Malarial diseases continue to risk the lives of more than 3 billion people in 97 countries in the world, causing sickness in several million people and death to half a million patients. The preponderate malaria causing apicomplexan protozoan parasite species *Plasmodium falciparum* and *Plasmodium vivax* have become genetically resistant to most of the approved antimalarial drugs, including the artemisinin-based combination therapies (ACTs). At this time, there is a vigorous need to make enough efforts to meet the challenge of combating multi-drug resistant malaria by (a) speeding up the trials in progress on relatively more effective, new and mechanistically different antimalarial pharmaceuticals, (b) production of effective vaccines against *falciparum* and *vivax* malaria, (c) devising of new ways to use the presently available anti-malarials such as by using three-drugs ACTs and by using the different two-drug and three-drug ACTs sequentially, and (d) induction of *Artemisia annua dry leaf therapy* (ALT) of recent origin, but of ancient precedent, as an effective treatment for acute and complicated malaria. Here, a perspective type review is presented of the: pre-ALT antimalarial drugs, methodology of their usage and consequences of resistance development; safety, efficacy, affordability, quality maintenance and resilience to resistance development aspects of ALT; and possibilities of ALT re-purposement for treating many infectious-metabolic disorder related- and cancerous-diseases. In conclusion, an urgent need is emphasized for pilot studies and clinical trials on ALT to attest its deployment as anti-malarial and cure for diseases beyond malaria.

**Keywords:** Antimalarial Drug-Resistance; Antimalarial Pharmaceuticals; Auto-Immune Diseases; Cancers; Infectious Diseases; Non-Artemisinic Secondary Metabolites.
as 2015, 2016), 3.5 billion people in 97 countries were at risk of getting infected with malarial parasite(s) (Fig. 1). Actually, in each of these years, several hundred million humans got malarial infections and about half a million patients, preponderantly young children, elderly, and pregnant women, succumbed to the disease. In 2016, about 90% of malaria in Southeast-cum-South Asia region was contributed by India (World 2016 Malaria report). In approximately last ten years, since the introduction of artemisinin combination therapy (ACT) as the treatment of malaria and regulation of parasite transmission, at least ten countries have become largely malaria free. During this period, due to success in control of the disease causing parasite by chemotherapeutic treatments, such as ACT, prophylaxis, and control of mosquito attacks by use of pyrethroid insecticide impregnated bednets and indoor insect repellants (Landier et al., 2016; Dondrop et al. 2017; Sluydts et al., 2017), the loss of life from malaria has been halved. For the last 72 years, from the time chloroquine was introduced as a substitute/alternative of quinine in malaria treatment, the disease has been contained by use of five classes of individual pharmaceuticals (aminoquinolones, aminoalcohols, antifolates, hydronaphthoquinone and endoperoxides) and their combinations. However, malarial parasites have developed genetic resistance against most (perhaps all) of the effective antimalarials and their combinations. Besides, the resistant parasites have become geographically widespread. The vector mosquitoes have also developed resistance to insecticides used to impregnate bednets. The new affordable antimalarial chemical compounds and vaccines undergoing tests and trials are thought to be at least a decade away (Dondrop, 2017; White, 2017; Kazmin et al., 2017; Lopaticki et al. 2017; Sissoka et al., 2017; Nasamu et al., 2017; Bisland et al. 2018; Cowell et al. 2018; Kisalu et al. 2018). All these factors have posed a grave challenge for the control of malarial disease worldwide in coming years. Discussion is in progress on ways to increase the life span of currently available pharmaceuticals by employing them in alternate combinations, to resist the resistance power in the parasite and combat parasite transmission. At this time, when new effective and affordable malarial treatments are being eagerly

Fig. 1: World map of malaria endemic areas: the green coloured areas = transmission rate of malaria is high; peach coloured areas: relatively lower malaria transmission rate; indigo colored areas = recently became malaria free; slate coloured areas = have been malaria free for long time (reproduced with requested permission: Rabinovich R N et al., Plos Med 14, e1002456)
awaited, a botanical treatment that appears to clear (artemisinin) resistant malaria has been recently described. Daddy et al. (2017) have reported success in curing 18 cases of severe malaria by administering to the patients tablets made of dry leaves of *Artemisia annua* (the natural rich source of the pharmaceutical artemisinin) plants. This treatment called *Artemisia annua* dry leaf therapy (ALT) was found to have cured malaria caused by parasites resistant to the currently used antimalarials, including artemisinin derivatives (Fig. 2). Perspectives of this novel, highly promising and innovative development are discussed in this review.

![Fig. 2: ALT tablets made from dry Artemisia annua cv Sanjeevani leaves. a = A plant of *A. annua* cv Sanjeevani at pre-flowering development stage; b = *A. annua* freshly harvested leaf; and c = Tablets made by compressing the dried *A. annua* leaves](image)

To delineate the importance of origin of ALT as a safe, efficacious and affordable antimalarial treatment, with multi-repurposing possibilities, this review (perspective) deals with several relevant subject matter areas. The first section defines characteristics of different species of malaria causing parasites, their vectors and endemicity along with several features of disease development. This is followed by chemical, biological and antimalarial properties of currently used (approved) antimalarials. Next are described the choices and methodologies of administration of antimalarials to adult, child, pregnant and lactating women patients of acute and complicated malarias. Nature of mutations that render parasites species resistant to individual and multiple antimalarial drugs and circumstances that allowed soft and hard sweeps of certain artemisinin resistant mutation(s) is discussed next. This is followed by a section on discussions taking place to devise strategies to combat drug resistant malaria, especially artemisinin resistance. The next section covers aspects about the origin, empirical basis and evidence on clinical efficacy of ALT. Following it are sections that discuss the quality maintenance and cost aspects of ALT. The final section brings into focus the possibilities of repurposing ALT as treatment for a variety of autoimmune, metabolic and cancerous diseases.

**Kinds of Malaria and Symptoms**

There are about 200 different unicellular eukaryotic apicomplexan obligate narrow host-range parasite species of the genus *Plasmodium*, transmitted by dipteran insect species, whose infection can cause various kinds of malarial diseases in a wide range of vertebrates. The five major species of *Plasmodium* that cause malaria in humans are *falciparum* (Pf), *knowlesi* (Pk), *malariae* (Pm), *ovale* (Po) and *vivax* (Pv). The important properties of these human malarial parasites are comparatively summarized (and the references concerned with this section are also given) in the Table 1. Among the human malarial parasites, Pk is known to be a zoonotic species whose infection in several species of macaque monkeys produces malaria-like symptoms. Recently, another zoonotic species- *Plasmodium simium* (Ps)- has been found to cause malaria in humans in the Atlantic Forest Area of Brazil. The natural hosts for Ps are monkeys of the genera *Aloulta, Brachyteles, Cebus* and *Sapajus* (Brasil et al., 2017).

The insect hosts of *Plasmodium* species are anopheline mosquitoes. Out of about 515 known species of *Anopheles*, approximately 70 are vectors of human malaria (Sinka et al., 2012). Each of Pf, Pk, Pm, Po, Pv and Ps is transmitted to humans by several to many *Anopheles* species, in the geographical areas of their occurrence. The genomes of human malarial *Plasmodium* species and of the major *Anopheles* vector species have been sequenced (Neafsy et al., 2015; Rufledge et al., 2017). The vector for Ps has been identified as *Anopheles kerteszcia cruzii* (Brasil et al., 2017). Phylogenetically,
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characters</th>
<th>Plasmodium falciparum (Pf)</th>
<th>Plasmodium vivax (Pv)</th>
<th>Plasmodium ovale curtisi (Poc)</th>
<th>Plasmodium wallikeri (Pow)</th>
<th>Plasmodium malariae (Pm)</th>
<th>Plasmodium knowlesi (Pk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(A) Features of (n=14) genome Size (Mb)</td>
<td>23.3</td>
<td>29.1</td>
<td>33.5</td>
<td>33.5</td>
<td>33.6</td>
<td>24.4</td>
</tr>
<tr>
<td>2</td>
<td>Estimated gene number</td>
<td>5355</td>
<td>6671</td>
<td>7165</td>
<td>6340</td>
<td>6559</td>
<td>5284</td>
</tr>
<tr>
<td>3</td>
<td>G+C content (%)</td>
<td>19</td>
<td>40</td>
<td>29</td>
<td>29</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>Pre-erythrocytic growth in hepatocytes (hepatic schizogony) (number of days = d)</td>
<td>5-7</td>
<td>6-9</td>
<td>8-9</td>
<td>14-16</td>
<td>14-16</td>
<td>6-9</td>
</tr>
<tr>
<td>5</td>
<td>Whether relapse causing hypnozoites are formed in liver ?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Incubation period (d)</td>
<td>8-15</td>
<td>10-21</td>
<td>12-20</td>
<td>18-60</td>
<td>10-12</td>
<td>10-12</td>
</tr>
<tr>
<td>7</td>
<td>Fever cycle (erythrocytic schizogony) (number of hours = h)</td>
<td>Tertian (48)</td>
<td>Tertian (48)</td>
<td>Tertian (48)</td>
<td>Quartan (72)</td>
<td>Quotidian (24)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Nature of red blood cells affected</td>
<td>All types of erythrocytes</td>
<td>Reticulocytes</td>
<td>Reticulocytes</td>
<td>Mature erythrocytes</td>
<td>All kinds of erythrocytes</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>(B) Features of life cycle in human host Size of parasitaemia (number of parasites per µL of blood (x10^3))</td>
<td>20-500</td>
<td>20-50</td>
<td>9-10</td>
<td>5-10</td>
<td>0.5-10</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Whether cytoadherence of parasite causes microvascular dysfunction ?</td>
<td>Yes</td>
<td>Rarely (if at all)</td>
<td>Rarely (if at all)</td>
<td>Rarely (if at all)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Whether severe malaria develops ?</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Whether recrudescence occurs ?</td>
<td>Yes (when treatment fails)</td>
<td>Yes (when treatment fails)</td>
<td>Rare</td>
<td>Yes (sometimes after 30 to 50 y from the primary attack)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Time of appearance of gametocytes (d after the start of parasitaemia)</td>
<td>8-14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>(C) Features of life cycle in mosquito host Transmission causing Anopheles mosquito vector species</td>
<td>Many species (&gt;70), most prominent are: gambiае, culicifacies and stephensi</td>
<td>Many species (&gt;71), most prominent are: aquasalis, culicifacies, stephensi, darlingi and dirus</td>
<td>Several species (~10), most prominent are: funestus, gambiae, stephensi, freeborni, dirus, farauti and atroparvus</td>
<td>Many species (&gt;30), most prominent are: culicifacies, aconitus, arabiensis, atroparvus and freeborni</td>
<td>Several species including: craccus, hackeri, latens and bala-bacensis</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1: Contd....

<table>
<thead>
<tr>
<th>16</th>
<th>(D) Major geographical areas of prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>World-wide tropical and subtropical areas (especially in Africa, Asia and Mediterranean)</td>
</tr>
<tr>
<td></td>
<td>World-wide subtropical areas (especially in Asia, Latin America and Africa)</td>
</tr>
<tr>
<td></td>
<td>Tropical regions of Africa and Asia and in Pacific islands, sympatrically (subspecies)</td>
</tr>
<tr>
<td></td>
<td>World-wide tropical and subtropical areas (including Pacific islands)</td>
</tr>
<tr>
<td></td>
<td>Southeast Asia and South Asia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>17</th>
<th>(E) Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Pf</em> is the preponderant cause of malaria. The <em>falciparum</em> malaria is the deadliest and if not treated timely</td>
</tr>
<tr>
<td></td>
<td>the acute (or uncomplicated) malaria turns into cerebral (or complicated) malaria</td>
</tr>
<tr>
<td></td>
<td><em>Pv</em> can cause severe disease and death due to splenomegaly. The Duffy blood group deficient in Africa when infected are often symptomless</td>
</tr>
<tr>
<td></td>
<td>In some cases relapse can occur as late as 4-5 years from initial inoculation</td>
</tr>
<tr>
<td></td>
<td>It is less life-threatening than vivax and falciparum malarials. However, it can cause chronic lifetime infection</td>
</tr>
<tr>
<td></td>
<td>This parasite is zoonotic, also causes malaria in the monkeys <em>Macaca fascicularis</em>, <em>M. nemestrina</em> and <em>Presbytis melalophos</em>.</td>
</tr>
<tr>
<td></td>
<td>The disease in humans is mild, but can be lethal (mortality H~2%).</td>
</tr>
<tr>
<td></td>
<td>The Duffy blood group people in West Africa are often insensitive to this parasite.</td>
</tr>
<tr>
<td></td>
<td>Transmission occurs from humans to monkey and vice versa. Human to human transmission is rare (perhaps via the vector <em>A. dirus</em>)</td>
</tr>
</tbody>
</table>

References: Antinori et al. (2017); Barber et al. (2017); Epstein et al. (2017); Rutledge et al. (2017); White (2017); York (2017); Anvikar et al. (2016); Camargo-Ayala et al. (2016); Doctor et al. (2016); Molina-Cruz et al. (2016); Sutherland (2016); Josling and Leianas (2015); Sharma et al. (2015); St. Laurent et al. (2015); Tainchum (2015); WHO (2015c); Naing et al. (2014); Visser et al. (2014); White et al. (2014); Hii and Rueda (2013); Singh and Daneshwar (2013); Boussena and Drakeley (2011); Pain et al. (2008); Collins and Jaffrey (2007 and 2005); Carlton et al. (2008); Kakkilaya 2003; Gardner et al. (2002); Silvestrini et al. (2000); Hansbook et al. (1996); Garnham (1966); www.antimicrobe.org/new/bo5.asp; www.cdc.gov/malaria/about/biology/parasites.htm; emedicine.medscape.com/article/221134; www.parasitesinhumans.org/plasmodium-falciparum-malaria.htm.
distance-wise the parasite species are related to each other as follows: Pf → Po → Pm → Pk, Pv and Ps (Hall, 2012; Brasil et al., 2017). In terms of the frequencies of malaria infections caused by them in humans, parasites fall in the following order: Pf > Pv > Po, Pm > Pk > Ps. The malarias caused by Pf, Pv and Pk can be fatal if not treated. The Po and Pm caused malarias are less severe and generally not lethal. Pv, Ps and Po caused infections can remain dormant in the liver for up to many months. Pm infection can remain latent for years. The Duffy blood group deficient (ackr1 = atypical chemokine receptor 1) humans (who are largely the inhabitants of West Africa) are resistant to infection by Pv and Pk because the parasites are unable to invade their Fy a b erythrocytes (de Carvalho and de Carvalho, 2011).

