

# ARTEMISIA AND ARTEMISININ, A STORY ABOUT TOXICITY

Chris J. van Boxtel, MD, PhD

*Emeritus Professor of Clinical Pharmacology, University of Amsterdam, Amsterdam, The Netherlands*

*This article is not intended to be more than a game of associations and the author does not want to imply that there is a real risk of artemisinin neurotoxicity.*



*Chris J. van Boxtel*

**M**alaria is still one of the major health problems in many tropical countries. The situation worsened with the increasing emergence of drug resistant strains of *Plasmodium falciparum*, the malaria parasite responsible for the 2 million people who die yearly from malaria. The problem with drug resistance is greatest in South-East Asia, where resistance or reduced sensitivity has been found against all antimalarial drugs including mefloquine, halofantrine and even quinine. Treatment of severe malaria relies now on the use of the latest antimalarials, the artemisinin derived agents<sup>1, 2</sup>.

## Artemisia, Qinghaosu and Wormwood

These compounds are derived from the herb *Artemisia annua*, in China known as 'qinghao' (Chinese for "from green herb"), which is a member of the 'Asteraceae' family. Qinghao, notwithstanding a bitter taste also known as 'sweet Annie' or 'sweet wormwood', is a fragrant annual herb, which now grows wild in a number of countries, including Australia, Argentina, Bulgaria, France, Hungary, Italy, Spain and the United States. It is native to Asia and has been used as antiparasitic therapy for malaria in Traditional Chinese Medicine (TCM) for more than 1,000 years. The earliest reference to the medicinal use of *Artemisia annua* in 'The Fifty Two Prescriptions' which was uncovered in ancient Chinese burial tombs during an archaeological dig in the 1970s, dates back more than 2,000 years. There are more than 300 species that comprise the genus *Artemisia*, many of which are sources of herbal medicines, spices or essential oils.

Chinese chemists isolated the primary active ingredient in *Artemisia annua* from the leafy portion of the plant in 1972 and called the crystalline compound, 'qinghaosu' - or 'artemisinin' in the West. Artemisinin is a sesquiterpene lactone that bears a peroxide group and unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. It is poorly soluble in water and decomposes in other polar solvents, probably by opening of the lactone ring. It is also poorly soluble in most apolar solvents and can thus only be administered orally. It shows a remarkable thermal stability. The peroxide moiety of artemisinin has appeared to be indispensable for chemotherapeutic activity<sup>2, 3</sup>. In vivo artemisinin is decomposed to dihydroartemisinin in which the integrity of the peroxide group is retained.

The mechanism of action of artemisinin and its derivatives is well known: artemisinin reacts in the parasite with iron (ferrous compounds)<sup>3,4,5</sup>. The haemoglobin within the infected erythrocyte is digested by the parasite, haem is released and neutralized by polymerisation into haemozoin with a high Fe<sup>2+</sup> content. When artemisinin comes into contact with high iron concentrations, a chemical reaction is produced which creates free radicals that attack cell membranes, breaking them apart and killing the single-cell parasite. The 'explosive' nature of this mechanism of action of artemisinin and its derivatives is of interest and gave rise to the hope that it could prevent any rapid occurrence of resistance. However, the Ukraine word for artemisia is 'Chernobyl' and we should bear in mind that the formation of free radicals has a potential for serious adverse reactions.

## Artemisinin and how it works

Artemisinin derivatives act as blood schizontocidal agents effectively inhibiting the late stage ring parasites and trophozoites. They kill the *Plasmodium* parasites more quickly than any other antimalarial agent and are toxic to the parasite at very low concentrations. Thus far no in-vivo resistance has been described, making them also effective in the treatment of multiresistant malaria<sup>2,5</sup>. However, out of precaution it is advised that the artemisinin drugs should be reserved for situations where problems of resistance or unwanted side effects of the available antimalarial drugs are expected.

In patients with severe malaria oral treatment is often impossible and thus parenteral formulations were required. Therefore water soluble artesunate, the hemi-succinate of dihydroartemisinin and the oil soluble arteether were developed by Chinese scientists for intravenous and intramuscular administration respectively<sup>6</sup>. Development of oil soluble artemether was promoted by WHO and the water-soluble artelinic acid by the Walter Reed Army Institute of Research.

