American Journal of Agricultural and Biological Sciences 6 (4): 511-516, 2011
ISSN 1557-4989

Erythropoietin-Like Effects of Dihydroartemisinin in Wistar Albino Rats

[1]Utoh-Nedosa Uchechukwu Anastasia,
[2]Nedosa Kenechi Stanislaus and [3]Onyedibe Ikenna Kenneth
[1]Department of Pharmacology and Toxicology,
Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University,
P.M.B. 5025, Awka, Anambra State, Nigeria
[2]Evangelical Churches of West Africa Hospital (ECWA),
Egbe, P.M.B., Kogi State, Nigeria
[3]Department of Clinical Microbiology,
University of Jos Teaching Hospital, Jos, Plateau State, Nigeria

Abstract: Artemisinin drugs were active during the intra-erythrocytic stage of malaria parasite infection. The activity of artemisinin and synthetic endoperoxides was related to their interaction with heme. The electrophillic intermediate formed from artemisinin in the presence of heme alkylates the protein portion of hemoglobin preferentially to the heme portion. **Problem statement:** Since there might be an interaction between artemisinin and the heme of the blood, we studied the effects of 5-day and 7-day oral Dihydroartemisinin (DHA) treatments with 5 dosage regimens of dihydroartemisinin on the blood and six vital organs of Wistar albino rats. **Approach:** The dosages of DHA tested on 5 test adult Wistar albino rats (weight = 106-140 grams) were 1, 2, 60 or 80 mg Kg^{-1} rat weight of DHA by oral intubation for 5 or 7 days. Four rats of similar weight which served as controls in each experiment were given distilled water equivalents of the administered doses of DHA. Another group of 5 test rats and four control rats (weight 75-90 gms) were given 1 mg kg^{-1} rat weight of DHA or distilled water for 5 or 7 days and were allowed to rest for one week after which the treatment was repeated. **Results:** The findings of the study showed that Dihydroartemisinin (DHA) had erythropoietin-like properties. In the study DHA produced dose, repetition and time dependent statistically significant increases in the Packed Cell Volume (PCV) ($P<0.01$-0.03) and the total White Blood Cell count (WBC) ($P<0.01$) of the DHA-treated rats which was absent in the controls. The 7-day DHA treatments produced lower statistically significant increases of the PCV ($P<0.01$-03) and the WBC ($P<0.01$) than the 5-day DHA treatments. **Conclusion:** This result suggested that the administered DHA inhibited its own stimulated statistically significant increases in the PCV and the WBC of the treated rats through an inhibitory (negative) feed-back effect. The structure and composition of the blood cell types like the presence of large numbers of reticlocytes and left-shifted neutrophils in the blood samples of 5-day DHA -treated rats but not in those of 7-day DHA treated rats indicated that new haemopoiesis was actively going on in the first 5 days of DHA treatment but had slowed down by the sixth and seventh day of treatment. The initial stimulation of haemopoiesis and later inhibition of haemopoesis by a negative feed-back effect on haemopoiesis suggest that DHA has erythropoietin-like properties.

Key words: Dihydroartemisinin (DHA), Packed Cell Volume (PCV), feed-back effect, left-shifted neutrophils, White Blood Cell Count (WBC), hemoglobin preferentially, wistar albino rats

INTRODUCTION

Artemisinin-heme adducts form in drug treated parasites (Meshnick *et al*., 1996). The electrophillic intermediate formed from artemisinin in the presence of heme alkylates the protein portion of hemoglobin preferentially to the heme portion[2]. The artemisinin-globin adduct formed in the presence of heme must be

Corresponding Author: Utoh-Nedosa Uchechukwu Anastasia, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria

of some significance. This study investigated the effect of Dihydroartemisinin (DHA) on the blood and six systemic organs of Wistar albino rats.

MATERIALS AND METHODS

Five adult Wistar albino rats which weighed 106-140 grams were given 1, 2, 60 or 80 mg Kg^{-1} rat weight of DHA by oral intubation for 5 or 7 days. Four rats of similar weight which served as controls in each experiment were given distilled water equivalents of the administered doses of DHA.

Five test rats and four control rats which weighed 75-90 gms were given 1 mg kg^{-1} rat weight of DHA or distilled water for 5 or 7 days and were allowed to rest for one week after which the treatment was repeated.