The Pf, Pv, Po, Pm, Pk and Ps malarias have differential distribution. Pv is the most widespread malaria; it is the major malaria causing parasite in subtropical areas of Asia, Americas and Africa. Nearly half of the malarial cases that occur outside of Africa are related to Pv infection. More dangerous than Pv malaria, Pf malaria is predominant in Africa, but also occurs in tropical regions of Asia and in Middle East. Pf malaria is responsible for 90% of malarial deaths in Africa. The distribution of Pm malaria is similar to that of Pf malaria except that it is much less frequent. Both Po and Pm are the cause of malaria in Pacific islands. There are two subspecies of Po called P. curtisi and P. wallekeri, both are cause of malaria in Africa and Asia, sympatrically. Together, Po and Pm account for about 10 million cases of new malaria each year. Malaria caused by Pk occurs largely in southeast- and south-Asia. The Ps malaria is limited to Brazil. In areas where frequency of occurrence of malarial infections is high, mixed infections of more than one Plasmodium species have been observed (Mayxay et al., 2004). Recently, a rare case of malaria caused by infection of Pf, Pv, Po and Pm has been reported from forest area in Central India (Krishna et al., 2017) which has high incidence of mixed infection.

Initial symptoms of malaria are often as non-specific as one or more of the following type of sickness: fever, chills, sweating, fast heart rate, sore throat, cough, pneumonia, headache, muscular pain, joint pain, fatigue, difficulty in swallowing, hyper-salivation, jaundice, nausea, weakness, vomiting, constipation and enlargement of spleen. Laboratory diagnosis is essential to confirm malaria. The most reliable diagnosis is the detection of parasite-infected red blood cells through microscopic examination of thick and thin blood films. The rapid diagnostic tests (RDTs), based on detection of parasite antigens, can be used, but should not substitute for the needed microscopic tests (Kakkilaya, 2003). Once diagnosed, a confirmed malaria patient should immediately begin receiving the WHO prescribed treatment at the earliest.

The findings of microscopic test/s are helpful in classifying malaria as uncomplicated or severe. In cases of the non-complicated malaria, the parasitemia (% of parasitized red blood cells) is lower than 2%. If parasitemia is 10%, the malarial patient is facing the severe form of disease. The symptoms of the severe malaria include high fever and one or more of the following conditions: renal impairment (dark urine and limited output) acidosis, hypoglycemia, spontaneous bleeding, breathing difficulties, severe anemia, prostration or coma. Young children and pregnant women are not only more vulnerable to malarial infection but also prone to developing severe malaria. Consequences of severe malaria in pregnant woman include miscarriage, stillbirth, premature birth and birth defects in neonates. Generally, all kinds of malaria cause bone loss due to chronic bone inflammation and adversely affect functioning of skeletal and heart muscles due to poor supply of nutrients and oxygen (Marrelli and Brotto, 2016; Lee et al., 2017). There occurs macrovascular dysfunction in Pf and Pk malaria due to adherence of infected cells to walls of blood vessels (White, 2017). The above kinds of deficits imposed by malaria, span of morbidity, possibility of death can all be checked by anti-malarial drug treatment which also aims to clear malarial parasites from the body of malarial patient such that malaria does not relapses and transmission to mosquitoes is blocked. Antimalarial drugs are also used as chemoprophylaxis, in mass drug administration campaigns to limit the spread of malaria in endemic areas, and to travelers visiting the malaria endemic areas.

**Currently Used Antimalarial Drugs**

The antimalarial drugs include; quinine, mefloquine,
halofantrine and lumefantrine (aryl aminoalcohols); chloroquine, amodiaquine and piperaquine (4-aminoquinolones); primaquine (8-aminoquinoline); pyronaridine (mannich base); atovaquone (napthaquinone); proguanil, pyrimethamine and sulfadoxine (antifolates); tetracycline, doxycycline and clindamycin (antibiotics); and artesunate, dihydroartemisinin and artemether (artemisinin derived endoperoxides). The Table 2 illustrates chemical structures, purpose and regimen of administration to malaria patients, biological effects on Plasmodium parasites and prescription properties. There are large inter-class and intra-class differences in the properties of the drugs. In vivo half-life of artemisinins is short (0.5 to few hours) as compared to that of lumefantrine, pyronaridine, pyrimethamine, sulfadoxine, piperaquine and chloroquine (3 to 60 days). Quinine and artemether are highly insoluble in water and are usable for parenteral application. Artemisinins are very fast acting drugs. Quinine, chloroquine, piperaquine and artemisinins are able to block transmission of parasites to mosquitoes. Primaquin too blocks transmission but also prevents Pv and Po malaria relapses. Quinine, mefloquine, lumefantrine, atovaquone and artemisinins do not allow multiplication of parasite in mosquitoes. Unlike proguanil, pyrimethamine, sulfadoxine and atovaquone target singular but different parasite functions, whereas artemisinin derivatives, chloroquine and quinine exemplify antimalarials which target multiple functions in the parasites.

To overcome deficiencies of individual chemotherapeutics and to slow down resistance development, anti-malarials are now used in combinations. The following combinatorial regimen have been recommended by WHO to cure various kinds of malaria (Table 2): chloroquine + primaquine (against Po and Pv malaria); quinine + tetracycline or clindamycin (against severe malaria); ACTs = artemether + lumefantrine or mefloquine, dihydroartemisinin + piperaquine, artesunate + pyronaridine or sulfadoxine + pyrimethamine or artesunate + amodiaquine (against uncomplicated malarias, especially those caused by Pf). Primaquine or alternatively tafenoquine is given additionally to stop relapses and transmission; both are contraindicated for G6PD deficient patient (Table 3: Elmes et al. 2008; Graves et al., 2018). The tertian malaria caused by Ps is curable by chloroquine + primaquine treatment (Brasil et al., 2017). The combinations used for chemoprophylaxis in endemic areas are atavoquone + proguanil and proguanil + chloromycetin. For chemoprophylaxis mefloquin and doxycycline are also used preferably singly.

Life Cycle Stages at which Plasmodia are Killed by the Anti-Malarial Drugs

The Fig. 3 gives a diagramme of the life cycle stages of malarial parasites in human host and mosquito vector. Table 1 has quantitative data about parasite life cycle stages in all the five kinds of malaria. Life cycle stages at which the anti-malarials kill the parasites are identified in Table 2. The references from which information is described in this section, and summarized in Fig. 1, Table 1 and Table 2, was collated are given in the figures and tables.

The human host is inoculated with a small number (>10) of sporozoites when an infected female mosquito bites to obtain a blood meal. Sporozoites reach liver through blood and lymphatic system and invade hepatocytes. There is no antimalarial drug that blocks this process. The parasites in liver cells develop into schizonts, wherein the parasite undergoes many divisions to produce thousands of merozoites. The liver tissue schizonts rupture and release the merozoites into the blood stream. There are no clinical symptoms of malarial illness all through this stage. Merozoites developing in their liver schizonts are killed by several antimalarial drugs: primaquine, atavoquone, proguanil, pyrimethamine, tetracycline, doxycycline and artemisinin derivatives (artemisinins). The liver stage is completed in about 5-9 days from inoculation, except that in Pm malaria, it takes up to 16 days. Some Pv and Po infected hepatocytes produce hypnozoites. These dormant parasites can get activated and cause relapsed malaria, any time up to 5 years. Hypnozoites are eliminated from the liver by the drug primaquine.

The merozoites released from liver invade red blood cells (RBCs). In the infected erythrocytes (RBCs), parasite forms schizonts in which it produces 16-32 merozoites per schizont. When these schizonts have ruptured and the released merozoites have invaded fresh erythrocytes, an erythrocytic-schizont cycle of parasite infection is completed. Only upon completion of 3-8 such cycles, the parasite density in blood reaches the pyrogenic level of 50/µL. At this stage febrile paroxysm lasting 8-12 h appears. In this period the body temperature progressively rises and
Fig. 3: Life cycle of the malaria causing parasites of Plasmodium species (adapted after Kumar et al. 2015). Five species of the unicellular protozoan Plasmodium that cause malaria in humans are falciparum, vivax, ovale, malariae and knowlesi. Genome sequences of all of these species are now known (Table 1). Humans get the transmitted malarial parasites
when infected anopheline female mosquito bites them to obtain blood as its food. There are more than 500 species of Anopheles mosquito of which more than 60 serve as malaria vector. Genome sequences of the entire major malaria vector Anopheles species have been described (Neafsey et al., 2015). Life cycle of malarial Plasmodium species is complex and consists of several stages in each of the vertebrate and mosquito hosts. There are two asexual stages (in liver and red blood cells) and a sexual stage (in red blood cells) of Plasmodium life cycle in humans. (a) Hepatic schizogony: In this asexual phase the parasites inoculated into human host by mosquito, in the form of sporozoites, multiply asexually in liver parenchyma cells to produce a huge population of merozoites. Each sporozoite produces a few thousands of merozoites in schizonts. Not shown in the figure is an alternative pathway taken by parasites of P. vivax and P. ovale at this stage, wherein parasites enter a dormant phase to become hypnozoites. These latent parasites are the cause of relapse malarias, after several to many months from the initiation of infection as they reenter hepatic schizogony. (b) Erythrocytic schizogony: The liver produced merozoites infect red blood cells, wherein they pass into schizont stage after completion of ring and trophozoite stages. Each erythrocytic schizont produces many merozoites via asexual cell proliferation. Thus parasite load on the host increases enormously which leads to the appearance of clinical symptoms of malaria. (c) Gametocytogenesis: The pre-merozoites present in fraction of red blood cell schizonts get committed to sexual differentiation. Two types of gametocytes are formed: macro (female)- and micro-(male). The gametocytes are precursors of gametes. Gametocytes get ingested by the female mosquitoes that bite infected humans to obtain their blood meal. There are four stages of parasite development in the mosquito host. (d) Gametogenesis: Gametocytes ingested from human host enter into gametogenesis in the lumen of mosquito gut. Microgamocyte exits erythrocyte and differentiates into a female gamete. The microgamocyte upon exit from erythrocyte undergoes divisions there to produce eight flagellated male gametes by a process that has been called exflagellation. Fertilization involves fusion between a male gamete and a female gamete (both haploid like merozoites and gametocytes) to form zygote(s). (e) Ookinetization: One or more than one zygote is formed, depending on the number of female gametocytes ingested by the mosquito. Each zygote turns into a motile ookinete, during this process its diploid nucleus undergoes meiosis to produce haploid nuclei. This is the stage wherein genetic recombination occurs resulting in the production of new genotypes of the parasite. (f) Oocyst development: Ookinete moves to form a cyst on the midgut epithelium. In the oocyst so produced, the haploid parasites multiply repeatedly to form thousands of sporozoites. (g) Programming of sporozoites: Oocyst ruptures and sporozoites get released into haemocoel. Sporozoites migrate to the salivary glands where they are programmed to eject and enter human skin at the time when infected mosquito carrying them probes the human host for a bite to obtain a blood meal. P. knowlesi has several macaque monkeys as vertebrate hosts in addition to humans. It has been experimentally shown that the following types of malaria transmission can occur: mosquito → monkey or human; monkey → monkey; human → human. However, there is little evidence for the natural human to human transmission of knowlesi malaria. Whereas the natural transmission of all kinds of malaria in human occurs when infected mosquito bites, malaria of all kinds can also be transmitted by mother to newborn, blood transfusion, organ transplanting or usage of contaminated needles or syringes.

