

## **An Update on Artemisinin- A Multifaceted Drug**

**Anu Gopalakrishnan\*, Varuna P. Panicker**

**College of Veterinary and Animal sciences, Mannuthy- 680651, Kerala, India.**

**\*Corres. Author: dranugvet@gmail.com  
Mobile no: 9446212684**

**Abstract:** Discovery of artemisinin from the Chinese herb '*Artemisia annua*' and establishment of its antimalarial activity by Chinese scientists during later half of 20<sup>th</sup> century marked one of the greatest discoveries in medicine. Artemisinin and its derivatives are now well established for the treatment of malaria in humans, even against highly drug resistant strains of *Plasmodium sp.* Artemisinins act more rapidly than other types of antimalarials, both in killing the parasites and in inhibiting their major metabolic processes, such as glycolysis, nucleic acid and protein synthesis. The naturally rare endoperoxide bridge (1,2,4-trioxane structure) present in the artemisinin molecule is believed to be responsible for the drug's mechanism of action. Potential of artemisinins can be extended to broader therapeutical applications such as anti-schistosomal, anticancerous and as antiviral agents. In the continuing absence of an effective malarial vaccine, the development of new antimalarial drugs most likely derived from, or inspired by, artemisinin will continue to be our primary weapon in the fight against malaria.

**Key words:** Artemisinin, Derivatives, *Artemisia annua*, Antimalarial, therapeutical applications.

### **Introduction**

Artemisinin is a naturally-occurring quadricyclic endoperoxide sesquiterpene lactone extracted from a herb, *qinghao* (*Artemisia annua*- sweet Annie, sweet wormwood), which was documented in traditional Chinese pharmacopoeia for the treatment of fevers over two millennia, has a longer history of use. Artemisinin and its derivatives are well established for the treatment of malaria in humans, including highly drug resistant strains of *Plasmodium sp.* Their efficacy also extends to phylogenetically unrelated parasitic infections such as Schistosomiasis. More recently, they have also shown potent and broad anticancer properties in cell lines and animal models.<sup>1</sup>



**Scientific name:** *Artemisia annua* L.

**Common name(s):** sweet wormwood, sweet annie, annual wormwood (English); qing hao (meaning 'blue-green' artemisia) (Chinese).

**Synonym(s):** *Artemisia chamomilla*.

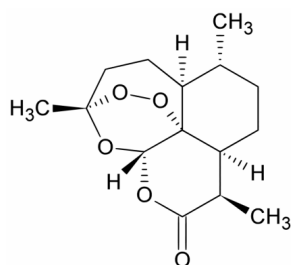
## Discovery of artemisinin

Discovery of artemisinin and establishment of its antimalarial activity by Chinese scientists represents one of the great discoveries in medicine in the latter half of the 20th century (1960s). During 20th century middle World Health Organisation (WHO) made successful attempts to eradicate malaria across the world using the powerful residual insecticides like DDT and the highly effective antimalarial drug of the time 'chloroquine'. By the end of 1950s the incidence of malaria had dropped dramatically but in late 1960s the disease reemerged due to chloroquine resistant *Plasmodium falciparum* strains and resistance of mosquito, the malarial vector to the insecticides. Under this circumstances, in 1967 Chinese government set up a secret military project during Vietnam war, known as 'Project 523' lead by the Pharmaceutical Institute of the Academy of Traditional Chinese Medicine, to find an adequate treatment for malaria by screening herbs used in Traditional Chinese medicine. They reinvestigated a 2000-year-old Chinese pharmacopoeia, Ge Hong's 'The Handbook of Prescriptions for Emergency Treatments' and identified sweet wormwood (*Artemisia annua*). The earliest records about *Artemisia* dates back to 200 BC, in the "Fifty-two Prescriptions" unearthed from the Mawangdui Han Dynasty tombs.<sup>2</sup> In the 1970s clinical studies with the plant extract on members of the Chinese army infected with the *Plasmodium* parasite demonstrated an outstanding therapeutic efficiency in almost all patients and was completely effective in treating chloroquine-resistant *Plasmodium falciparum* malaria. From the cold ethereal extracts of *Artemisia*, artemisinin-'qinghaosu' ('principle from qinghao' in Chinese) - stable and easily crystallisable compound - was isolated and characterised in 1972 and the results of the study was published in the 'Chinese Medical Journal' in 1979. Project 523 developed, in addition to artemisinin, a number of products that are used in combination with artemisinin, including lumefantrine, piperaquine, and pyronaridine.<sup>3</sup>