The starting dose of the 1 mg Kg^{-1} DHA for both the single dosage regimen treatment and the repeated dosage regimen treatment was 2 mg Kg^{-1}.

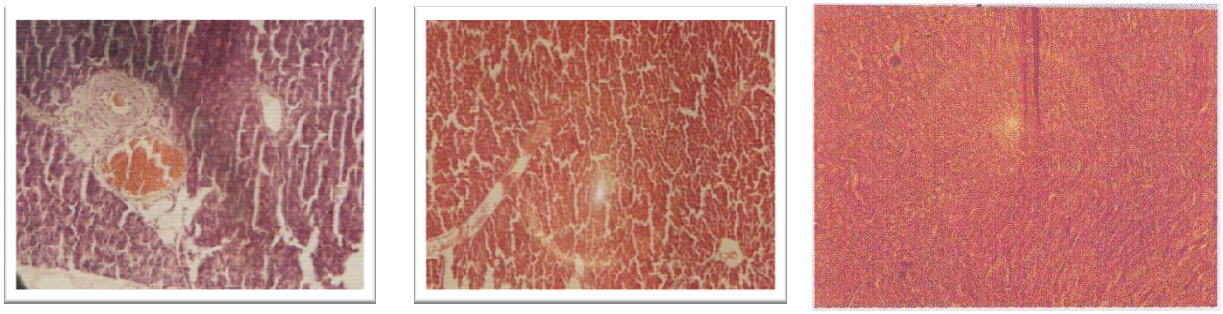
The test and control rats were sacrificed 24 hours after the administration of the last dose of each dosage regimen; after the collection of their whole blood samples for haematological investigations in EDTA anti-coagulant containing bottles. The lungs, heart, liver, intestine, spleen and kidney of the test and control rats were collected for histological studied after they had been examined gross anatomically.

Conventional procedures were employed in the production of photomicrographs of the lungs, heart, liver, intestine, spleen and kidney of the DHA-treated and control rats. Standard haematological procedures were also used to obtain the packed cell volume, the total white blood cell count and other relevant haematological indices of the collected blood samples.

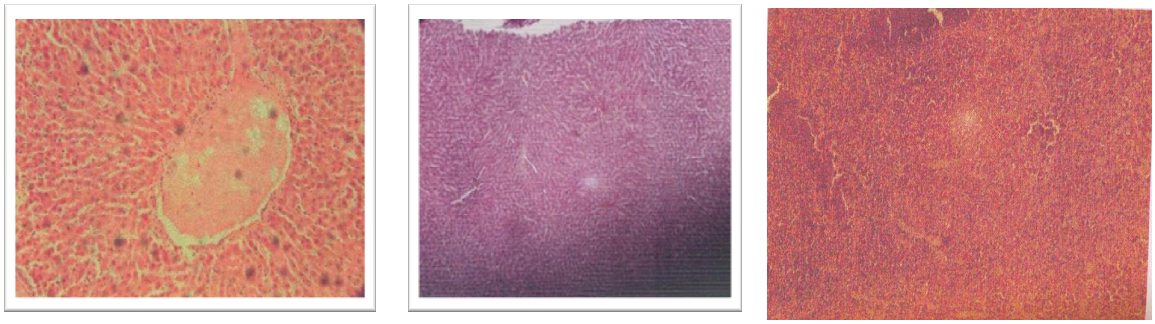
RESULTS

The results of the study showed that DHA produced dose, repetition and time dependent statistically significant increases in the Packed Cell Volume (PCV) ($P<0.01, 0.03$) and the total white blood cell count ($P<0.01$) in both the 5 day and the 7day DHA-treatment rats; which were absent in the controls. The PCV of the 5-day treatment rats was in the range of 45-48% and their total WBC count ranged from 10,500 mm^3-13,800 mm^3.The PCV of the 7-days DHA-treated rats ranged from 35-39% and their total WBC count ranged from 7,050-10,950. These figures compared against the PCV value of 42% and the total WBC count of 4.800-5,200 mm^3 of the control rats. These results show that DHA stimulated new haemopoiesis. The hemopoeisis stimulation effects of DHA were observable gross anatomically and histologically.