References
Barber et al., 2017; White, 2017; Doering et al., 2015; Josling and Llianas, 2015; Guttery et al., 2012; Janse et al., 1986
Table 2: Structure, activity and related features of different classes of antimalarials, that are presently in use against various developmental stages of *Plasmodium falciparum* (*Pf*), *Plasmodium vivax* (*Pv*), *Plasmodium ovale* (*Po*), *Plasmodium malariae* (*Pm*) and *Plasmodium knowlesi* (*Pk*) caused human malaria(s) and genetic markers of resistance detected against the antimalarials in *P. falciparum* and *P. vivax*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antimalarial compound (drug)</th>
<th>Prescription-cum-administration</th>
<th>Malarial parasite lifecycle stage(s) on which known to be active</th>
<th>Current knowledge about mechanism of action on parasite(s) and indication (purpose) of usage</th>
<th>Genetic markers, in <em>Plasmodium falciparum</em> (<em>Pf</em>) and <em>P. vivax</em> (<em>Pv</em>) parasites, for the resistance that has developed against the antimalarial</th>
<th>Remarks</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(A) Aryl amino-alcohols</td>
<td>Quinine</td>
<td>Parenteral or oral</td>
<td>Blood schizontidal, gametocytocidal and sporontocidal</td>
<td>Inhibits heme detoxification; used to treat malaria unresponsive to other drugs and to control transmission</td>
<td>Single nucleotide polymorphisms (SNPs) in <em>Pfmdr1</em>, <em>Pfcrtb</em>, <em>Pfalc</em> and gene amplification in <em>Pfmdr1</em></td>
<td>The drug is not to be given to the patients of tinnitus and optic neuritis</td>
</tr>
<tr>
<td>2</td>
<td>Mefloquine</td>
<td>14-18 d Oral 250mg tablets (Larium; is relatively expensive)</td>
<td>Blood schizontidal and sporontocidal against <em>Pf</em>, <em>Pv</em>, <em>Po</em> and <em>Pm</em> malaria</td>
<td>Inhibits heme detoxification and parasite's ribosomes; used in treatment of acute uncomplicated malaria</td>
<td>SNPs and copy number increase in <em>Pfmdr1</em>; SNPs in <em>Pvmdr1</em></td>
<td>It is not to be given to pregnant women and neuropsychiatric patients</td>
<td>Cowman et al. (1994); Sullivan et al. (1998); Price et al. (2004); Lelievre et al. (2012); Gargano et al. (2012); Gogtay and Ferner (2015); Grigg et al. (2016); Livezey et al. (2016)</td>
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<tr>
<td>3</td>
<td>Halofantrine</td>
<td>1-90 h Oral 250mg tablets (Halfan; is relatively expensive), 1500mg in six doses over 18h period</td>
<td>Blood schizontidal in <em>Pf</em>, <em>Pv</em>, <em>Po</em> and <em>Pm</em> malaria</td>
<td>As above</td>
<td>As above</td>
<td>It is contra-indicated for cardiac disease patients</td>
<td>Croft et al. (2007); Friedman and Caflisch (2009); Oluyomi et al. 2009; Pluczinski et al. (2015)</td>
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<td>4</td>
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<td>Lumefantrine</td>
<td>3-6 d</td>
<td>Oral (artemisinin-based combination therapy = ACT) tablets of 120mg lumefantrine and 20mg of artemether (Coartem); four tablets to be taken twice a day for 4 days</td>
<td>Blood schizonticidal and sporontocidal Inhibits heme de-toxification; used together with artemether to treat acute malaria</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>4-</td>
<td></td>
<td>Chloroquine</td>
<td>30-60 d</td>
<td>Oral tablets of 250 and 500 mg (Aralen; is the cheapest antimalarial drug); 2.5g is administered in 48h</td>
<td>Blood schizonticidal in Pf, Pv, Po &amp; Pm malaria, and gametocytocidal in Pv malaria</td>
</tr>
<tr>
<td></td>
<td>(B)</td>
<td>4-</td>
<td>Amino-</td>
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<td></td>
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<td>quinolines</td>
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<td>6</td>
<td></td>
<td></td>
<td></td>
<td>Amodiaquine</td>
<td>5h</td>
<td>Oral tablets of 200mg; 25-35mg/kg body weight over 3d period</td>
<td>Blood schizonticidal treatment of acute uncomplicated malaria and alongwith sulfadoxine plus pyrimethamine for prophylaxis in travellers</td>
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<tr>
<td>7</td>
<td>Piperaquine</td>
<td>30d</td>
<td>Oral 18mg/kg body weight of Piperaquine plus 4mg/kg body weight of dehydroartemisinin (ACT) for 3d</td>
<td>Blood schizonticidal; given in combination with dehydroartemisinin to treat acute malaria and for transmission control and prophylaxis</td>
<td>Inhibits heme detoxification; the ACT is safe and effective against acute malaria in adults and children</td>
<td>Contraindicated in pregnancy</td>
<td>Davis et al. (2005); Moore et al. (2008); Dalessandro (2009); Friedman and Caflisch (2009); Naobya et al. (2010); Rijken et al. (2011); Delves et al. (2012a,b); Levelievre et al. (2012); Stack et al. (2012); Agarwal et al. (2013); Hodel et al. (2013); Masanja et al. (2013); Amato et al. (2016); Witkowski et al. (2017); Hohlund et al. (2017); Bassat and Menendez (2017); Thanh et al. (2017)</td>
</tr>
<tr>
<td>8</td>
<td>(C) 8-Aminoquinoline</td>
<td>7h</td>
<td>Oral 15mg and 26.3mg tablets</td>
<td>Eliminates hypnozoites, liver stage schizonts, and gametocytes and has very weak blood stage activity against <em>Pf</em> malaria</td>
<td>Impairs mitochondrial functions; to cure and prevent relapse of <em>Pv</em> and <em>Po</em> malaria and prevention of <em>Pv</em> malaria; stops transmission from humans to mosquitoes</td>
<td>Resistance is known but not the markers</td>
<td>Contraindicated for pregnant and breast feeding women and glucose 6 phosphate dehydrogenase (G6PD) deficient individuals</td>
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<td>9</td>
<td>(D)</td>
<td>Mannich base</td>
<td>Pyronaridine</td>
<td>&lt;3-8d</td>
<td>Oral (ACT) tablets of 180 mg Pyronaridine and 60 mg Artesunate (Pyramax); given one/d for 3d</td>
<td>Blood inhibits β-haematin formation</td>
<td>SNPs in Pfmrp1</td>
</tr>
<tr>
<td>10</td>
<td>(E)</td>
<td>Atovaquone</td>
<td>Napthaquinone</td>
<td>59h</td>
<td>Oral tablet of 1g Atovaquone and 400mg Proguanil (Malarone); one tablet/d for 3d, lower doses for children and 25% strength tablet for prevention</td>
<td>Liver stage and blood stage schizonticidal, and sporonticidal</td>
<td>Acts against mitochondrial bc1 complex and inhibits parasite's respiration process; given in combination with Proguanil to treat acute malaria and for prophylaxis</td>
</tr>
<tr>
<td>11</td>
<td>(F)</td>
<td>Antifolates</td>
<td>Proguanil</td>
<td>24h</td>
<td>As above</td>
<td>Mild liver stage schizonticidal and blood stage schizonticidal for Pf, Pv, Po malaria</td>
<td>Inhibits dihydrofolate reductase (DHFR) synthesis or folate synthesis; the drug is administered together with Atovaquone or Chloroquine to treat acute malaria and for prophylaxis</td>
</tr>
<tr>
<td></td>
<td>Pyrimethamine</td>
<td></td>
<td></td>
<td>Oral tablet of 25mg Pyrimethamine and 500mg of Sulfadoxine (Fansidar; is a cost effective drug); adults to take 2-3 tablets/week</td>
<td>Mild liver stage schizonticidal, blood stage schizonticidal and sporontocidal</td>
<td>Inhibits dihydrofolate reductase (DHFR) synthesis and thereby folate synthesis; it is given along with sulfadoxine to cure acute malaria, even in pregnant women</td>
<td>As above</td>
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<td>12</td>
<td>Pyrimethamine</td>
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<td></td>
<td>&lt;3-&gt;4 d</td>
<td>Oral tablet of 25mg Pyrimethamine and 500mg of Sulfadoxine (Fansidar; is a cost effective drug); adults to take 2-3 tablets/week</td>
<td>Mild liver stage schizonticidal, blood stage schizonticidal and sporontocidal</td>
<td>Inhibits dihydrofolate reductase (DHFR) synthesis and thereby folate synthesis; it is given along with sulfadoxine to cure acute malaria, even in pregnant women</td>
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<td>13</td>
<td>Sulfadoxine</td>
<td></td>
<td></td>
<td>&lt;4-&gt;8 d</td>
<td>As above</td>
<td>Blood stage schizonticidal in Pf malaria but relatively ineffective in Pv malaria</td>
<td>Inhibits the dihydropteroate synthesis (DHPS) and thereby folate synthesis; indication as above</td>
</tr>
<tr>
<td>14</td>
<td>(G) Tetracycline</td>
<td></td>
<td></td>
<td>8-11h</td>
<td>Oral 250mg tablets (Sumycin and other brands; is cost effective)</td>
<td>Slow acting liver and blood stage schizonticidal</td>
<td>Protein synthesis inhibitor; given along with Quinine</td>
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<td>I</td>
<td>II</td>
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<td>15</td>
<td>Doxycycline</td>
<td>15-25h</td>
<td>Oral 250mg tablets; is highly cost effective</td>
<td>As above</td>
<td>Inhibitor of protein synthesis; given along with fast acting anti-malarial to cure acute malaria and as prophylaxis for travellers</td>
<td>As above</td>
<td>Not to be given to pregnant women</td>
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<tr>
<td>16</td>
<td>Clindamycin</td>
<td>3h</td>
<td>Oral 75mg to 900mg capsules; is cost effective</td>
<td>Blood schizonticidal</td>
<td>Inhibition of protein synthesis, given along with Quinine to patients unable to tolerate Tetracycline</td>
<td>As above</td>
<td>Not to be given to patients of intestinal disease</td>
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<tr>
<td>17</td>
<td>(H) Artesunate Endoperoxides</td>
<td>0.5-1.4h</td>
<td>Oral as ACT@ 4mg/kg body wt of Artesunate with 10mg/kg body wt of Amodiaquine or 25mg/kg body wt of Mefloquine or 25mg/kg body wt of each of Sulfadoxine and Pyrimethamine; all for 3d; or Artesunate as suppository</td>
<td>Fast acting liver and blood stage schizonticidal, incomplete gametocytocidal and sporontocidal; given along with combination ACT in acute malaria; parenterally and as suppository in cerebral malaria; calcimycin calcium ATPase and heme metabolism; prescribed to cure acute malaria as ACT partner and in severe malaria</td>
<td>Fe (II) present in heme proteins clears the peroxide bond, the free radicals thus generated react with essential parasite proteins inactivating them, such as sarcoplasmic endoplasmic reticulum calcium SNPs in Pf kelch-13; (the haplotypes C580Y is undergoing hard selective sweep in Southeast Asia); amplification of Pfmdr1</td>
<td>Not to be given to the patients of respiratory disease, and pregnant and lactating women</td>
<td>Pukrittayakamee et al. (2000); Dondorp et al. (2009); Rottman et al. (2010); Charman et al. (2011); Zhang et al. (2012); Barber et al. (2013); Delves et al. (2013); Klonis et al. (2013); Ashley et al. (2014a); Ashley et al. (2014b); Arney et al. (2014); Henriques et al. (2014); White (2014); Gopalkrishnan and Kumar (2015); Strainer et al. (2015); Cui et al. (2015); Tilley et al. (2016); wwwwarn (2016); Antony and Panja (2016); Al-Zaydani et al. (2016); Kremsner et al. (2016); Hemming-Shroeden and Lo (2017); Imwong et al. (2017); Abraham (2017)</td>
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<td>18</td>
<td>Dihydroartemisinin</td>
<td>0.5-0.75h</td>
<td>As shown at serial no. 7</td>
<td>Fastest acting Artemisinin derivative that does not allow trophozoite formation and is gametocytocidal and sporontocidal</td>
<td>Mode of action as above; treatment of acute malaria as an ACT component</td>
<td>As above</td>
<td>As above</td>
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<tr>
<td>19</td>
<td>Artemether</td>
<td>4-11h</td>
<td>As shown at serial no. 4; the drug is lipid soluble and is used orally, rectally and intramuscularly</td>
<td>Mode of action like Artesunate; As an ACT it is a frontline drug for acute malaria</td>
<td>As above</td>
<td>As above</td>
<td>As above</td>
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</table>

*a-i = Antimalarial resistant drug alleles: a, mdr1 = Multi-drug resistance gene on chromosome 5; b, crt = Chloroquine resistance transporter gene on chromosome 7; c, nhe1 = Sodium/hydrogen exchanger gene on chromosome 13; d, mrp1 = Multi-drug resistance associated proteins gene on chromosome 1; e, plasmepsin-2, -3 = Aspartyl (protease plasmepsin gene located on chromosome 4; f, cyt b = cytochrome B gene on mitochondrial genome; g, dhfr = Dihydrofolate reductase gene on chromosome 8; h, dhps = Dihydropteroate synthetase gene on chromosome 4; i, kelch-13 = Kelch 13 propeller gene located on chromosome 13, (the gene product has three domains an apicomplex domain, a BIB/POZ domain and a β-propeller Kelch domain)*
bone marrow and only the final stage is accomplished in the blood stream. Generally, the gametocytes are short-lived, except that in Pf malaria, once formed they remain viable for months. The gametocytes are not responsible for any clinical symptoms. The following anti-malarials are able to block gametocyte formation: quinine and chloroquine in Pv and Pm malarials; and primaquine and artemisinins in all kinds of malarials. The artemisinins do not eliminate gametocytes completely.