It took more than a decade for artemisinin to make its appearance on the global stage because access to the purified drug and the plant from which it was extracted were restricted by the Chinese government. Furthermore some Western scientists were sceptical about the stability of 1,2,4-trioxane ring embedded in the structure of artemisinin and questioned its usefulness as a drug. In the late 90s, Novartis bought a new Chinese patent for a combination treatment with artemether and lumefantrine, providing the first artemisinin-based combination therapies (ACTs) (Coartem) at reduced prices. In 2001 Coartem became the first fixed dose artemisinin-based combination therapy to meet the WHO's pre-qualification criteria for efficacy, safety and quality. In 2002 artemisinin was placed in WHO's 'essential drug' list for malaria treatment. In 2006, after artemisinin had become the treatment of choice for malaria, WHO called for an immediate halt to single-drug artemisinin preparations in favour of combinations of artemisinin, to reduce the risk of resistance development by parasite.<sup>21</sup> Two main scientists involved in the discovery are Zhenwing Wei and Youyou Tu, in 2011 the latter received the prestigious Lasker award in clinical medical sciences.<sup>4,5</sup>

## Structure Activity Relationship

Chemical structure of artemisinin



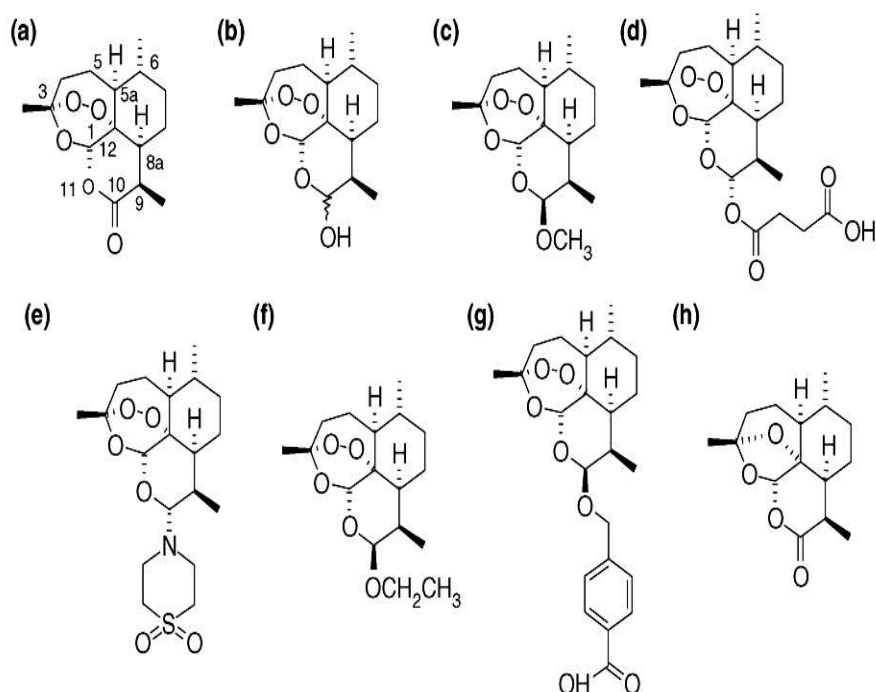
Chemical formula- C<sub>15</sub>H<sub>22</sub>O<sub>5</sub>, Mol. Mass-282.332 g/mol.