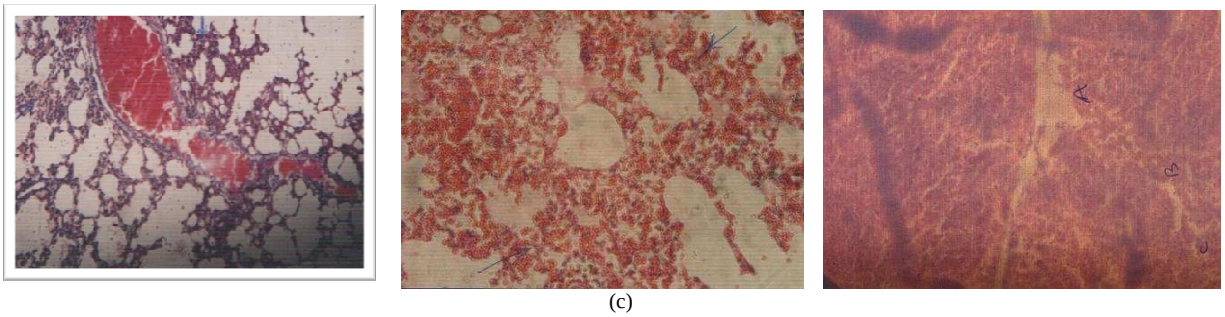
The haemopoeitic effects of 5 days oral DHA treatments and those of 7 days oral DHA treatments on the heart, liver, lungs and kidney obtained in the study are shown in Fig. 1a-c.



(a)



(b)



(c)

Fig 1: Illustration of the haemopoetic effects of 5 and 7 days oral DHA treatment on (a) the lungs; (b) the heart; (c) the liver and the lungs of Wistar albino rats

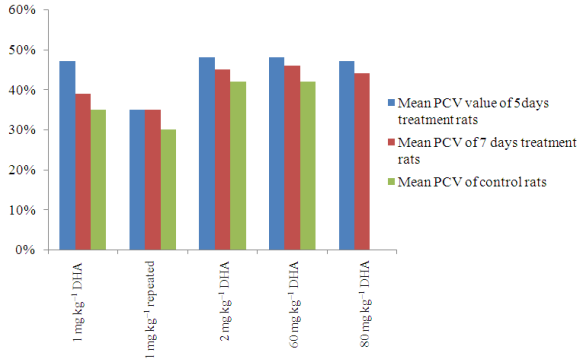


Fig. 2: The effect of 5days and 7 days oral dihydroartemisinin treatment on the packed cell volume of Wistar albino rats

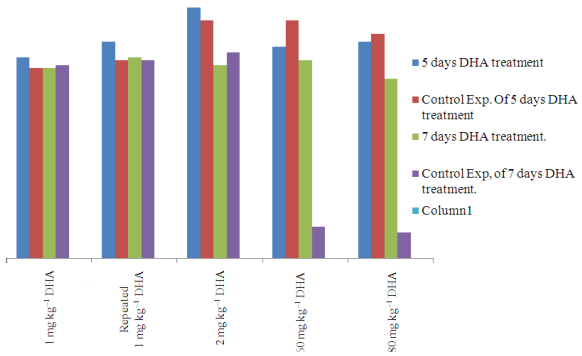


Fig. 3: The effect of 5 days and 7 days oral dihydroartemisinin treatment on the total white blood cell count of Wistar albino rats in mm^3

The red blood cells of the 5-days DHA-treated rats were normocytic and normochromic. They were also characterized by polychromasia and the presence of a large number of reticlocytes and left-shifted neutrophils. A smaller proportion of the red blood cells of the 7-days DHA-treated rats had normocytic normochromic red blood cells and a few of them had left- shifted neutrophills and no reticlocytes. Majority of the erythrocytes of the 7-day DHA treatment rats were mildly hypochromic and normocytic.

The effects of the tested doses of DHA produced dose, repetition and time dependent differences in the staining of the smooth/cardiac muscles of the heart, lungs, liver, intestine, spleen and kidney. Samples of such muscles affected by DHA treatment are shown in Fig. 1a-c for the heart, liver and the lungs as examples. These red blood cell staining effects of DHA on the cardiac muscles of the heart and those on the smooth muscles of the lungs, liver and kidney, heart, spleen and intestine followed the same pattern of dose, repetition and time (5 day or 7 day treatment) dependence as the pattern of the its PCV and WBC count elevation effects of the 5 and 7 days DHA treatment in Fig. 2 and 3.

DISCUSSION

The results of this study suggest that DHA has self-regulatory stimulatory effects on haemopoiesis. The inhibitory feedback effect of DHA produced the lower PCV value increases and the lower WBC count increases obtained with 7day DHA-treated rats incomparism with those obtained with the 5day DHA-treated rats.