The route of malaria spread in human is human → female mosquito → human. Mosquitoes transmit the gametocytes from infected humans as a part of their blood meal. In the mosquito gut, the gametocytes exit erythrocytes and undergo gametogenesis. A female gametocyte develops into a female gamete. A male gametocyte divides into 8 male gametes, each male gamete is flagellated. The gametes, gametocytes, merozoites and sporozoites are all haploid (n = 14). The haploid male and female gametes fuse to form a zygote (2n = 28) which differentiates into an oocyst. The oocyst undergoes meiosis and subsequently the haploid products in it multiply to produce thousands of sporozoites. Upon rupture of oocysts, the sporozoites migrate to salivary glands where they get programmed for inoculation into human host via carrier mosquito’s bite. The sporogony cycle from gametocyte formation to sporozoite production in oocysts and their accumulation in salivary glands is blocked by several antimalarial drugs, including the following: quinine, mefloquine, lumefantrine, atavaquone, proguanil, primaquine, methylene blue and pyromethamine.

**Treatments Based on Currently Used Antimalarial Drugs**

The Table 3 presents a summarized account of first line treatments, for acute and severe malaria caused by different species of malaria parasites in adults, pregnant women and young children, recommended by WHO. The recommendations are a result of the scores of trials carried out, for the cure of different kind of malaria in endemic areas owing to their occurrence in Africa, Americas, Pacific islands, Southeast-and South-Asia, on adult men and women, pregnant and lactating women and young children. Some of the references on which the WHO recommendations are based are given at the bottom of the Table 3. The dosages of drugs for children are to be adjusted with the body weight. Some drugs are prescribed when malarial patients suffer from concurrent ailments or inherited metabolic deficiencies, the prescriptions for each of the antimalarial drugs are given in the Table 2. Most importantly, primaquine is not to be administered to pregnant and/or breast feeding women. Because severe malaria patients can suffer from blockage in blood flow, filling up of fluid in lung’s air sacks, clotting in blood vessel, renal failure, and/or seizures etc., they must be treated under intensive care environment. Any concurrent bacterial infection in malaria patients should receive immediate attention, along with malaria treatment.

The options for the treatment of uncomplicated malaria in adult men and women are: artemether + lumefantrine; dihydro-artemisinin + piperaquine; atavaquone + proguanil; quinine + doxycycline. Along with a drug combination, a dose of primaquine ensures control of transmission. The treatment for Pv, Po or mixed malaria is one of the following: artemether + lumefantrine; dihydro-artemisinin + piperaquine; chloroquine. For Pm and Pk malaria, the drug recommended is chloroquine. For Pf malaria in adult men and women are: artemether + lumefantrine; dihydro-artemisinin + piperaquine; atavaquone + proguanil; primaquine, methylene blue and pyromethamine. Chloroquine is the drug recommended for children against Pf malaria are: artemether + lumefantrine; dihydroartemisinin + piperaquine; atavaquone + proguanil; quinine + clindamycin. Chloroquine is the drug recommended for children against Pv, Po, Pm and Pk malaria. The pregnant women afflicted with any kind of malaria are recommended to use quinine + clindamycin or artemether + lumefantrine and those having non-Pf malaria are also recommended chloroquine. The treatment options for the patients with severe malaria in adult men and women caused by all kinds of parasites are: intravenous artesunate for one or more days until the patient can swallow tablets, but not more than 5 days, followed by a full course of artemether + lumefantrine, dihydro-artemisinin + piperaquine or quinine + tetracycline; or intravenous quinine for 2 days or until the patient can begin to swallow tablets, followed by a full course of quinine + doxycycline. In the severe malarial cases caused by Pv or Pk, the intravenous treatments are to be followed by full course of chloroquine. The pregnant women patients with severe Pf malaria are to be given intravenous artesunate or quinine treatments, like that for adult...
Table 3: The prevalent antimalarial treatment regimens against un-complicated and complicated malaria(s) in adults, pregnant and breast feeding women and children

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Indicative malarial condition</th>
<th>Malaria caused by</th>
<th>Plasmodium falciparum</th>
<th>P. vivax, P. ovale, P. malariae or P. knowlesi</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>(A) Uncomplicated malaria: (i) in adults</td>
<td>Oral: Artemether + Lumefantrine (the drug(s) of choice); Dihydroartemisinin + Piperaquine (not to be administered to patients suffering from cardiac condition(s))&lt;sup&gt;a&lt;/sup&gt;; Atovaquone + Proguanil (known to produce pronounced gastrointestinal side effects); Quinine + Doxycycline&lt;sup&gt;b&lt;/sup&gt;; and a single dose of 0.25mg/kg body weight of Primaquine on the first day or 15 mg/Kg of Methylene blue for three days</td>
<td>Oral: Artemether + Lumefantrine&lt;sup&gt;a&lt;/sup&gt;; Dihydroartemisinin + Piperaquine&lt;sup&gt;b&lt;/sup&gt; or Chloroquine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Oral: Chloroquine&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>(ii) in pregnant women</td>
<td>Oral: Quinine + Clindamycin (in: all trimesters)&lt;sup&gt;e&lt;/sup&gt;; or Artemether + Lumefantrine (in all trimesters)</td>
<td>As in column 2; item 2; or Chloroquine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>As in column 3; item 2</td>
</tr>
<tr>
<td>3</td>
<td>(iii) in children (&lt; 12 years)</td>
<td>Oral: Quinine + Clindamycin&lt;sup&gt;g&lt;/sup&gt;; Atovaquone + Proguanil&lt;sup&gt;h&lt;/sup&gt;; Artemether + Lumefantrine&lt;sup&gt;i&lt;/sup&gt;; or Dihydroartemisinin + Piperaquine&lt;sup&gt;j&lt;/sup&gt;</td>
<td>Chloroquine</td>
<td>Chloroquine</td>
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<tr>
<td>4</td>
<td>(B) Severe or complicated malaria: (i) in adults</td>
<td>Intravenous Artesunate for 24h or more (or until the patient can swallow tablets, but not more than 5 days)&lt;sup&gt;k&lt;/sup&gt;, followed by a full course of Artemether + Lumefantrine&lt;sup&gt;a&lt;/sup&gt; or of Dihydroartemisinin + Piperaquine or Intravenous Artesunate treatment followed by a full course of Quinine + Doxycycline&lt;sup&gt;b&lt;/sup&gt;, or alternatively Intravenous Quinine for 48 hours or until the patient is able to swallow tablets, followed by oral Quinine + Doxycycline&lt;sup&gt;c&lt;/sup&gt;</td>
<td>As in column 2; item 4; or intravenous artesunate treatment followed by full course of Chloroquine</td>
<td>As in column 2; item 4</td>
</tr>
<tr>
<td>5</td>
<td>(ii) in pregnant women</td>
<td>Intravenous Artesunatei, followed by Artemether + Lumefantrine or Dihydroartemisinin + Piperaquine as in item 4 above; or Intravenous Quinine followed by a course of Quinine + Clindamycinl</td>
<td>Oral: Chloroquine (in all trimesters); Artemether + Lumefantrine (in all trimesters)</td>
<td>As in column 2; item 4</td>
</tr>
<tr>
<td>6</td>
<td>(iii) in children (&lt; 12 years)</td>
<td>A rectal suppository dose of upto 100mg (10mg/kg body weight) Artesunate followed by intravenous Artesunate or Quinine and thereafter dihydroartemisinin + Piperaquine or Quinine + Clindamycin; as for adults with dosage adjusted as per body weight</td>
<td>As in column 2</td>
<td>As in column 2</td>
</tr>
<tr>
<td>7</td>
<td>Relapse (prevention): (i) in adults</td>
<td>Not applicable</td>
<td>Primaquineo (not to be administered to Glucose-6-phosphate dehydrogenase = G6PD deficient)&lt;sup&gt;p&lt;/sup&gt;</td>
<td>As in column 3</td>
</tr>
<tr>
<td>8</td>
<td>(ii) in pregnant and breast feeding women</td>
<td>As above</td>
<td>Chloroquine&lt;sup&gt;q&lt;/sup&gt; followed by Primaquine upon withdrawal of breast feeding</td>
<td>As in column 3</td>
</tr>
</tbody>
</table>

<sup>a</sup> = 4 tablets (such as of Coartem) followed by 4 tablets at 0, 8, 24, 36, 48 and 60 hours; <sup>b</sup> = 3 or 4 tablets (such as of Malarone) daily for 3 days; <sup>c</sup> = 4 tablets (such as of Eurartesim) daily for 3 days; <sup>d</sup> = 600mg Quinine sulphate every 8h for 5-7 days and 200mg Doxycycline daily; <sup>e</sup> = 600 mg Quinine sulphate every 8 h plus 450 mg Clindamycin every 8 hours for 7 days; <sup>f</sup> = 1 to 4 Malarone paediatric tablets (as per body weight from = 10 kg to = 40 kg); <sup>g</sup> = 1-4 tablets at 0, 8, 24, 36, 48 and 60 hours (as per body weight from = 15 kg to = 35 kg); <sup>h</sup> = ½ to 3 tablets followed by equal amount at 24 and 48 hours (as per body weight = 10 kg to = 60 kg); <sup>i</sup> = 2.4 mg/kg body weight injection of Artesunate at 0, 12 and 24 h and thereafter daily; <sup>j</sup> = starting dose of 20mg/kg body weight of Quinine hydrochloride in 5% dextrose over a 4h period, followed by 10mg/kg body weight of Quinine hydrochloride every 8h for up to 48h and later every 12h; <sup>k</sup> = 600mg Quinine sulphate three times a day a day for 5 to 7 days from the start of Quinine therapy, plus oral 200mg of Doxycycline each day for 7 days; <sup>l</sup> = Intravenous Quinine therapy to be followed by oral quinine, like in k, except in place of Doxycycline, Clindamycin (450mg) will be administered 3 times a day a day for a period of 7 days; <sup>m</sup> = 620mg at 0h, 310mg at 8h and 310mg on day2 and 3; <sup>n</sup> = 10 mg starting dose, then 5mg/kg at 8h and also on day 2 and 3; <sup>o</sup> = 15 to 30mg/day or 0.2-0.5 mg/kg body weight/day
for 14 days depending on body weight; p = The G6PD deficient may be administered 0.75mg/kg of Primaquine per week for 8 weeks; q = 500mg each week

**References:** Dicko et al. (2018); Abraham (2017); Antinori et al. (2017); Dellicour et al. (2017); Phuc et al. (2017); Plucinski et al. (2017); Thanh et al. (2017); Tine et al. (2017); Baird et al. (2016); Gutman et al. (2016); Herchline (2016); Kakara et al. (2016); Kremser et al. (2016); Laloo et al. (2016); Pekyi et al. (2016); Sirima et al. (2016); Verlinden et al. (2016); White (2016); Kheng et al. (2015); Kumar et al. (2015); Shanks et al. (2015); Sharma et al. (2015); Eziefu et al. (2014); White et al. (2014); WHO (2014); Barber et al. (2013); Cordel et al. (2013); Delves et al. (2013); Gogtay et al. (2013); Singh and Daneshwar (2013); Eder et al. (2012); Keating (2012); Ojango and Juma (2012); Achan et al. (2011); Dauly et al. (2011); Rijken et al. (2011); Kiszewski et al. (2011); Donderop et al. (2010); RCQG (2010); Achan et al. (2009); Sinclair et al. (2009); Hatz et al. (2008); Mc Gready et al. (2008); Hill et al. (2006); Karunajeewa et al. (2006); Kumar and Srivastava (2005); Mistland et al. (2005); Baird and Hoffman (2004); Donderop et al. (2005); Newton et al. (2013); van Hansbrock et al. (1996); White et al. (1983); WHO-HTM-GMP-2017.9-org.pdf; www.ctrmmcrb.org/new/bo.5.asp; www.cdc.gov/malaria/diagnosis_treatment/treatment.html; www.who.int/malaria/publications/atoz/9789241548528/en; www/apps.who.int/iris/bitstream/10665/181162/1/9789241509244_eng.pdf

men and women. Whereas artesunate treated pregnant women patients are to be given a full oral course of artemether + lumefantrine or dihydro artemisinin + piperaquine, those who received intravenous quinine will be given full oral course of quinine + clindamycin. The severely ill pregnant women, with any non-Pf malaria, will be given a full course of oral chloroquine or atemether plus lumefantrin, irrespective of the trimester of pregnancy. Young children with complicated malaria are to be first treated with artemunate given rectally, followed by the treatments (with dose adjustment according to the patient’s body weight) recommended for severely ill adult patients.