Artemisinin is chemically a sesquiterpene (15carbon) lactone formed by combination of three isoprene (five-carbon) molecules. The molecule contains an endoperoxide bridge (1,2,4-trioxane structure). This peroxide bridge is believed to be responsible for the drug's mechanism of action. Very few natural compounds are known with such a peroxide bridge.<sup>6</sup>

## Artemisinin Derivatives

Since artemisinin itself has physical properties such as less water solubility, less lipid solubility and hence poor bioavailability that limit its effectiveness, semisynthetic derivatives of artemisinin have been developed. Modification is done on the lactone ring oxygen atom of artemisinin to produce various derivatives. Dihydroartemisinin, artemether, artesunate and arteether were prepared in the 1970s. Reduction of artemisinin by sodium borohydride produced dihydroartemisinin in which the lactone group had been converted to a lactol (hemi-acetal) group. Artemisone representative of a new class of artemisinin known as amino-artemisinins, is curative in clinical trials at one-third the dose regimen of artesunate. It is characterized by low toxicity. Artelinate was prepared at the Walter Reed Army Institute of Research but was withdrawn because of toxicity concerns. A synthetic compound with a similar trioxolane structure as that of artemisinin, named Arterolane showed promise in in vitro testing and is under clinical trial. Deoxyartemisinin lacking the peroxide bridge is biologically inert.<sup>7,8</sup> Artemisinin together with its derivatives are collectively called artemisinins.

## Chemical structures of artemisinins



(Adopted from; Review Trends in Pharmacological Sciences Vol.29 No.10 522)

(a) Artemisinin; (b) Dihydroartemisinin(DHA); (c) Artemether; (d) Artesunate; (f) Arteether; (e) Artemisone; (g) Artelinate; (h) Deoxyartemisinin

## Pharmacokinetics

Artemisinins can be administered by several routes. Artemisinin can be given orally, intramuscularly or as suppository, artemether orally and intramuscularly. The water soluble derivative artesunate can be given by the oral, intramuscular, intravenous and intrarectal routes. They are rapidly absorbed and widely distributed and are converted in the liver to active metabolite dihydroartemisinin. The half life of artemisinin is about 4h, of artesunate 45min and of artemether 4-11h noted in humans.<sup>9</sup>

## Pharmacodynamics

Artemisinins act more rapidly than other types of antimalarial, both in killing parasites and in inhibiting their major metabolic processes, such as glycolysis, nucleic acid and protein synthesis. Artemisinins also attack the broadest age range of parasites, from the tiniest rings that have recently invaded erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts. Furthermore, artemisinins inhibit the ability of maturing parasites to make the red cell surface sticky (cytoadherence to endothelial cells) much more

effectively than most other antimalarials. As cytoadherence is a recognised virulence determinant, inhibiting it more efficiently may confer a comparative advantage over other classes of drug.

There is considerable debate regarding the mechanisms of antimalarial action of artemisinins. As peroxides are generally reactive entities of artemisinins, a link has been drawn between the mechanism of action of artemisinins and the generation of reactive oxygen species (ROS)-hydroxyl, alkoxyl, (protonated) superoxide or peroxy radicals-within the parasitized erythrocyte. Formation of ROS can be enhanced through the Fe<sup>2+</sup>-dependent Fenton process. For many years, artemisinins have been proposed to act on parasite haemoglobin-digestion processes within the 'food vacuole'. Other studies have indicated that artemisinins could also target the parasite mitochondrion or the translationally controlled tumour protein (TCTP) and PfATP6, a parasite-encoded sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA).<sup>11</sup>

Hence the various proposed molecular mechanisms/cellular targets through which artemisinin acts include:

### Haem pathway

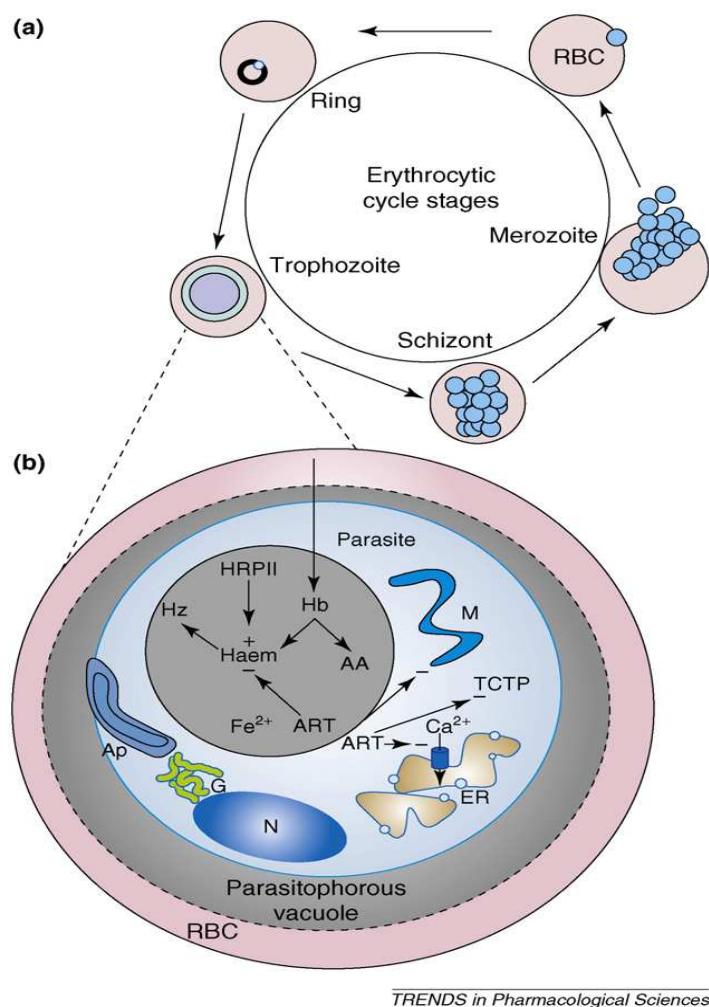
Haemoglobin is digested within a food vacuole of *Plasmodium* results in the formation of haem. As the haem is formed, it associates via one of the peripheral carboxyl groups with the Fe<sup>3+</sup> of an adjacent haem to form insoluble parasitic pigment haemozoin (Sanjeev *et al.*, 2008). It has been proposed, although not proven, that this process is aided by a protein termed the histidine-rich protein II (HRP-II). The endoperoxide bridge of artemisinins is proposed to be activated by ferrous iron to generate free radicals (of the oxy or C-centred variety) in vitro experiments and, subsequently, to alkylate haem<sup>10</sup>. As iron is the principal element deposited in haemozoin, digestion of haemoglobin by parasites is suggested to render them susceptible to killing by locally activated artemisinins.<sup>11, 12</sup> However in many cases the plasmodial stages most susceptible to the activity of artemisinins are too young to manifest the visible pigment. Further, their effectiveness against non-pigment-producing apicomplexan parasites also suggest alternative molecular targets in parasite for artemisinins.

### PfATP6 calcium ATPase inhibition

This mechanism is more target specific compared to haeme pathway. Synthetic trioxolanes, such as OZ277 carrying the endoperoxide bridge like that of artemisinins were shown to inhibit PfATP6 calcium ATPase activity when expressed in *Xenopus* oocyte model at low ( $\mu$ M) concentrations.<sup>13, 14</sup> This led to the identification of PfATP6 (*Plasmodium falciparum* SERCA-Smooth Endoplasmic Reticulum Calcium ATPase-orthologue) as a target for artemisinins. Evidence from transfection into parasites of DNA encoding PfATP6 that have altered sensitivity to some artemisinins provided suitable genetic tests for the PfATP6 hypothesis. Also another study from French Guiana showed a clear association between mutation(s) in PfATP6 and decreased susceptibility to artemether, particularly with position 769 (Ser769Asn substitution) supported the hypothesis. Parasites with Ser769Asn had a median IC<sub>50</sub> value >20-times higher for artemether (indicating artemether resistance) compared with parasites without this mutation.<sup>15, 16, 29</sup>

### Other targets

Recent studies indicate that mitochondrial membrane potential can be disrupted by artemisinin when grown in nonfermentable conditions. The TCTP (translationally controlled tumour protein) orthologue of *P. falciparum* was identified as a protein alkylated by radiolabelled artemisinin.<sup>1</sup> However, the relevance of these observations to antimalarial activity of artemisinins is unclear.