This inhibition of further stimulation of haemopoiesis showed in the blood picture as the absence of reticlocytes and left-shifted neutrophils which are usually associated with new hemopoiesis.

The mild hypochromic normocytic red blood cells of these 7day DHA-treated rat blood samples also suggest that mobilization of iron and chromium for new red blood cell production had slowed down by the seventh day of DHA administration.

Heme catalyses the breakdown of artemisinin and also forms a covalent complex with it which retains the heme iron structure and seems to have lost the

artemisinin structure (Meshnick *et al*., 1996; Asawamahaskada *et al*., 1994a; 1994b). A study found that the artemisinin –heme adduct forms in drug treated parasites but is unlikely to be related to the mechanisim of action of the drug as in vitro, there was no effect of artemisinin treatment on hermozoin synthesis or on its degradation in parasites in culture even in concentrations which might inhibit hypoxanthine incorporation (Asawamahaskda *et al*., 1994).

The findings of this study suggest that the artemisinin-heme adduct was utilized in the formation of new red blood cells in the artemisinin treated rats. In vitro, heme and iron catalyse the conversion of artemisinin and its derivatives into free radicals in the same way they catalyse the decomposition of hydrogen peroxide into free radicals (Asawamahaskda *et al*., 1994).

Since artemisinin alkylates the protein portion of haemoglobin and not the haem portion (Meshnick *et al*., 1996), the author elucidate that dihydroartemisinin formed adducts with heme (forms of iron stored in the body) including the heme in haemoglobin and myoglobin and then alkylated globin. These actions of DHA stimulated erythrocyte and leucocyte stem cells in germinal sites of erythropoietic sites of the DHA-treated rats. The stimulated stem cells grew, proliferated and matured into the new erythrocytes and new white blood cells obtained in this study.

Heme and artemisinin form covalent adducts with molecular weights 856 and 871 when they are mixed in solutions (Meshnick *et al*., 1996). These adducts seem to contain one heme molecule and one artemisinin molecule (Meshnick *et al*., 1996). One of the artemisinin-heme adducts probably later initiates the formation of new hemoglobin for incorporation into maturing new erythrocyte stem cells. Another artemisinin-heme adduct probably forms free radical for per oxidation of parasites, pathogens and even cancer tumor cells as anti-tumor cell activity of dihydroartemisinin and artesunate have been demonstrated in various studies (Efferth *et al*., 2001; Woerdebag *et al*., 1993; Lai and Singh,1995; Singh and Lai, 2001; Ponmee *et al*., 2007).

A study found that artemisinin "loses its antimalarial activity" on prolonged exposure to erythrocytes especially α-thalassemic erythrocytes (Meshnick *et al*., 1996). According to the study, the major artemisinin inactivating factor in cytosol of normal erythrocytes was found to be heat labile but a heat stable factor from α-thalassemic erythrocytes which was shown to be released from haemoglobin also played a significant role in reducinging artemisinin effectiveness[13]. In the study, investigation of fractionated lysate from genetically normal erythrocytes revealed that the protein fraction with molecular weight greater than 100 kDa was capable of reducing artemisinin effectiveness more than the lower molecular weight fraction (Meshnick *et al*., 1996). Catalase and Hb A but not selenoprotein glutathione peroxidase were capable of reducing artemisinin effectiveness [hemin (ferriprotoporphyrin) IX reduced artemisinin effectiveness in a concentration and time dependent manner (Meshnick *et al*., 1991; Benoit-Vical *et al*., 2000). Thus this study found that heme and heme-containing compounds are largely responsible for reducing artemisinin effectiveness. In the light of the present study, "reduction of artemisinin effectiveness" as used in the above cited study, means involvement of artemisinin in execution of other actions other than "alkylation of malaria parasite proteins". The stimulation of haemopoiesis by dihydroartemisinin is one such action of artemisinin.

When blood is exposed to various drugs or oxidizing agents in vitro or *in vivo*, the ferrous ion (Fe^{2+}) in the heme of hemoglobin is converted to ferric ion (Fe^{3+}) forming methemoglobin[3]. Methemoglobin is dark coloured and when it is present in large quantities in the circulation it causes a dusky discoloration of the skin resembling cyanosis (Ganong, 2001). In the present study Dihydroartemisinin not only interacted with the heme of haemoglobin to form methemoglobin but also interacted with the globin of hemoglobin to initially stimulate and later inhibit new haemopoiesis.