**Natural Selection of Genetic Resistance to Currently Used Antimalarial Drugs, in Malarial Parasites**

Like other organisms, human malarial parasite species are endowed to evolve, by induction of new mutations, amplification of variation by genetic recombination and selection of variant genotypes, for improved adaptation to changing environmental conditions, which in their case include; lethal anti-malarial drugs used singly and in combinations. The genetic variants of parasites become resistant to a drug when the gene product(s) targeted by the drug have become modified or the drug does not reach the site of its action because the process of its transport is disabled or it is effluxed out from its site of action. Drug resistance is acquired via selection of mutations that result in over expression and/or increased activity of one or more of target gene function(s) or deactivation of one or more host protective gene function(s). Several life cycle characteristics of malarial parasites, such as these mentioned below, are favourable for selection of drug resistance imparting mutations: (a) rapid rate of genome replication (Agarwal et al., 2017); (b) high mutation rate per genome replication cycle (Hamilton et al., 2017; William et al., 2017); (c) large population size within infected human and mosquito hosts (Table 1; Churcher et al., 2017); and (d) enormity of overall population size of parasites in humans and mosquito hosts in malaria endemic areas (Duffy et al., 2017). Besides, selection of drug resistant mutations also depends on the drug pharmacokinetics, purity and dosage; irregular and sub-optimal intake of drug favours selection of genetic drug resistance in parasite (Anyangwu et al., 2017). The combinatorial use of drugs with differing pharmacokinetics and mode of action, such as in ACTs, delay development of resistance against the drugs (Dondorp et al., 2009). Drug resistance in malarial parasites is known to have arisen faster for those drugs that targeted only one gene function in them. For example, resistance against proguanil, atavquone, pyrimethamine and sulfadoxine was selected and became observable within one or two years of their large scale usage (Paloque et al., 2016). Contrariwise, resistance against artemisinins, which have multiple targets in parasites, was observable 26 years after their large scale therapeutic adoption (Paloque et al., 2016).

The malarial parasite cells have in them a nuclear genome (n=14), and copies of mitochondrial and apicoplast organelle genomes (Gardner et al., 2002; Vaidya and Mather, 2009; Rai et al., 2017). The comparison of genome sequences of the sensitive and resistant isolates (clones) of parasites have allowed identification and mapping of molecular markers (point mutation, deletion and insertion polymorphisms) associated with anti-malarial
resistance. The molecular markers for resistance to different drugs have been found to be located on several different nuclear chromosomes and resistance to atovaquone to be located on the mitochondrial genome (Sharma, 2005; Antony and Parija, 2016; Menard and Dondorp, 2017). In the Table 2, the column VIII gives information about the target(s) of the 19 drugs; and the column IX lists molecular markers found associated with resistance selected in parasites for 15 drugs. The chromosomes in which drug resistant mutations are located are identified in the legend of Table 2.

Resistance of parasites towards tetracycline, doxycycline and clindamycin has not been observed and the identity of gene polymorphism(s) responsible for resistance against primaquine is yet to be established. It will be seen that mutations in the CRT and MDR1 genes render the parasite variably resistant to several drugs. The Pf crt polymorphism affects response of the parasite to quinine, amodiaquine, piperaquine and lumefantrine in addition to giving resistance to chloroquine. Pf mdr1 poses resistance to chloroquine, quinine, mefloquine, halofantrine, lumefantrine and artesiminin. There are examples of different mutant alleles in the same gene inducing differential responses in the parasite; mutations in the amino terminal and carboxy terminal of Pf MDR1 are of this type (Cui et al., 2015; Antony and Parija, 2016; Menard and Dondorp, 2017). Mutational polymorphism in the Pf kelch 13 gene has been responsible for resistance to artesiminin (Ashley et al., 2014; Miotto et al., 2015; Takala-Harrison et al., 2015; Amato et al., 2016; Li et al., 2017); 20 SNPs are known to have arisen independently in different locations – an example of multiple origin of soft sweep (adaptation to ART; Pennings and Hermisson, 2006). Among the Pf k 13 mutations, the C580Y allele not only imparted ART resistance to Plasmodium falciparum, but made the parasite fitter (Imwong et al., 2017). It got selected in the background of Pf crt, Pf dhps, PfΔhfr and Pfmdr1 mutations and its hard sweep has spread the allele widely in southeast Asia (WHO-multidrug-resistant-lineages--; Hemming-Schroeder and Lo, 2017; Imwong et al., 2017; Thanh et al., 2017). A symptom of ART resistance due to a C580Y type mutation is recrudescence or reappearance of Pf infection upon depletion or removal of artesiminin.

Many ring stage intra-erythrocytic C580Y parasites enter a quiescent state, because upregulation of the UPR (unfolded protein response) signaling pathway in them allows repair of proteins that underwent alkylation or oxidation, by free radicals released by disintegration of the peroxide bridge in ART when it comes in contact with iron from haemoglobin. The quiescent parasites are supported by minimal energy metabolism taking place in the parasite’s apicoplast and mitochondria. Quiescent parasites breakout as soon as ART is depleted (Mbengue et al., 2015; Paloque et al., 2016). Recently (Rossi et al., 2017), emergence of P. falciparum strains with pfmdr1, pfplasmepsin2 and pfkelch13 580Y triple mutations has been recorded in Cambodia.

There is emergent danger of this (C580Y type) and other multi-drug resistant strains getting entry into India and Africa. There is also evidence for de novo ACT resistance development in African areas of high Pf malaria endemicity (Lu et al., 2017; Daddy et al., 2017). Another observation of great concern is that artesiminin-resistant Pf clinical isolates, including perhaps the ACT resistant ones, can infect diverse mosquito species that transmit malaria in Southeast Asia and Africa (St. Laurent et al., 2015). WHO has developed a global technical strategy to control and eliminate malaria worldwide by 2030 (WHO:GST_Malaria_Eng.pdf). Some leading workers in this field have proposed additional strategies for malarial treatment and vector control and there has been emergence of a new treatment- the A. annua dry leaf therapy (ALT) for treatment of drug resistant malaria, which are discussed below.

**Strategies Proposed to Treat and Control Multidrug-Resistant Malaria**

The studies summarized in table 2 shows that resistance has developed against antimalarial drug in current use singly or in two drug combinations. It is visualized that in the absence of new drugs and vaccines (Kazmin et al., 2017; Zhang et al., 2018) in the near future, there is an urgent need to use the existing drugs in better ways and new combinations. The two treatments advised for chloroquine resistant Pf malaria are: (a) dihydroartesiminin + piperaquine with a dose of primaquine (White, 2016), and (b) administration of verapmil, the calcium channel blocker which serves as a chemo sensitizer, along
with chloroquine to improve drug efficiency (Verlinden et al., 2016). The possible treatments advised for ACT resistant Pf malaria are: (a) A new ACT combination of artemunate + pyronaridine to be introduced as treatment (White, 2016). (b) ACTs such as dihydroartemisinin + piperaquine and artesunate + mefloquine be used rotationally (Dondorp et al., 2017; Menard and Dondorp, 2017). (c) The period of use of prevalent ACTs to be extended from 3 days to up to 7 days (Dondorp et al., 2017; Menard and Dondorp, 2017). (d) ACTs to be used as combinations of a artemisinin drug with two partner drugs, such as artemether + lumefantrine + amodiaquine, and dihydroartemisinin + piperaquine + mefloquine (Shanks et al., 2015; Verlinden et al., 2016; Dondorp et al., 2017; Menard and Dondorp, 2017). (e) The double and triple drug ACTs to be used sequentially (Verlinden et al., 2016). (f) The combination of fosmidomycin and piperaquine to serve as a sure cure (Mombo-Ngoma et al. 2017). Another important suggestion is administration of a dose of the drug ivermectin in endemic areas along with the ACT or singly periodically on a mass scale (Dondorp et al., 2017; Menard and Dondorp, 2017). Ivermectin taken by mosquitoes along with the blood meal of ivermectin administered humans will have killing effect on them, thereby drastically controlling the malarial transmission (Chaccour et al., 2013; Kumar et al., 2015).

An entirely new strategy to treat multi-drug (ACT) resistant malaria has been developed, wherein tablets made of dried leaves of the A. annua plant (natural resource of artemisinin drugs) are used (Daddy et al., 2017). The origin and essential features of this highly affordable malaria therapy are discussed below.

**Artemisia Annua Dry Leaf Antimalarial Therapy (ALT)**

The ALT has been earlier called as the whole Plant based Artemisinin Combination Therapy (pACT; Weathers et al., 2014a). pACT was called a combination therapy because of the involvement of artemisinin and other metabolites present in the leaves of A. annua in the antimalarial therapeutic effect of Artemisia annua dry leaves (Weathers et al., 2011, 2013, 2014b a and b; Elfawal et al. 2012; Onimus et al., 2013; Weathers and Towler, 2014; Desrosiers and Weathers, 2016; Daddy et al., 2017). ALT is unlike the conventional ACTs (mentioned in Tables 2 and 3), in which the artemisinin component, extracted from A. annua (Kumar et al., 2000; Misra et al., 2014) or artemisinin synthesizing transgenic tobacco or Physcomitrella patens whole plant (Malhotra et al., 2016; Ikram et al., 2017), or semi-synthesized from Artemisia annua produced natural precursor(s) (Paddon et al., 2013; Paddon and Keasling, 2014; Ikram and Simonsen, 2017; Kung et al. 2018) is present in its derived pharmaceutical forms such as artesunate, arteether and dihydroartemisinin. ALT is a non-pharmaceutical antimalarial treatment that depends on artemisinin and many other metabolites naturally biosynthesized and present in the leaves of Artemisia annua plant, but for many of which the mode(s) of antimalarial action remains to be revealed. To get WHO recommendation, ALT has to go through extensive, essential, fundamental and clinical research which needs to demonstrate that ALT is safe, efficacious and would not promote development of resistance to artemisinin in malarial parasites (WHO, 2012).

ALT uses standardized tablets as the antimalarial drug prepared by compressing the dried pulverized leaves, harvested from cultivated plants of specific variety(ies) of Artemisia annua, which contain ≥1% artemisinin (Weathers and Towler, 2014; Daddy et al., 2017). The origin of ALT, as a dependable medicine against multi-drug-resistant malaria, is based on information from historical texts and a number of experimental findings. Some of the important empirical basis for ALT is annotated below:

(a) There is recorded evidence that the Chinese people have been using A. annua material as a remedy for fever and chills, such as those associated with malaria. One of the effective material consumed as traditional medicine was the consumption of juicy extract of water soaked A. annua leafy stems. The chinese traditional medicine literature does not report any case of resistance development against A. annua treatment used (Bensky and Barolet, 1990; Dhingra et al., 1999; Hongwen and Shouming, 2002; Hsu, 2006).

(b) The A. annua plant material has been used by human populations in various parts of the world, where the species existed naturally for various
purposes, including for medicinal uses and as an item of food for livestock and humans, without notice of any harmful effects (Bansky and Barolet, 1990; Brisibe et al., 2009; Yimer and Sahu, 2016) and therefore the species has been granted the GRAS (Generally Recognized As Safe) rating. Accordingly, *A. annua* leaves in amounts d” 30 g dry weight / day can be safely consumed (Duke, 2001; Wall and Watson, 2017).

(c) In a study, batches of healthy mice were orally fed on one hand, with an amount of artemisinin in its pure form, and on the other, were fed an equal amount of artemisinin in the form of *A. annua* dried leaves. The blood stream of mice fed with dry leaves contained > 40 times more artemisinin, as compared to mice fed with pure artemisinin. Mice were required to be fed with > 45 fold more of pure artemisinin (as a component of the normal mouse food) than artemisinin in dry *A. annua* leaves so that artemisinin could be detected in mouse blood stream (Weathers et al., 2011).

(d) In another study, it was observed that oral administration of the *Artemisia annua* leaves to the *Plasmodium chabaudi* - infected mice killed the parasite without causing toxicity to mice (ICIPE 2005; Elfwal et al., 2012; Onimus et al., 2013). It was further found that parasitaemia in the infected mice was reduced at least five fold more by a single dose of *A. annua* leaves as compared to an equivalent dose of pure artemisinin, and the effect of dry leaves lasted longer than that of pure artemisinin (Elfwal et al., 2012). The experiments at c and d above suggested that the presence of metabolites other than artemisinin in the dry leaves of *A. annua* improved both the bioavailability of artemisinin in the blood stream and therapeutic efficacy of artemisinin in the infected red blood cells. These possibilities were evidenced by correlating the phytochemistry of *A. annua* leaves with the response of healthy and parasite infected mice to feeding of pure artemisinin versus *A. annua* leaves, as above and below in e and f. Recently, using CaCo-2 model of intestinal transport, the digestates of *A. annua* dried leaves were found to improve the artemisinin transport by 37 % (Desrosiers and Weathers 2018).