### a. The complex life cycle of *Plasmodium falciparum*

### b. The intraerythrocytic parasite and proposed targets of artemisinins

Abbreviations: AA, amino acids; Ap, apicoplast; ART, artemisinins; DV, digestive vacuole; ER, endoplasmic reticulum; G, Golgi apparatus; Hb, haemoglobin; Hz, haemozoin; HRP-II, histidine-rich protein II; M, mitochondrion; N, nucleus; RBC, red blood cell; TCTP, translationally controlled tumour protein.

(Adopted from; Review Trends in Pharmacological Sciences Vol.29 No.10 522)

## Uncomplicated malaria and severe malaria

Artemisinins can be used alone, but this leads to a high rate of recrudescence (return of parasites) and other drugs are required to clear the body of all parasites and prevent recurrence. The WHO has recommended artemisinin combination therapies (ACT) be the first-line therapy for *P. falciparum* malaria worldwide. Combinations are effective because the artemisinin component kills the majority of parasites at the start of the treatment, while the more slowly eliminated partner drug clears the remaining parasites. Several fixed-dose ACTs are now available containing an artemisinin component and a partner drug which has a long half-life, such as mefloquine (ASMQ), lumefantrine (Coartem), amodiaquine (ASAQ), piperaquine (Duo-Cotecxin), and pyronaridine (Pyramax).<sup>19, 20</sup> Increasingly, these combinations are being made to GMP standard. Artemisinins are not generally used for malaria prophylaxis (prevention) because of the extremely short activity (half-life) of the drug (White, 2008). To be effective, it would have to be administered multiple times each day. Artesunate is now recommended by the WHO for treatment of all cases of severe malaria. For malaria usual dosage is 3 grams in humans, (about 50mg/kg) administered over a 3 to 5 day period. It is especially useful in the treatment of drug resistant malaria.<sup>25, 21</sup>

## Against Helminths and other parasites

Artemisinin was effective against schistosomes, the human blood flukes, which are the second-most prevalent parasitic infections, after malaria. Artemisinin and its derivatives are all potent anthelmintics. Artemisinins were later found to possess a broad spectrum of activity against a wide range of trematodes, including *Schistosoma japonicum*, *S. mansoni*, *S. haematobium*, *Clonorchis sinensis*, *Fasciola hepatica*, and *Opisthorchis viverrini*. Clinical trials were also successfully conducted in Africa among patients with schistosomiasis.<sup>1</sup>

*A. annua* extracts, semi-synthetic and synthetic artemisinin preparations orally showed good efficacy in treating kinetoplastid infections in BALB/c mice. Artemisinin found to reduce the severity of coccidial infection induced by *Eimeria tenella* and *E. acervulina* in chicken.<sup>22</sup> The infected chickens were treated with doses of 1 or 2.5 mg/kg per day artemisinin orally via plant extract. In another study it is also found out to inhibit *Babesia gibsoni*, *B. bovis*, *B. equi* *in vitro*.<sup>24</sup>

## Against Cancer

Since the late 1980s, anticancer properties of artemisinins have been assayed *in vitro*. After more detailed studies, artemisinins such as artesunate were found to be active against a variety of unrelated tumour cells lines, from the most common types such as colon, breast and lung cancers to leukaemias and pancreatic cancer. Peroxide lactone group in its structure when comes into contact with high iron concentrations (common in cancerous cells), the molecule becomes unstable and releases reactive oxygen species. It has been shown to reduce angiogenesis and the expression of vascular endothelial growth factor (VEGF) in some tissue cultures.<sup>1</sup> Artemisinins inhibit proliferation, migration and tube formation of human umbilical vein endothelial cells (HUVEC), inhibit vascular endothelial growth factor (VEGF) binding to surface receptors on HUVEC and reduce expression of VEGF receptors Flt-1 and KDR/flk-1 on HUVECs.<sup>27</sup>

## Adverse Effects

Artemisinins are generally well tolerated at the doses used to treat malaria. The side effects from the artemisinin class of medications may include transient heart block, nausea, vomiting, anorexia, and dizziness. Mild blood abnormalities have also been noted, rarely allergic reaction. One case of significant liver inflammation has been reported in association with prolonged use of a relatively high dose of artemisinin for an unclear reason. The drugs used in combination therapies can contribute to the adverse effects experienced by those undergoing treatment.<sup>20</sup> Adverse effects in patients with acute *P. falciparum* malaria treated with artemisinin derivatives tend to be higher. In animal studies artemisinin causes an unusual injury to some brain stem nuclei, particularly those involved in auditory function but no such report in human upto now.