The findings of this study therefore suggest that dihydroartemisinin has erythropoietin-like properties and that it employed these properties in stimulating and subsequently, inhibiting new haemopoiesis in the lungs, heart, liver, intestine, spleen and kidney of Wistar albino rats.

Erythropoietin is a circulating glycoprotein that contains 165 amino acid residues and four oligosaccharide chains which are necessary for its activity *in vivo* (Ganong, 2001). The circulating blood level of erythropoietin is markedly increased in anemia and decreased when the red cell volume is increased above normal by transfusion (Ganong, 2001). Erythropoietin increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors and subsequently to mature erythrocytes (Ganong, 2001). The receptor for erythropoietin is a linear protein with a single transmembrane domain that is a member of the cytokine receptor superfamily (Ganong, 2001). The erythropoietin receptor has tyrosine kinase activity and activates a cascade of serine and threonine kinases resulting in growth and development of its target cells

(Ganong, 2001). It is likely that Dihydroartemisinin bound to the erythropoietin receptor on target red and white blood cell stem cells to stimulate the new erythropoiesis obtained in our study

In a study, lethally irradiated mice were injected with marrow cells obtained from mice that had received phenylhydrazine plus control IgG or with marrow cells obtained from mice that had received phenylhydrazine plus ACK2. In parallel experiments, normal murine marrow cells were treated in vitro with control IgG or with ACK2 and were injected into lethally irradiated mice. The fraction of BFU-E and CFU-GM retrieved from the marrow and spleen of the recipient mice 4 hours later was reduced by approximately 75% when progenitor cells had been exposed to ACK2, in comparison with control IgG.

The results were interpreted by the researchers to mean that c-kit receptor function may be required for optimal response to acute erythropoietic demand and that erythropoiesis in the splenic microenvironment is more dependent on SCF/c-kit receptor interaction than is erythropoiesis in the marrow microenvironment and that interaction of SCF with the c-kit receptor affects the homing behavior of hematopoietic progenitor cells in the adult animal (Broudy *et al*., 1996). These results confirm our findings that dihydroartemisinin interacted with a receptor to stimulate haemopoiesis in the six organs of wister albino rats.

Another study obtained data which provided compelling evidence that tumor-derived VEGF displays a profound effect on the hematopoietic system and suggested that tumor-derived VEGF enters into the circulation and acts on either endothelial cells and/or hematopietic progenitor cells to modulate hematopoiesis. The findings of this study provide further evidence the endogenous or exogenous substances can stimulate haemapoietic progenitor cells (Carmeliet *et al*., 1996; Xue *et al*., 2009a; 2009b; Chen *et al*., 2003; Stockmann *et al*., 2008; Lyden *et al*., 2001; Pan *et al*., 2007; Collins and Hurwitz, 2005; Woodrow *et al*., 2005).

Some drugs are more toxic for earlier haemopoietic progenitor cells than for the more mature cells. In the treatment, of mice with such a toxic drug there was also a subsequent significant decrease of the RBC count, accompanied by a marked increase of the marrow CFU-E concentration. The icreases in the PCV recorded in our studies show that dihydroartemisinin effect on homopoietic stem cell was a healthy and beneficial effect for the test rats (Robert and Meunier, 1998; Nowrousian and Schmidt, 1982).

CONCLUSION

This study concludes that dihydroartemisinin has erythropoietin-like properties and that it employed these properties in stimulating and subsequently, inhibiting new haemopoiesis in the lungs, heart, liver, intestine, spleen and kidney of Wistar albino rats.

REFERENCES

Asawamahaskada, W., I. Ittrat, C.C. Chang, P. McElroy and S.R. Meshnick, 1994a. Effects of antimalarials and protease inhibitors on plasmodial hemozoin production. Mol. Biochem. Parasitol., 67: 183-191. DOI: 10.1016/0166-6851(94)00128-6

Asawamahaskda, W., I. Ittratt, Y.M. Pu, H. Ziffer and S.R. Meshnick, 1994b. Reaction of antimalarial endoperoxides with specific parasite proteins. Antimicrob. Agents Chemother, 38: 1854-1858.