Oral formulations of artemisinin and its derivatives are absorbed rapidly but incompletely with considerable interindividual variability. Peak plasma concentrations are reached in 1-2 hours and most compounds have a short elimination half-life of 1-3 hours after oral intake. Artesunate acts like a prodrug with fast transformation into dihydroartemisinin and it has an elimination half-life of less than half an hour. Intramuscular and rectal dosing exhibit slower and more variable absorption and elimination. For arteether an elimination half-life of 23 hours has been reported in dogs and also in healthy subjects after a single intramuscular dose<sup>7</sup>. It is thus predictable, and it has also been shown that arteether will cumulate after multiple doses.

A striking pharmacokinetic phenomenon in multiple dose studies of some artemisinin analogs is a time dependent decrease in plasma concentrations, probably caused by autoinduction. Related to the short elimination half-lives of most artemisinin drugs is the occurrence of recrudescences when they are given in short course monotherapy regimens. Therefore combination with a longer acting antimalarial drug is usually recommended.

Characteristic for the artemisinin drugs is their swift onset of action with clearance of parasites from the blood within 48 hours in most cases. A meta-analysis showed a survival-benefit

with artemisinin drugs compared to quinine in the treatment of severe (complicated or cerebral) malaria. The conclusions of a recent Cochrane review were as follows<sup>8</sup>: "The evidence suggests that artemisinin drugs are effective and safe for treating uncomplicated malaria. There is no evidence from randomised trials that one artemisinin derivative is better than the others. In areas where there is mefloquine resistance, combination therapy with an artemisinin derivative appears to improve sustained parasite clearance compared with either drug alone."

It can be concluded that artemisinin and its derivatives are a major breakthrough in the fight against a very prevalent and deadly disease<sup>9</sup>.

These drugs have surprisingly few adverse effects<sup>10</sup>. Regimens of artemisinin derivatives given to patients with acute falciparum malaria as single agents were associated with the following mild side effects; acute nausea (16%), vomiting (11%), anorexia (34%), and dizziness (15%). Nevertheless, repeatedly concern has been expressed centering on possible neurotoxicity<sup>11</sup>. In all experimental mammals tested (rats, dogs, primates), intramuscular injections of the oil-soluble antimalarial artemisinin derivatives artemether and arteether have produced an unusual pattern of selective damage to brain stem centers predominantly involved in auditory processing and vestibular reflexes. Neurological findings included gait disturbance, loss of spinal and pain response reflexes, and prominent loss of brain stem and eye reflexes. Artemether dose-dependent neuropathologic damage to the brain stem was shown in the mouse<sup>12</sup>. The neurons in the lower brain stem trapezoid nucleus, the gigantocellular reticular nucleus, and the inferior cerebellar peduncle were the most sensitive to the toxic effects of artemether. All mice with neuropathologic changes also showed behavioural changes. Most importantly, in vitro it was shown that Artemisinin induces oxidative stress in cultured neurones, as concluded from an increase of reactive oxygen species and extensive lipid peroxidation<sup>13</sup>. Especially in brain stem cultures a markedly deficient induction of antioxidant enzymes was observed.

Malaria itself can cause cerebral symptoms. Cerebral malaria induces changes in mental status and coma and is the main cause of death in Plasmodium falciparum malaria. The histopathological hallmark of this encephalopathy is the sequestration of red blood cells in cerebral capillaries and venules and it has been shown that reduced red cell deformability contributes to the derangement of the microcirculation<sup>14,15</sup>. High concentrations of artemisinin cause oxidation of red blood cell membrane proteins and then also decrease red cell deformability, especially in the presence of exogenous haem<sup>4,16</sup>. It can therefore not be excluded that these agents may affect plasma membrane function in infected erythrocytes.

Registration in Europe of several artemisinin drugs for the treatment of malaria was realized in the late nineties of the previous century. Just to explain our interest in these drugs, the Department of Clinical Pharmacology at the University of Amsterdam was heavily involved in the development of the fixed dose combination of artemether with lumefantrine<sup>17</sup>. Arrangements were made by the manufacturer of this combination, which have led to a differential price for developing countries relative to developed countries. In the Western world it is marketed under the name Riamet. The manufacturer has agreed to provide the product at a non-profit price to WHO under the name of Coartem. At the meeting in April 2002 of the 12th Expert Committee on the Selection and Use of Essential Medicines, artemether/lumefantrine was added to the WHO Model List of Essential Drugs.