(e) The leaves of *A. annua* plants are known to contain a number of classes of secondary metabolites including; artemisinic compounds other than artemisinin (Bhakuni et al., 2001; Brown, 2010; Mesa et al., 2015; Towler and Weathers, 2015; Weathers et al., 2017). Many of these possess varying levels of anti-plasmodial activity, albeit much weaker than in artemisinin. The non-artemisinin, antimalarial compounds affect the survival of parasites via mechanisms that are independent of that for artemisinin, or which determine the availability or activity of artemisinin at its site(s) of action. Some of the metabolites of *Artemisia annua* characterized for possession of their own kind of anti-*Plasmodium* activity, according to their chemical class, are as follows: artemisinic compounds = arteannuin B, artemisinic acid, dihydroartemisinic acid (Fig. 4; Allen et al., 1997; Suberu et al., 2013; Weathers et al., 2014); coumarin = scopoletin (Fig. 5; Ezcokonkwo and Obidoa, 2001; Ferreira et al., 2010; Malik et al., 2011); flavonoids = artematin, casticin, ciscineole, chrysoplenetin, chrysophenol-D, eupatorin, kaempferol, luteolin, myrcetin, quercetin (Fig. 6; Elford et al., 1987; Liu et al., 1992; Ching-Shue et al., 1992; Lehane and Saliba, 2008; Ferreira et al., 2010); phenolic acids = chlorogenic and rosmarinic acids (Fig. 5; de Magaehaes et al., 2012; Suberu et al., 2013; Towler and Weathers, 2014; Daddy et al., 2017); saponins (Fig. 5; Francis et al., 2002); sulfated polysaccharides (Xiao et al., 1996; Clark et al., 1997; Francis et al., 2002; Andrews et al., 2005); terpenes = artemisia alcohol, artemisia ketone, borneol, camphene, camphor, caryophyllene, 1, 8-cineole, germacrene D, limonene, myrcene, nerolidol, α-pinene, phytol, sabinene, spathulenol, α-terpineol (Fig. 7; Goulart et al., 2004; van Zyl et al., 2006; Grace et al., 2012; Suberu et al., 2013; Bilia et al., 2014; Weathers et al., 2014c; Towler and Weathers, 2015; Daddy et al., 2017). The flavonoids and phenolic acids in general inhibit the cytochrome enzymes, present in liver and intestine, that metabolize artemisinin to deoxyartemisinin, thereby increasing the bioavailability of artemisinin in the blood stream.
Perspectives of the Artemisia annua Dry Leaf Therapy (ALT) for Malaria

The methoxylated flavonoids increase the activity of artemisinin against the intra-erythrocytic plasmodia, by blocking the conversion of heme bound to artemisinin to hemozoin, such that there is an enhancement in the release of free radicals from the clearance of the peroxide bridge and eventually in the

(Elford et al., 1987; Liu et al., 1999; Svensson et al., 1999; Svensson and Ashton, 1999; Sanella et al., 2007; Lehane and Saliba, 2008; Sergent et al., 2009; Choi et al., 2011; de Magalhaes et al., 2012; Suberu et al., 2013; Towler and Weathers, 2015). The methoxylated flavonoids
killing of parasites (Rodriguez et al., 1972; Elford, 1987; Bilia et al., 2002 and 2008). The finding, that cytochrome enzymes, which metabolize artemisinin to deoxyartemisinin (Whirl-Carrillo et al., 2012) in the liver of the infected animals, are suppressed by flavonoids and phenolic acids was indicated in an animal-experiment by Weathers et al. (2014b). Separate batches of mice infected with *P. chabaudi* were fed with pure artemisinin and with artemisinin in *A. annua* dry leaves @ 100mg/ kg body weight and monitored for 2h for the presence of artemisinin and deoxyartemisinin in the blood stream. In mice fed with pure artemisinin, the
drug was detectable in the blood only after 60 min, whereas, artemisinin content in the blood constantly increased over the 120 min period in mice fed with artemisinin in *A. annua* dry leaves. In the leaf-fed mice, the content of deoxyartemisinin in the blood stream was much lesser than that in pure artemisinin-fed mice.

(f) ALT was shown to be effective against artemisinin resistant malarial infections and its
treatment resilient to resistance development in the animal model systems (Elfawal et al., 2015). Administration of a single oral dose of *A. annua* dry leaves (24 mg artemisinin/kg body weight) to rodents infected with artemisinin resistant *P. yoelli* cured their parasitaemia, whereas, an equivalent dose of pure artemisinin proved to be ineffective on corresponding animals. It was further shown that the stable resistance to *A. annua* dry leaf treatment, in *P. chabaudi* infected mice occurred 2.7 times slower than acquirement of resistance to pure artemisinin. Achievement of resistance to dry *A. annua* leaf treatment in *P. chabaudi* infected mice was found to be 1.6 times lower than that for the treatment with artesunate + mefloquine (ACT).

(g) The clinical use of ALT treatment on human patients with severe Pf malaria in the Democratic Republic of Congo proved the efficacy of ALT (Daddy et al., 2017). For ALT treatment, tablets of 500 mg weight, each containing 5.5 mg artemisinin, were prepared by compressing powdered dry leaves of Anamed-A3 variety of *A. annua*. The patients administered with the ALT treatment were 6 males and 12 females, of 14 months to 60 years of age, whose malaria did not get cured, neither with artemether + lumefantrine, nor with intravenous artesunate treatment. The malaria patients had entered the severe phase which included such symptoms as loss of consciousness, convulsions, frustration, shock, respiratory distress, pulmonary edema, bleeding, gastric distress and jaundice. Among the patients, the adults were administered one tablet twice daily for 5 days, children of 5-15 kg body weight and 15-30 kg body weight, were respectively given quarter and half tablet twice daily for 5 days, and those in coma or too young to swallow tablets, the tablet-dose was crushed, mixed with water and delivered via nasogastric tube. All the patients got cured of their malarial disease and there were no adverse side effects. In ALT treatment on another set of patients, rectal administration of dried pulverized leaves of *Artemisia annua* was found effective in curing Pf malaria (Abolaji et al., 2016). More extensive studies are needed that will cover 28 days of follow up after treatment with ALT.

From the evidence described above, about the roles of diverse phytochemicals present in the leaves of *A. annua* in augmenting the inhibitory/lethal effects of artemisinin in ALT on infections of *Plasmodium* species on animal model systems and about clinical efficacy and safety of ALT on human malaria patients, it is possible to conclude that ALT is an inexpensive but safe and effective option for treating acute and severe malaria. Since multiple secondary metabolites with independent lethal mode of action on malarial parasite are involved in the efficacy of ALT, it is possible to further conclude that it will take considerable time period before any resistance evolves against ALT treatment in malarial parasites or via it against artemisinin. It has been advised that safety of ART treatment in pregnant women be evaluated and that nausea resulting from oral intake of dry leaf tablets may be controlled by encapsulation or use of anthelmentics or sweet substances (Yarnell, 2014). Should there be recrudescence, the ALT treatment may be repeated or alternatively a triple ACT treatment be given.

**ALT: Establishment of the Compositional Consistency of Tablets**

Like for pharmaceuticals, stringent control over the quality of *A. annua* dry leaf tablets, during their manufacturing process, is essential for ALT’s inclusion in the first line antimalarial therapeutics. To achieve this objective in practice, all individual steps of the process must be standardized. To obtain leaves of high artemisinin content, only the identified genotypes of *A. annua* should be grown under consistent and specified cultivation conditions (cultivation_of_Artemisia_in_Africa_and_Asia.pdf; Ferreira et al., 1997; Kumar and Srivastava, 2005). To retain the secondary metabolites in high concentrations, the harvested shoots of field grown plants must be dried under clean and ambient conditions (Ferreira et al., 1992; Laughlin, 2002) to retain the secondary metabolites present in them in high concentrations. From the dry shoots, leaves are to be mechanical separated from stem on clean surface, the dry leaves produced from different fields should be homogenized, sieved, pulverized using a blade cutter or equivalent instrument, characterized and converted into tablets of standard weight, size and content of artemisinin and a few flavonoids and terpenes, under hygienic conditions (Weathers and Towler, 2014; Weathers et
The *A. annua* crops can be cultivated in temperate and sub-tropical agroenvironments, such as those available in the countries of central and southern Europe, Central Asia, Southeast Asia, South Asia, East Africa, South America and in Australia. Several genetically improved and bred varieties of wide adaptability, whose leaves upon drying contain 0.7 to 1.2% artemisinin, are readily available, including Anamed (A3), Jeevanraksha, Arogya and Sanjeevani (de Magalhaes *et al.*, 1997; Kumar *et al.*, 1999; Delabays *et al.*, 1993 and 2001; Ferreira *et al.*, 2005; Kumar and Srivastava, 2005; Simonnet *et al.*, 2008; Paul *et al.*, 2010; Gupta *et al.*, 2016). Besides, several to many seed industry bred varieties of *A. annua* are also available.

*A. annua* is a short day-flowering, open-pollinated annual shrubby species that completes its life cycle in up to one year time. The sowing and harvesting times of *A. annua* crops to obtain high quality produce of leaves have been prescribed according to the agro-climates of country-wise geographical locations of cultivation and variety(ies) (Ferreira *et al.*, 1992 and 1994; Laughlin, 1994 and 2002; Ram *et al.*, 1997; Gupta *et al.*, 2002; Kumar *et al.*, 2002 and 2004; de Magalhaes *et al.*, 2004; Brisibe *et al.*, 2009; Luo *et al.*, 2009; Goel *et al.*, 2011; Jiang *et al.*, 2013; Pop *et al.*, 2017). The nursery grown plants of 1 month or more age are transplanted in fields @ 20-70 thousand plants/ha, depending on the plant architecture and average field duration of plant population of the variety used. Nursery plants are raised by spreading the seeds on a wet soil surface, in a farmyard manure fertilized field. The amount of seeds required for planting 1 ha of crop is 3-5 g. Fields of sandy to sandy loam soil type are used and fertilized with manure and fertilizers @ N:P:K::60:40:40 kg/ha. The transplanted *A. annua* crop, to produce dry leaves for ALT, is harvested before flowering occurs on plants. The plant shoots are dried at temperatures <40°C, in field, under shade or in specially designed temperature controlled chambers. The desirable moisture content in the dried leaves is 10-12%. Dry leaves are stored and transported in the form of large blocks by compressing leaves in moulds.

*Artemisia annua* has been in commercial cultivation by farmers in India for more than 15 years, under the public-institution assisted farmer-company partnerships (Fig. 8; Kumar *et al.*, 2015). In recent years, such farmer-company partnerships have covered 2500 h/y, largely in the North-West India and in this region preponderantly in the Indo-Gangetic plains area. According to the agro-climate of the Indo-Gangetic plains, the most suitable time for the sowing of nursery is 15 December to 15 January. Seedlings are transplanted into the fields vacated by potato crops between 20 February and 1 March. This summer crop of *A. annua* is harvested between May 28 and June 5 (several weeks before the onset of monsoon rains) and shoots are dried under shaded conditions. Alternatively or additionally, the plants growing in nursery are transplanted in fields vacated by wheat crop from 15 May onwards, and the resulting crop is harvested between 21 September and 1 October (after the withdrawal of monsoon rains and with the

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**Fig. 8:** *Artemisia annua* cv Sanjeevani crops in farmer’s fields in the village Shivpura of district Unnao in the state Uttar Pradesh of the Indo-Gangetic plains area of India [Pictures shared by Sanjay Kumar of CIMAP, Lucknow; Kumar *et al.*, 2015]
onset of inflorescence development, but before flowering occurs). The autumn crop is dried in temperature controlled chambers. The yield of dry leaves, respectively, from the summer and autumn harvested crop is 2.5 and 3.5 T/ha, with 0.8 to 1.2% artemisinin content, depending on the variety used; highest levels of ART (1-1.4%) are present in the leaves harvested from crops of Sanjeevani variety (Sanjay Kumar, Ramesh Srivastava and Anil Gupta, personal communication).

Need is felt internationally for new genotypes of A. annua and for methodologies of plant population propagation, such that the individual plants under cultivation have the same genotype or largely similar genotypes. Since A. annua is an open pollinated crop, individual plants in populations of its registered varieties Anamed (A3), Jeevanraksha, Sanjeevani and others demonstrate phenotypic differences arising from segregation of alleles of thousands of genes which are present in heterozygous condition. A genomic study has confirmed presence of heterozygosity at a large number of protein coding genes, amongst 63226 genes identified in A. annua (Shen et al. 2018). The quality of dry leaf tablets from any available variety is the result of an average phenotype of its cultivated populations. In the future it is desirable to have ALT tablets from plants of a single genotype. There are several possibilities to pursue this aim. One of these is to develop elite inbred lines through selfing in existing varieties for 6 or more generations. The seeds of chosen inbred line will be always produced in isolation. Second, F1 hybrids of two selected inbred lines, selected for heterosis, may be chosen for cultivation. Again, F1 seeds will be produced from co-cultivation in isolation of the parental inbred lines whose own seeds will be produced in isolation. Special genotypes, an important one being photo-period independent early flowering, could be developed in the background of chosen singular genotype(s). When suitable genotype(s) have become available for mono-genotype-culture, an alternative method to produce planting material on a mass scale could be the deployment of micro-propagation procedures (Mathur and Kumar, 1996; Paul et al., 2012; Gopinath et al., 2014; Fei and Weathers, 2015, 2016). Any one selected plant from Jeevanraksha, Sanjeevani or Anamed (A3) could become a clonal variety with the use of micropropagation for genotype multiplication.

