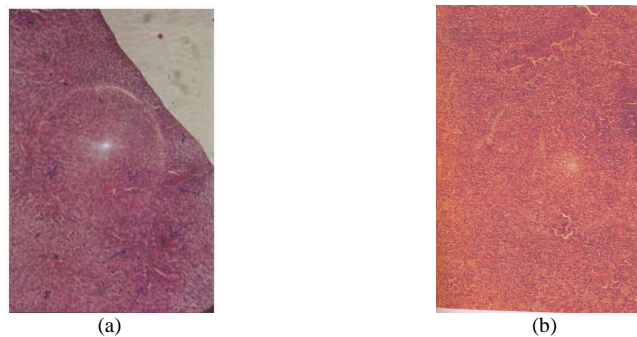


Fig. 3: Dark-stained sites containing large numbers of nuclei of cells at sites of new haemopoiesis in DHA-treated rat liver (a) which are absent in that of the control (b) show that DHA treatment produced new haemopoiesis in the test rats

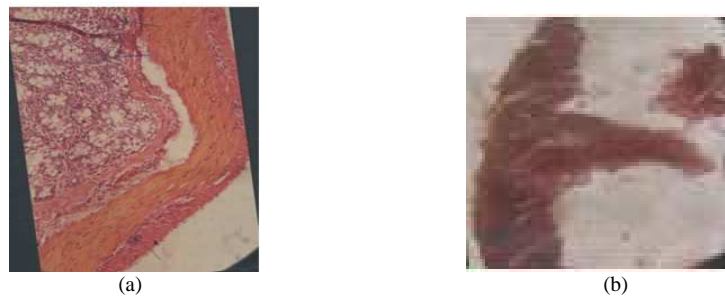


Fig. 4: Dark red stained proliferated cells seen in photomicrographs of DHA-treated rat (a) but not in that of a control rat (b), indicated that DHA stimulated new haemopoiesis in the test rats

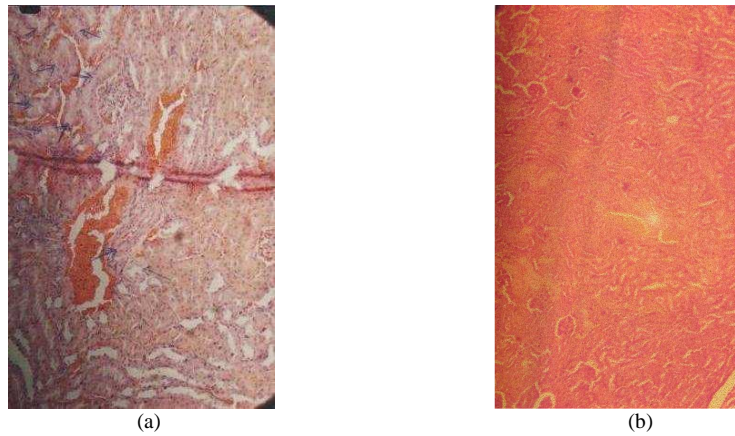


Fig. 5: Dark red heavily stained sites which contain many cells which are present in the photomicrographs of the kidney of DHA-treated rat (a) and absent in those of control rat (b) indicate that DHA stimulated new haemopoiesis in the kidney of the test rats

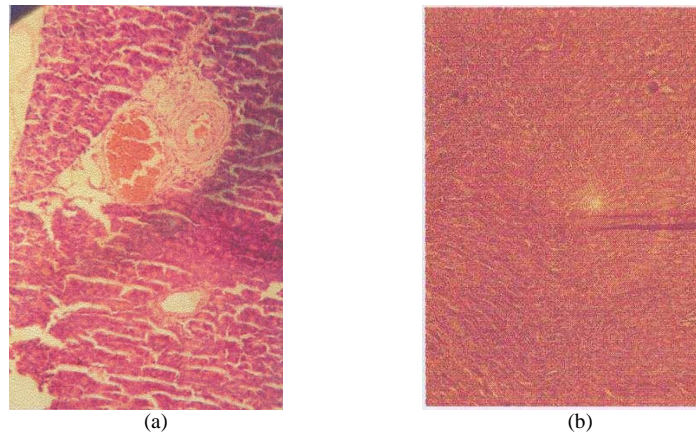


Fig. 6: Dark red stained proliferated cells seen in photomicrographs of DHA-treated rat spleen (a) which were absent in that of a control rat (b) shows that DHA treatment produced new haemopoiesis in the heart of the test rats