## Resistance

Clinical evidence for artemisinin resistance in patients was first reported in 2008 and subsequently confirmed by a detailed study from western Cambodia and Thailand in 2012. The resistance is characterized by a slower elimination of parasite (clearance rate, CR) after treatment with artemisinin. Different studies were carried out to find a major Genome region underlying artemisinin resistance in Malaria, in Southwest Asia.<sup>26</sup> These studies identified three areas of possible association of this resistance, on chromosomes 6, 13 and 14. The region located on chromosome 13, contains several candidate genes and is associated with the variation in the clearance rate.<sup>23, 28</sup>

## Conclusion

Artemisinins are now firmly established in combination therapies to treat drug-resistant malaria in humans. Several ongoing studies have come up with synthetic artemisinin analogues carrying the endoperoxide ring structure but, because of the complexity of the target compound, none is commercially competent enough with the natural compound, upto now. These include OZ277, OZ439 and RKA 182 which are currently being tested to see how effective they are. Synriam™ is a recently launched fixed drug combination of artemether maleate and piperaquine phosphate, the two antimalarial agents. Artemether is a synthetic trioxalane which has been proved effective to kill the malarial parasite in blood rapidly, providing fast relief from symptoms of malaria. It was launched in Indian market by Ranbaxy Laboratories Limited on 25 April, 2012 and was approved by Drug Controller General of India.<sup>30</sup> Micropropagation (tissue culture) of *A. annua* and thereby extensive *in vitro* production of artemisinin are goals that are intended to achieve in near future. Genetically

modified *A. annua* variety which is expected to have more yield of the reactive moiety is also under studies.<sup>18</sup> In the continuing absence of an effective malaria vaccine, the development of new antimalarial drugs most likely derived from, or inspired by, artemisinin will continue to be our primary weapon in the fight against malaria. Artemisinin's potential can be extended to broader applications such as an anti-schistosomal agent, against different types of cancers and as antiviral agent also.