Cost-effectiveness of ALT Treatment

The ALT treatment in comparison to ACT treatment is highly cost effective. In the Indo-Gangetic plains area, the cost of cultivation, harvesting and processing of harvested shoots to obtain dry leaves of A. annua var Jeevanraksha, Arogya or Sanjeevani (all genetically related), and profit for farmers, under the farmer-private company partnership scheme, for two hectares of crop yield of 50 tons of dry leaves is ~ Rs. 2,00,000 (or ~US$ 3,500). The cost of producing 10 million tablets of 500 mg dry leaves each can therefore be speculated as ~ Rs 5,00,000 (or ~ US$ 8500). Considering the expenditure of all kinds on supply chain of ALT tablets, the cost of a “10 tablets treatment” of an adult is estimated as less than Rs 1 (or less than US Cents 17). The ALT treatment in India will be at least 60 to 150 fold less costly than an ACT treatment. It is possible to conclude that large scale adoption of ALT treatment as advised above can tremendously advance the aim of WHO and 97 malaria endemic countries, including India, to significantly reduce or eliminate the burden of malaria by 2030 (www.rollbackmalaria.org/about-rbm/aim-2016-2030; nvbdcp.gov.in>Doc>National-framework-for-malaria-elimination-in-India-2016-2030). ALT capsules have the added advantage of use as suppositories.

Possibilities of Using ALT Beyond Malaria

A variety of disease conditions in humans and livestock are known to respond curatively to artemisinic-, terpenoid-, and flavonoid-compounds present in A. annua leaves. There is thus a strong possibility that ALT tablets may prove to be of therapeutic value against many diseases beyond malaria. From a search of the enormous body of publications on the subject of artemisinin (including derivatives)- and A. annua-therapeutics, the tables 4 and 5 provide a short list of disease conditions which are controlled by treatment with artemisinin and/or its derivatives (artemisinins), A. annua’s dry leaf powder and/or leaf extracts (termed here as artemisannua). The Tables 4 and 5 also summarize some representative robust evidence which demonstrates that many virus-, bacteria-, fungi, protozoa-, and helminths-caused infectious diseases on the one hand and autoimmune-, and digestive systems/metabolic-disorders, and cancers on the other hand are attenuated/prevented by the treatment with artemisinins and artemisannua.
Perspectives of the Artemisia annua Dry Leaf Therapy (ALT) for Malaria

Table 4: Summarized evidence which shows that Artemisia annua leaf powder and/or leaf extract, artemisinin and/or artemisinin derivatives are inhibitory and putatively curative against a variety of infectious diseases in humans and livestock animals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Disease(s)</th>
<th>Causal infectious organism/agent</th>
<th>Host</th>
<th>Nature of study</th>
<th>Therapeutic test material(s)/compound(s) used</th>
<th>Observation(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Virus caused:</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Hepatitis B</td>
<td>Hepatitis B virus (HBV)</td>
<td>Human derived Hep.G2/2.2.15 cell line</td>
<td><em>In vitro</em></td>
<td>Artemisinin and artemunate</td>
<td>Both the drugs strongly inhibited viral replication without causing cytotoxicity</td>
<td>Romero et al. (2005)</td>
</tr>
<tr>
<td>2</td>
<td>Hepatitis C</td>
<td>Hepatitis C virus</td>
<td>Human derived HuH-2 cell line (HCV)</td>
<td><em>In vitro</em></td>
<td>Artemisinin</td>
<td>Viral replication was inhibited; this effect was potentiated by iron donated by hemin, without cytotoxicity</td>
<td>Paesbuiis et al. (2006)</td>
</tr>
<tr>
<td>3</td>
<td>Cytomegalovirus</td>
<td>Human cytomegalovirus</td>
<td>Human fibroblast cells</td>
<td><em>In vitro</em></td>
<td>Artesunate</td>
<td>Viral replication was inhibited, very strongly in the presence of iron, without toxicity</td>
<td>Kaptein et al. (2006)</td>
</tr>
<tr>
<td>4</td>
<td>Herpesvirus</td>
<td>Human herpesvirus 6A (HHe-6a)</td>
<td>Cultured human cells</td>
<td><em>In vitro</em></td>
<td>As above</td>
<td>Viral early and late gene expression and replication was inhibited and thereby viral multiplication was arrested</td>
<td>Milbrandt et al. (2009)</td>
</tr>
<tr>
<td>(B) Bacterial caused:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>Tuberculosis</td>
<td>Mycobacterium tuberculosis H37Rv</td>
<td>MGI 960 system, and Ogawa slant medium assay</td>
<td><em>In vitro</em></td>
<td>As above</td>
<td>A single dose strongly inhibited bacterial growth measured 21 days after treatment</td>
<td>Choi (2017)</td>
</tr>
<tr>
<td>6</td>
<td>As above</td>
<td>Sprague Dawley rats</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>3.5mg/kg dose a day for 4 weeks</td>
<td>As above cured the rats of infection without causing toxicity</td>
<td></td>
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<tr>
<td>(C) Fungus caused:</td>
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</tr>
<tr>
<td>7</td>
<td>Aspergillosis</td>
<td>Aspergillus fumigatus</td>
<td>Fungus</td>
<td><em>In vitro</em></td>
<td>Artemisinin</td>
<td>The drug killed the fungus by targeting fungal oxidative phosphorylation and cell wall and ergosterol synthesis pathways</td>
<td>Gautam et al. (2011)</td>
</tr>
<tr>
<td>(D) Protozoan caused:</td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>a) Acanthamoebiasis</td>
<td>Acanthamoeba castellani (free living amoeba)</td>
<td>Cultured trophozoites</td>
<td><em>In vitro</em></td>
<td>Artemether</td>
<td>Amoebae were killed</td>
<td>Deng et al. (2015)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Acanthamoeba castellani 309 and Ac32</td>
<td>As above</td>
<td><em>In vitro</em></td>
<td>Artemisia annua leaves-water, alcohol or chloroform extract</td>
<td>Amoebae were killed</td>
<td>Derda et al. (2016)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>BALB/C mice (Mus musculus)</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>As compared to untreated infected mice, the treated infected mice survived for 2 to 4 fold longer time period</td>
<td>As above</td>
</tr>
<tr>
<td>11</td>
<td>b) Cocci-diosis</td>
<td><em>Eimeria tenella</em></td>
<td>Domesticated chicken (<em>Gallus domesticus</em>)</td>
<td><em>Artemisia annua</em> dry leaves</td>
<td>Addition of dried leaves @ 20g/kg to feed was coccidostatic as well as growth promoter</td>
<td>Brisbe <em>et al.</em> (2008); Bosselman and Gylling (2013)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>As above</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>Addition of dried leaves @ 1.5% eliminated coccidiosis</td>
<td>Dragan <em>et al.</em> (2014)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>c) Leishma-niasis</td>
<td><em>Leishmania donovani</em> (Visceral infection)</td>
<td>Infected human macrophages</td>
<td><em>Artemisinin</em></td>
<td>Parasite load was reduced; the anti-leishmanial activity was via apoptosis of parasites</td>
<td>Sen <em>et al.</em> (2007 &amp; 2010)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>As above</td>
<td>BALB/C mice</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>Leaves and seeds of <em>Artemisia annua</em> or their hexane extract</td>
<td>Islamuddin <em>et al.</em> (2012); Mutiso <em>et al.</em> (2011)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>As above</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td>Essential oil of leaves of <em>Artemisia annua</em></td>
<td>Intra-peritoneal administration of the essential oil reduced the parasite burden in spleen and liver by 90% without toxicity to test animals</td>
<td>Islamuddin <em>et al.</em> (2014)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>As above</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td><em>Artemisinin</em></td>
<td>Administration of the drug loaded nanoparticles reduced the parasite burden and spleen- and hepato-megaly</td>
<td>Want <em>et al.</em> (2015)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><em>Leishmania major</em> (cutaneous infection)</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>Lesion size was reduced via induction of apoptosis in promastigotes</td>
<td>Ghaffarifar <em>et al.</em> (2015)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>As above</td>
<td>As above</td>
<td><em>In vivo</em></td>
<td><em>Artemisinin ointment</em></td>
<td>Ulcers were healed</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td><em>Leishmania panamensis</em> U-937 macrophage cell line (cutaneous infection)</td>
<td><em>In vitro</em></td>
<td><em>Artemisia annua</em> leaf powder</td>
<td>Amastigotes were inhibited without toxicity to macrophages and genotoxicity to lymphocytes</td>
<td>Mesa <em>et al.</em> (2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>As above (Mesocricetus auratus)</td>
<td><em>Hamster</em></td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>Intracellular amastigotes present in the ulcers were killed and 5 out of 6 treated hamsters were cured after 30 days treatment with 500mg/kg/day</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>As above</td>
<td>Humans</td>
<td><em>In vivo</em></td>
<td>As above</td>
<td>Two patients were cured after 45 days of treatment with 30g of leaf powder (666mg/day)</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>d) Toxi-plasmosis</td>
<td><em>Toxoplasma</em> Human foreskin fibroblast (HFF) (Obligate apicomplexan parasite)</td>
<td><em>In vitro</em></td>
<td><em>Artesunate</em></td>
<td>Infected cells killed much like intra-erythrocytic malarial parasites</td>
<td>Gomes <em>et al.</em> (2012)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>As above</td>
<td>CDI, OFI, Kunming <em>Mus musculus</em></td>
<td><em>In vivo</em></td>
<td><em>Artesunate, dihydroartemisinin and their combination</em></td>
<td>All three treatments reduced the infection and improved survival time period of the diseased animals</td>
<td>Sarciron <em>et al.</em> (2000)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>e) Trypa-no-somiasis</td>
<td><em>Trypanosoma brucei</em> (African sleeping sickness)</td>
<td><em>In vivo</em></td>
<td><em>Artemether</em></td>
<td>5 days of treatment eliminated the parasite</td>
<td>Akande and Fagbemi (2011); Yimar and Sahu 2016</td>
<td></td>
</tr>
</tbody>
</table>
Table 4 summarizes results of some studies on the effects of artemisinins and artemisinannua on viral-, bacterial-, fungal-, protozoan- and helminth-infections, in cell lines in vitro and/or on model animals or humans in vivo. The drugs artemisinin and artemesunate have been found to inhibit replication/multiplication of hepatitis causing hepatitis B (HBV) and C (HCV) viruses and sore inducing herpes virus and its close relative cytomegalovirus in cultured human cells. The in vitro growth of Mycobacterium tuberculosis (the bacterium which causes tuberculosis in humans), as well as the tubercular bacterial growth in infected mice, has been found to be arrested by artemesunate. Addition of artemisinin to the culture of Aspergillus fumigatus (which causes aspergillosis in human) has been observed to stop the growth of fungus. Artemether and extracts of A. annua leaves have proved lethal to in vitro growing Acanthamoeba castellanii (a cause of amoebiasis in humans). Treatment of mice infected with Acanthamoeba with water-, alcohol- or chloroform- extract of Artemisia annua leaves was observed to have increased the life span of diseased animals. Feeding of A. annua leaves to the broiler chickens infected with Eimeria tenella parasites saved the infected animals from development of coccidiosis disease. Growth of both visceral and cutaneous leishmaniasis causing Leishmania parasites, in human macrophage cultures, was found to be attenuated by the treatment of artemisinin. Analogously, the leishmania infections in model animals were also observed to have been arrested by treatment with artemisinin or A. annua

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism</th>
<th>Cell Line</th>
<th>Treatment</th>
<th>Infection Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oluyomi et al. (2009)</td>
<td>Rattus rattus</td>
<td>In vivo</td>
<td>Artemisinin</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Mishina et al. (2007)</td>
<td>Cultured trypanomastigotes</td>
<td>In vitro</td>
<td>Artemisinin</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Akande and Fagbemi (2011); Olivera et al. (2015)</td>
<td>Cultured in Vero cells (kidney epithelial cells of African green monkey)</td>
<td>In vitro</td>
<td>Artesunate</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Utzinger et al. (2000); Kaiser and Utzinger (2007)</td>
<td>Trypanosoma cruzi (Chagas disease)</td>
<td>In vitro</td>
<td>Artemether</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Li et al. (2012)</td>
<td>BALB/C mice</td>
<td>In vivo</td>
<td>Dihydroartemisinin</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>(E) Metazoan caused:</td>
<td></td>
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</tr>
<tr>
<td>Utzinger et al. (2000); Kaiser and Utzinger (2007)</td>
<td>Schistosoma mansoni (Helminth flatworm; common in Africa and South America)</td>
<td>In vivo</td>
<td>Artemether</td>
<td>Prevention of parasite's infection</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Li et al. (2012)</td>
<td>BALB/C mice</td>
<td>In vivo</td>
<td>Dihydroartemisinin</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
<tr>
<td>Utzinger et al. (2000); Kaiser and Utzinger (2007)</td>
<td>Schistosoma japonicum (common in Southeast Asia)</td>
<td>In vivo</td>
<td>Artemether, artesunate and dihydroartemisinin</td>
<td>Inhibition of parasite growth</td>
<td>Life span extended</td>
</tr>
</tbody>
</table>
### Table 5: Curative effect of *Artemisia annua* leaf extracts, artemisinin or its derivatives on the diseases of human /model animal immune and digestive systems and cancers of various organs