### An artistic detour

By coincidence, the Metropolitan Museum of Art had in February of 2002 the exhibition 'Orazio and Artemisia Gentileschi: Father and Daughter Painters in Baroque Italy'. We will now leave

science for a short while and look a little bit over the boundaries of our professional discipline because, apart from the obvious association with Artemis, the Greek goddess of the hunt - who also defends women in labour, children, and small animals, artemisia can be linked to other fascinating figures in mythology, art and even religion. Orazio Gentileschi (1563-1639) and his daughter Artemisia (1593-1652) were among the most important Italian baroque painters who worked in Rome during the first decades of the 17th century. To demonstrate that especially Orazio is still not forgotten we can mention that to illustrate the CD-Rom with the Hutchinson Encyclopaedia of Music the Helicon Company selected from the thousands of famous paintings in art history that have a bearing on music a work by Orazio Gentileschi.

In the autumn of 1998 in Paris the movie 'Artemisia; the Passion of Painting' was released which tells the story of Artemisia Gentileschi. In short, Artemisia accused her teacher Agostino Tassi of raping her when she was nineteen but then the court tried to force her by torture to withdraw her accusations. As a direct aftermath of this incident Artemisia produced some of her most famous paintings, paintings which seem to have some affiliation with artemisinin as we will see.

When we talk about the passion of painting we inevitably also have to turn to Vincent van Gogh whose passion for painting turned into madness. There are many speculations about the etiology of this madness but an attractive hypothesis is that it was caused by the neurotoxicity of absinth, especially in combination with turpentine. Vincent van Gogh's fondness of absinth is well



Self-portrait by Vincent Van Gogh

known. Less known is the fact that he used to lick his brushes and thus ingested considerable amounts of turpentine. Turpentine is a volatile mixture of hydrocarbon isomers obtained either from pine gum or pinewood. It is a skin, eye, mucous membrane, and upper respiratory tract irritant in humans and may cause gastrointestinal, urinary tract and also central nervous system effects. Ingestion of turpentine causes, among others, excitement, ataxia, confusion, stupor and seizures. Actually van Gogh's own doctor, Dr Gachet of Auvers-sur-Oise, suggested that he was suffering from the effects of too much southern sun and of turpentine poisoning.

Absinth is made with an extract from artemisia absinthia which is known under the names of 'wormwood' or 'annual wormwood'. It is often assumed that Linnaeus named the plant family of which artemisia absinthia is the most common European species, after Artemis, the Greek goddess of the hunt who was called Diane by the Romans. It is therefore not amazing when Shakespeare makes a reference to artemisia absinthia in 'A Midsummer Night's Dream' he talks about "Dian's bud" (Act IV Scene I). However it is much more plausible that Linnaeus was not thinking of Diane but of Artemisia, the

wife of the Corinthian king Mausoleus, because the main interest of this Artemisia were plants and her botanical gardens while nowhere in mythology any link can be found between the goddess Diane and herbs, plants or flowers.

### The rise of absinth

The pharmacological activity of wormwood was already known in ancient history. Hippocrates recommended absinth for jaundice, rheumatism, anemia, and menstrual pains. The Roman scholar Pliny the Elder called it *apsinthium* in the first century A.D. and noted that it was customary for the champion in chariot races to drink a cup of absinth leaves soaked in wine to remind him that even glory has its bitter side. Absinth derives from the Greek word *apsinthion*, which means 'undrinkable', presumably because of its bitter taste. Wormwood is mentioned in the Judeo-Christian Bible at least a dozen times. It is noted for its intense bitterness (Deuteronomy 29:18; Proverbs 5:4; Jeremiah 9:15; Amos 5:7) and in the symbolical language of the bible wormwood stands for bitterness, affliction, remorse and punitive suffering. During the day of his crucifixion Christ is offered twice a drink of vinegar, made of light wine rendered acid, the common drink of Roman soldiers. While He was on the cross the gospels tell us: "Now there was set a vessel full of vinegar: and they filled a sponge with vinegar, and put it upon hyssop, and put it to his mouth." (John 19:29). Rembrandt made a series of etchings of Christ on the Cross and on one of them the soldier with the sponge drenched in acid wine is depicted. However already earlier, when they arrived at Golgotha we can read in the gospel of Matthew: "They gave him vinegar to drink mingled with gall: and when he had tasted thereof, he would not drink." (Matt 27:34), or, according to Mark (15:23), "mingled with myrrh". Both expressions mean the same thing, namely, that the vinegar was made bitter by the infusion of wormwood, usually given, according to a merciful custom, as a sedative to those who were crucified. However, Christ, knowing this, refuses to drink it. Tintoretto probably shows this first offering on his most famous painting of the crucifixion.