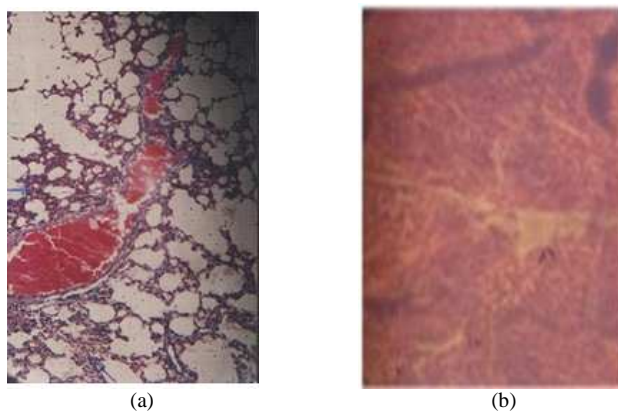


Fig. 7: Dark stained multiplied blood cells seen in Photomicrograph Of the lungs of DHA-treated rat (a) and that of control rat (b) which show that DHA produced haemopoiesis in the DHA-treated rats

Figure 1 a shows dark red coloration of the heart, lungs, liver, spleen and kidney of DHA-treated rats in which DHA caused new haemopoiesis. Figure 1b shows equivalent organs of a control rat.

The erythropoietic sites stimulated by DHA to produce the increases in the blood seen in the gross anatomical display in Fig. 1 are indicated by the pointing arrows in the micrograph of the kidney (Fig. 2a), the liver (Fig. 3a), the intestine (Fig. 4a) the heart (Fig. 5a); the spleen (Fig. 6a), the lungs (Fig. 7a). The equivalent micrographs of the kidney, liver, intestine, heart, spleen and lungs of control rats are shown in (Figs. 2-7b) respectively.

### DISCUSSION

Some drugs have been shown to be able to protect blood cells from damage while other drugs have effectively managed cancer of the blood (Erer *et al.*, 2000; Tuan *et al.*, 1992; Limoli *et al.*, 2003; Reiter *et al.*, 1997; Vijayalaxmi *et al.*, 1996; 1999; Easton, 1974; Chun *et al.*, 1991; Lyytikainen *et al.*, 1996). The multiplicity of substances which affect the blood cells or the bone marrow show that a therapeutic agent like dihydroartemisinin that controls a disease of the blood [malaria can have profound effects on the haemopoietic system. The statistically significant increases in the PCV ( $P < 0.5$ ) and the WBC ( $P < 0.01$ ) of the DHA-treated rats (and not in the control rats) show that DHA stimulated the production of large numbers of new red and white blood cells. The presence of large numbers of reticulocytes and left-shifted neutrophils in the blood of DHA-treated rats but not in those of control rats suggests that new haemopoiesis took place.

The dose; repetition and time dependent blood congestion observed gross anatomically and the hyperplasia of blood cells observed histologically, provide additional evidences of new haemopoiesis in DHA-treated rats as these were absent in the controls.

From a number of studies, it was concluded that when artemisinin reacts with haemoproteins, it alkylates the protein portion and not the haem portion as there was no reaction in solution between globin and dihydroartemisinin but there was marked reaction between haemoglobin and dihydroartemisinin. In this reaction most of the drug molecules bound to the protein (globin) and not to the haem. The dihydroartemisinin-myoglobin complex and the dihydroartemisinin-globin complex produced the new haemopoiesis seen as DHA dose dependent dark red colorations of the tissue photomicrographs of the different organs of DHA treated rats (Fig. 2-7).

The results of our study suggest that the dihydroartemisinin-myoglobin complex resulted in the dose, repetition and time dependent stimulation, proliferation and maturation of the haemopoiesis stem cells in the lungs, the liver, the intestines, the heart, the spleen and the kidney of the DHA-treated albino rats. The maturation of the haemopoietic stem cells resulted in the similarly dose, repetition and time dependent statistically significant increases in the packed cell volume and total white blood cell counts obtained in the DHA-treated rat but not in the controls.

### CONCLUSION

The findings of this study show that dihydroartemisinin stimulated new haemopoiesis in the lungs, the heart, the liver, the intestines, the spleen and the kidney of Wistar albino rats suggesting that each of these organs is an extra-bone marrow site of haemopoiesis. The results of this study also illustrated why dihydroartemisinin effects are affected *in vivo* and *in vitro* by hermin, porphyrin and metalloporphyrins.

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