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of disease(s)</th>
<th>Organism(s)/ system(s) used for testing</th>
<th>Disease condition: origin/method by which induced</th>
<th>Therapeutic agent tested for its efficacy</th>
<th>Observation(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td></td>
<td><strong>A. Autoimmune</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Rheumatoid arthritis</td>
<td>Rat</td>
<td>Collagen</td>
<td>Artesunate</td>
<td>The inflammatory symptoms were attenuated by intra-peritoneal treatment with the drug (3-5 mg/kg/d)</td>
<td>Li et al. 2013</td>
</tr>
<tr>
<td>2.</td>
<td>Crohn’s disease (Inflammatory bowel)</td>
<td>Mouse</td>
<td>Variously</td>
<td>As above</td>
<td>The symptoms of disease were ameliorated by administration of the drug @ 150 mg/kg/d</td>
<td>Yang et al. 2012</td>
</tr>
<tr>
<td>3.</td>
<td>Allergic asthma</td>
<td>BALB/C mouse</td>
<td>Ovalalbumin</td>
<td>As above</td>
<td>The treatment suppressed both the inflammation and oxidative damage associated with severe asthma</td>
<td>Cheng et al. 2011</td>
</tr>
<tr>
<td>4.</td>
<td>Lupus</td>
<td>B6D2F1 and Pristane DBA/2 mice</td>
<td>Artemisinin</td>
<td>As above</td>
<td>The drug relieved the symptoms of the diseases</td>
<td>Wu et al. 2010</td>
</tr>
<tr>
<td>5.</td>
<td>Uveitis</td>
<td>Long-Evans rat</td>
<td>Lipopolysaccharide induction</td>
<td>Artemisinic acid</td>
<td>The drug suppressed the uveitis</td>
<td>Wang et al. 2011</td>
</tr>
<tr>
<td></td>
<td><strong>B. Digestive system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Obesity</td>
<td>C57BL/6 mouse</td>
<td>Nutrition rich diet</td>
<td>Boiled water extract of <em>Artemisia annua</em> dry leaves</td>
<td>Oral administration of the extract reduced the weight of animals without affecting their appetite</td>
<td>Baek et al. 2015</td>
</tr>
<tr>
<td>7.</td>
<td>As above</td>
<td>Sprague Dawley rats</td>
<td>As above</td>
<td><em>Artemisia annua</em> leaves</td>
<td>Body weight, adipose tissue mass, adipocyte cell size, total cholesterol level were decreased</td>
<td>Song et al. 2017</td>
</tr>
<tr>
<td>8.</td>
<td>Fatty liver</td>
<td>CB7BL/6J mouse</td>
<td>High fat diet</td>
<td>Dehydrated water extract of <em>Artemisia annua</em> leaves</td>
<td>Twelve weeks treatment prevented hepatic fibrosis, obesity and inflammation of liver by reducing the accumulation of lipids</td>
<td>Kim et al. 2011</td>
</tr>
<tr>
<td>10.</td>
<td>As above</td>
<td>Zebrafish, mouse, human diabetic pancreatic islet</td>
<td>Type I diabetes impairment</td>
<td>Artemether</td>
<td>The glucagon producing pancreatic α cell got transformed into insulin producing β cells via activation of GABA receptors by loss of Arx function and thereby insulin production got restored</td>
<td>Li et al. 2017</td>
</tr>
<tr>
<td></td>
<td><strong>E. Cancers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11.</td>
<td>Squamous cell carcinoma of oral cavity</td>
<td>Human cells</td>
<td>Gingival epithelial cancerous (1HGK) cells</td>
<td>Dihydroartemisinin</td>
<td>The drug was apoptotically cytotoxic to cancerous cells</td>
<td>Yamachika et al. 2004</td>
</tr>
<tr>
<td>No.</td>
<td>Tumor Type</td>
<td>Cell Type</td>
<td>Cell Lines/Details</td>
<td>Treatment Effect</td>
<td>References</td>
<td></td>
</tr>
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</tr>
<tr>
<td>12</td>
<td>Pancreatic cancer</td>
<td>Human cells</td>
<td>PANC-1, Bx Pe-8 and CFPAC-1 pancreatic cancer cell lines and HL-7702 normal hepatic cell line</td>
<td>The drug caused oncosis-like cell death multi-fold more on cancer cells than on normal cells</td>
<td>Du et al. 2010</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>As above</td>
<td>Human cancer cell xenograft on mouse</td>
<td>Panc-1 tumor xenograft in mouse</td>
<td>Artesunate caused dose dependent tumor regression</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Hepatocellular cancer</td>
<td>Human</td>
<td>Hep G-2 and BWTG-3 cells</td>
<td>Artesunate reduced the cancer cell viability in a dose dependent manner</td>
<td>Vandewynckel et al. 2012</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>As above</td>
<td>Mouse</td>
<td>Diethyl nitrosamine induced tumor in liver</td>
<td>Tumor burden was reduced without hepatotoxicity</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Gastric cancer</td>
<td>Human cells</td>
<td>SGC-7901, BGC-823 and AG5 gastric cancer and GES-1 normal cell lives</td>
<td>Cancer cells were killed by oncosis like process, but there was no significant effect on non-cancerous cells</td>
<td>Zhou et al. 2013</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>As above</td>
<td>Human tumor xenograft on mouse</td>
<td>Gastric tumors were xenografted on nude mice</td>
<td>The treatment regressed the tumors without detriment to animals</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Colorectal cancer</td>
<td>Human</td>
<td>Patients</td>
<td>Administration of 200 mg drug daily for 14 days cured 8 out of 9 patients</td>
<td>Magalhaes et al. 2012 ; Krishna et al. 2014</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Gall bladder cancer</td>
<td>As above</td>
<td>GBC-SD and NOZ gall bladder cancer cell lines</td>
<td>The treatment stopped cell proliferation and caused cell killing by apoptosis</td>
<td>Jia et al. 2016</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>As above</td>
<td>As above</td>
<td>The xenograft of above cancers on BALB/C mice</td>
<td>The xenograft growth was inhibited by drug treatment</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Renal cancer</td>
<td>As above</td>
<td>Renal cell carcinoma cell lines: Caki-1, 786-O and SN12C-GFP-SR Lu 2</td>
<td>Cancer cells were killed by oncosis via ROS generation and ATP depletion</td>
<td>Jeong et al. 2015</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>As above</td>
<td>Human cancer xenograft on mice</td>
<td>Xenograft of 786-o-Luc cells planted subcutaneously</td>
<td>Intra-peritoneal administration of the drug repressed the tumor (growth, metastasis and angiogenesis were all inhibited)</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Cervical cancer</td>
<td>Human</td>
<td>HPV-39 inhibited- Artemisinin treated cervical cancer cells</td>
<td>The cancerous cells stopped proliferating and were killed apoptotically by the effect of the drug</td>
<td>Mondal and Chatterji 2015</td>
<td></td>
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</table>
leaf powder. Artesunate was observed to inhibit the Toxoplasma gondii infection of cultured human cells and of mice in vivo. Trypanosomiasis (human African sleeping sickness) like disease caused by Trypanosoma brucei infection in experimental mice and rats was found to have been cured by the artemether treatment. Artemisinin and artesunate treatments given individually inhibited the growth of T. brucei and T. cruzi (the cause of chagas disease in humans) in cultured human cells. Infection in humans and in experimental mice of Schistosoma mansoni as well as S. japonicum (both the species are cause of schistosomiasis disease) was observed to get inhibited by the treatment with each of these drugs- artemether, dihydroartemisinin, and artesunate.

A summary of the results of some representative studies on the effects of artemisinins and artemisannua on rheumatoid arthritis, Crohn’s disease, asthma, lupus and uveitis (autoimmune disorders), obesity and diabetes (metabolic disorders) and eight kinds of cancers is listed in the table 5. In different studies, artesunate was found to cure/ suppress and relieve symptoms of collagen induced rheumatoid arthritis, Crohn’s disease, ovalabumin induced asthma, and lipopolysaccharide induced uveitis, all in model animals. Obesity and fatty liver diseases caused by consumption of high fat/ nutrition diet in experimental animals were found to be cured by treatment with A. annua leaf extracts. The A. annua leaf extracts also cured alloxan induced diabetes in rats. It was found that artemether treatment, to type1 diabetic zebrafish, mice and rats and human pancreatic islets, transformed the pancreatic α cells into ß cells such that insulin synthesis started relieving the type 1 diabetes symptoms. Cells of human cell lines of pancreatic-, hepatocellular-, gastric-, colorectal- and renal- cancer stopped proliferating and got killed by an oncosis- like process upon treatment with artesunate. Also the xenographs of pancreatic-, hepatocellular-, gastric- and renal-cancers in animal models were found to regress upon treatment with artesunate. The artemisinin treatment produced analogous results on in vitro and in vivo gall bladder cancer and on in vitro cervical cancer. The experimental findings, that artemisannua controlled obesity and diabetes in model animals strongly suggest ALT as a treatment for these diseases in humans.

Clearly, the above discussion suggests that the mechanisms of biological actions of artemisinins and artemisannua are such that these agents serve as broad spectrum therapeutics so as to cure a variety of human diseases. These observations raise the possibility that perhaps ALT can substitute for artemisinins and artemisannua and ALT could be a therapy for multiple diseases beyond malaria. In view of the above, the need for pilot studies and clinical trials on quality controlled ALT tablets for studying the response of their administration to patients of each of the different non-malarial, as well as malarial diseases, that respond to artemisinins and artemisannua, can not be overemphasized.

Concluding Remarks

In the approximately last ten years, the incidence of malaria disease was reduced by 20% and mortality among malaria patients by 30%. This was mainly achieved by use of two-drug ACTs and chloroquine in the treatment, respectively, of falciparum and vivax malaria and by use of primaquine treatment to block the transmission of parasites from humans to mosquitoes (Table 3). However, the falciparum and vivax malarial parasites have developed genetic resistance against a large majority of the approved antimalarial pharmaceuticals in some of their populations in malaria endemic areas, thereby making the drugs ineffective (Table 2). There has been independent development of artemisinin resistance in Southeast Asia and Africa; consequently ACT treatments too have become ineffective in parts of these geographical areas. To meet the challenge of multi-drug resistant falciparum malarial strains, treatment with three-drug ACTs has been advised. This year, a new treatment (ALT) has been added to cure the acute and complicated malaria caused by ACT-resistant falciparum parasites. The ALT treatment comprises of capsules filled with or tablets made from A. annua dry leaf powder, derived from cultivated plants of specific variety(ies) bred for e” 1% artemisinin content and a combination of other therapeutically active metabolites naturally present. A regimen of two 500 mg leaf powder tablets a day for 5 days was found to cure adults suffering from ACT resistant complicated falciparum malaria that was unresponsive to ACT or iv artesunate (most likely artemisinin resistant). The ALT treatment’s malaria curing property has been related to antimalarial
activities of artemisinin, several other artemisinic compounds, many terpenes and flavonoids and other types of molecules present in the dry *A. annua* leaves. ALT is safe and seems resilient against artemisinin drug resistance development. The cost of an ALT-treatment was estimated to be about 100 fold lower than that of an ACT-treatment. Extensive putative use of ALT has gained importance since a recent policy statement of WHO (September 10, 2017) emphasizes on the importance of affordability for everyone of safe, efficacious and quality medical products (http://odishatv.in/health/afforability-medicine-who-wants-bold-steps-237875/). The ALT, besides being an efficacious antimalarial treatment has properties which raise possibilities of its multi-repurposment as a treatment against all those diseases which respond curatively to artemisinin, its derivatives and *A. annua* leaf powder or its extracts. This list includes diseases as diverse as hepatitis, tuberculosis, leishmaniasis, toxoplasmosis, trypanosomiasis, schistosomiasis, asthma, rheumatoid arthritis, diabetes, and cancers of various body organs. There is now an urgent need for (a) further evaluation of artemisinin efficacy against several of the listed diseases *in vivo* models, and (b) pilot studies and clinical trials to attest ALT treatment for varied malaria and diseases beyond malaria for which artemisinin efficacy has been experimentally established, for the benefit of billions of patients of above listed diseases.

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