*Crucifixion by Tintoretto*

Modern absinth allegedly was invented in 1792 by a French doctor called Pierre Ordinaire, who fled the revolution in France to settle in Couvet, a small village in western Switzerland. Like most country doctors, he prepared his own remedies, and being acquainted with the use of wormwood in ancient times, he began experimenting with it. After his death, he supposedly left a secret recipe, which ultimately came in the hands of a man named Henri-Louis Pernod. Thus begins the origins of the first product made by the famous Pernod label in Switzerland and France. Originally, Pernod's product was given to French soldiers in Algeria as a medicine against fever. This happened to be a well known indication for wormwood and in many countries in Europe, also in Holland, fever in general and malaria in particular were treated with a herbal medicine obtained from *artemisia absinthia*.



*Absinth by Degas*

By the mid to late 1800s, the reputation of absinth was at its peak. Some very famous and popular painters and writers of the day such as Van Gogh, Toulouse Lautrec, Picasso, Verlaine, Oscar Wilde, and Baudelaire claimed to have received their inspirations while drinking absinth. However, *artemisia absinthium* contains an active narcotic derivative, the monoterpene thujone, which can cause central nervous system disorders and generalised mental deterioration<sup>18</sup>. A very clear description of the effects of absinth is given by Oscar Wilde: "After the first glass, you see things as you wish they were. After the second, you see things as they are not. Finally, you see things as they really are, which is the most horrible thing in the world."<sup>19</sup>

From 'the green fairy' absinth turned into 'the green curse of France'. The havoc that was caused by absinth in the late 19th century, as portrayed by for example Degas in his painting 'Absinth', resulted in a strong anti-absinth campaign and between



*Judith and Holofernes by Artemisia Gentileschi*

1905 and 1913 Belgium, Switzerland, the United States, and Italy banned absinth. The French government banned it officially in 1915. While the thujone content of old absinth was up to 260 ppm, currently available versions of absinth have a thujone content of 8-9 ppm, still within the European Commission's upper limit of 10 ppm.

## Adverse reactions

We now need to go back to Artemisia Gentileschi and her most famous painting, which shows the beheading of Holofernes by Judith. The Old Testament narrates the episode of Judith who saved her city (Judith Ch. 10-13). Judith was a Jewish widow whose town, Bethulia, was under siege by the army of the Assyrian general, Holofernes. She went to Holofernes' tent as an emissary and seduced him. Helped by her maid, Abra, Judith then decapitated Holofernes in his sleep and smuggled his head back to Bethulia. If we remember that Artemisia was raped by her teacher, it is not difficult to imagine that in Judith she is probably painting herself and that this painting is a revenge. Can we then see this painting as a symbol of the power of artemisia against malaria or should we look at it as a warning for the potential neurotoxicity of artemisinin derivatives?

In conclusion, we have seen that at least one species of artemisia can have serious adverse neurological reactions in humans. Furthermore, the mechanism of action of artemisinin through the formation of free radicals is potentially toxic in nature. In experimental animals neurological damage could be induced with several artemisinin derivatives and in vitro artemisinin, through an increase of reactive oxygen species, induces oxidative stress in cultured neurones. Finally, it is not excluded that artemisinin derivatives may influence the deformability of red blood cells and that therefore their possible neurotoxicity could masquerade as cerebral malaria.

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Still life with absinthe by Van Gogh