Benoit-Vical, F., A. Robert and B. Meunier, 2000. *In vitro* and *in vivo* potentiation of artemisinin and synthetic endoperoxide antimalarial drugs by metalloporphyrins. Antimicrob. Agents Chemother, 44: 2836-2841. DOI: 10.1128/ AAC.44.10.2836-2841.2000

Broudy, V.C., N.L. Lin, G.V. Priestley, K. Nocka and N.S. Wolf, 1996. Interaction of stem cell factor and its receptor c-kit mediates lodgment and acute expansion of hematopoietic cells in the murine spleen. Blood, 88: 75-81.

Carmeliet, P., V. Ferreira, G. Breier, S. Pollefeyt and L. Kieckens *et al*., 1996. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature, 380: 435-439. PMID: 8602241

Chen, H.H., H.J. Zhou and X. Fang, 2003. Inhibition of human cancer cell line growth and human umbilical vein endothelial cell angiogenesis by artemisinin derivatives *in vitro*. Pharmacol. Res., 48: 231-236. PMID: 12860439

Collins, T.S. and H.I. Hurwitz, 2005. Targeting vascular endothelial growth factor and angiogenesis for the treatment of colorectal cancer. Semin Oncol., 32: 61-68. PMID: 15726507

Efferth, T., H. Dunstan, A. Sauerbrey, H. Miyachi and C.R. Chitambar, 2001. The anti-malarial artesunate is also active against cancer. Int. J. Oncol., 18: 767-773. PMID: 11251172

Ganong, W.F., 2001. Review of Medical Physiology. 20th Edn., McGraw Hill, New York, ISBN-10: 0838582826, pp: 870.

Lai, H. and N.P. Singh, 1995. Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett., 91: 41-46. PMID: 7750093

Lyden, D., K. Hattori, S. Dias, C. Costa and P. Blaikie *et al*., 2001. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat. Med., 7: 1194-1201. PMID: 11689883

Meshnick, S.R., A. Thomas, A. Ranz, C.M. Xu and H.Z. Pan, 1991. Artemisinin (qinghaosu): The role of intracellular hemin in its mechanism of antimalarial action. Mol. Biochem. Parasitol., 49: 181-190. PMID: 1775162

Meshnick, S.R., T.E. Taylor and S. Kamchonwongpaisan, 1996. Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemotherapy. Microbiol. Rev., 60: 301-315.

Nowrousian, M.R. and C.G. Schmidt, 1982. Effects of cisplatin on different haemopoietic progenitor cells in mice. Br. J. Cancer, 46: 397-402. PMID: 6889884

Pan, Q., Y. Chanthery, W.C. Liang, S. Stawicki and J. Mak *et al*., 2007. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. Cancer Cell, 11: 53-67. PMID: 17222790

Ponmee, N., T. Chuchue, P. Wilairat, Y. Yuthavong and S. Kamchonwonpaisan, 2007. Artemisinin Effectiveness in erythrocytes is reduced by heme and heme-containing proteins, Biochem. Pharmacol., 74: 153-160. PMID: 17498668

Robert, A. and B. Meunier, 1998. Is alkylation the main mechanism of action of the antimalarial drug artemisinin? Chem. Soc. Rev., 27: 273-280.

Singh, N.P. and H. Lai, 2001. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sci., 70: 49-56. PMID: 11764006

Stockmann, C., A. Doedens, A. Weidemann, N. Zhang and N. Takeda *et al*., 2008. Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. Nature, 456: 814-818. DOI: 10.1038/nature07445

Woerdebag, H.J., T.A. Moskal, N. Pras, T.M. Malingre and F.S. El-Feraly *et al*., 1993. Cytotoxicity of artemisinin-related endoperoxides to Ehrlich ascites tumor cells. J. Nat. Prod., 56: 849-859. PMID: 8350087

Woodrow, C.J., R.K. Haynes and S. Krishna, 2005. Artemisinins. Postgrad. Med. J., 81: 71-78. DOI: 10.1136/pgmj.2004.028399

Xue, Y., F. Chen, D. Zhang, S. Lim and Y. Cao, 2009b. Tumor-derived VEGF modulates hematopoiesis. J. Angiogenesis Res., 1: 9-9. PMID: 20076778

Xue, Y., N. Petrovic, R. Cao, O. Larsson and S. Lim *et al*., 2009a. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. Cell Metab., 9: 99-109. DOI: 10.1016/j.cmet.2008.11.009 PMID: 19117550