## References

1. Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing importance in medicine. *Trends Pharmacol Sci* 2008; 29: 520-7.
2. Youyou, T. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nat Med* 2011; 17: 19-22.
3. Miller LH. and Su X. "Artemisinin: discovery from the Chinese herbal garden". *Cell* 2011; 146: 855-8.
4. Klayman DL, Lin AJ, Acton N, Scovill JP, Hoch JM, Milhous WK, Theoharides AD, Dobek AS. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. *J Nat Prod* 1984; 47:715-7.
5. Hsu E. Reflections on the "discovery" of the antimalarial qinghao. *Br J Clin Pharmacol.* 2006; 61:666-70.
6. O'Neill PM. Enantiomeric 1,2,4-trioxanes display equivalent in vitro antimalarial activity versus *Plasmodium falciparum* malaria parasites: implications for the molecular mechanism of action of the artemisinins. *Chembiochem* 2005; 6:2048-54.
7. Li G-Q, Guo X-B, Fu L-C, Jian H-X, Wang X-H. Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans R Soc Trop Med Hyg* 1994; 88:S5-S6.
8. Hien TT. An overview of the clinical use of artemisinin and its derivatives in the treatment of falciparum malaria in Viet Nam. *Trans R Soc Trop Med Hyg* 1994; 88:S7-S8.
9. Rang HP, Dale MM, Ritter JM, Flower RJ and Henderson G. Rang and Dale's Pharmacology. 7th edn. Elsevier Churchill Livingstone, 2007. pp 658-663.
10. Cui L, Wang Z, Jiang H, Parker D, Wang H, Su XZ, Cui L. "Lack of Association of the S769N Mutation in *Plasmodium falciparum* SERCA (PfATP6) with Resistance to Artemisinins". *Antimicrob Agents Chemother* 2012; 56: 2546-52.
11. Krishna S, Pulcini S, Fatih F, Staines H. "Artemisinins and the biological basis for the PfATP6/SERCA hypothesis". *Trends Parasitol* 2010; 26 (11): 517-23.
12. Krishna S, Woodrow CJ, Staines HM, Haynes RK, Mercereau-Puijalon O. Re-evaluation of how artemisinins work in light of emerging evidence of in vitro resistance. *Trends Mol Med* 2006; 12, 200-5.
13. Creek, D.J. et al. Relationship between antimalarial activity and heme alkylation for spiro- and dispiro-1,2,4-trioxolane antimalarials. *Antimicrob Agents Chemother* 2008; 52, 1291-6.
14. Cumming JN, Ploypradith P, Posner GH. "Antimalarial activity of artemisinin (qinghaosu) and related trioxanes: mechanism(s) of action". *Adv Pharmacol* 1997; 37: 253-97.
15. Eckstein-Ludwig U, Webb RJ, Van Goethem IDA, East J M, Lee AG, Kimura M, O'Neill PM, Bray PG, Ward SA, Krishna S. "Artemisinins target the SERCA of *Plasmodium falciparum*". *Nature* 2003; 424: 957-961.
16. Pandey A.V. Artemisinin, an endoperoxide antimalarial, disrupts the hemoglobin catabolism and heme detoxification systems in malarial parasite. *J Biol Chem* 1999; 274:19383-8.
17. Wang J, Huang L, Li J, et al. "Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation". *PLoS ONE* 2010; 5 (3): e9582.
18. White NJ. 2008. Qinghaosu (Artemisinin): The Price of Success. *Science* 320; 330-4.
19. Vugt MV, Wilairatana P, Gemperli B, et al. "Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria". *Am J Trop Med Hyg* 1999; 60 (6): 936-42.
20. Haynes RK. Artemisinin and derivatives: the future for malaria treatment? *Curr Opin Infect Dis* 2001; 14: 719-26.
21. World Health Organisation (WHO). Guidelines for the Treatment of Malaria. 2nd ed., Geneva, Switzerland: WHO Publications. 2010, pp. 8-60.
22. Arab HA, Rahbari S, Rassouli A, Moslemi MH and Khosravirad F. Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Trop Anim Health Prod* 2006; 38:497-503.

23. Cheeseman H. A major genome region underlying Artemisinin resistance in malaria. *Science* 2012; 336: 79-82.
24. Nagai A, Yokoyama N, Matsuo T, Bork S, Hirata H, Xuan X, Zhu Y, Claveria FG, Fujisaki G and Igarashi I. Growth-Inhibitory Effects of Artesunate, Pyrimethamine, and Pamaquine against *Babesia equi* and *Babesia caballi* in in vitro Cultures. *Antimicrob Agents Chemother* 2003; 47: 800-3.
25. Krudsood S, Looareesuwan S, Tangpukdee N, et al. "New Fixed-Dose Artesunate-Mefloquine Formulation against Multidrug-Resistant *Plasmodium falciparum* in Adults: a Comparative Phase IIb Safety and Pharmacokinetic Study with Standard-Dose Nonfixed Artesunate plus Mefloquine". *Antimicrob Agents Chemother* 2010; 54 (9): 3730–7.
26. Sanjeev K, Anne-Catrin U and Richard KH. Artemisinins: mechanisms of action and potential for resistance. *Drug Res Update* 2004; 7: 233-44.
27. Efferth T, Dunstan H, Sauerbrey A, Miyachi H, Chitambar CR. The anti-malarial artesunate is also active against cancer. *Int J Oncol* 2001;18(4):767–73.
28. White NJ. Antimalarial drug resistance. *J Clin Invest* 2004; 113, 1084–92.
29. Golenser J, Judith HW, Krugliak M, Hunt NH, Grau GE. Current perspective on the mechanism of action of Artemisinins. *Int J Parasitol* 2006; 36:1427–41.
30. Gautam A1, Ahmed T, Sharma P, Varshney B, Kothari M, Saha N, Roy A, Moehrle JJ, Paliwal J. Pharmacokinetics and pharmacodynamics of arterolane maleate following multiple oral doses in adult patients with *P. falciparum* malaria. *J Clin Pharmacol* 2011; 51(11):1519-28.

\*\*\*\*\*