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Integrated use of *Artemisia annua* to control malaria: follow-up longitudinal study to confirm preliminary observations and complementary investigations

Proposal for funding

EXTRACTS

October 2012

1. Background

Malaria is the world's most important parasitic protozoan infection caused mainly by *Plasmodium falciparum*. It is endemic in over one third of the world (~ 500 million infections annually) and results in more than 2 million deaths. It is also causally associated with kidney diseases, hyperactive splenomegaly, Burkitt's lymphoma, anaemia and a myriad of other chronic health consequences. Africa accounts for ~90% of the global deaths resulting from the disease (WHO, 2008), where children under the age of five and pregnant women are especially at risk. The infection in the continent is transmitted mainly by *Anopheles gambiae* complex (Greenwood *et al.* 2002; Guerra, *et al.* 2008). Within the complex, the highly anthropophilic *An. gambiae s.s.,* that prefers to feed on humans, is considered the most efficient malaria vector (Coetzee, 2004). The negative societal impact of malaria in Africa is high, particularly on the overall growth and performance of school-age children. In economic terms, the annual cost of malaria is estimated to range in billions of dollars (Sachs and Malaney, 2002). Lifting this burden range would thus have significant positive effects on African socio-economic growth.

Two broad strategies (and an integration of these) have been deployed to minimise the malaria burden in Africa. One has targeted the malaria vector seeking to reduce their numbers and/or contact with human hosts. Recently, use of bed nets conventionally treated with an insecticide (ITNs) or factory-treated long-lasting nets with insecticides incorporated within or bound around the net fibres (LLITNs). However, a number of factors have raised concerns on the sustained efficacy of these vector control methods, including heterogeneous distribution and low usage rates by members of different communities (Seidein et al., 2012), shifts in mosquito feeding behaviour (outdoor feeding during earlier part of the nights) and rapid resistance development against the insecticides leading to resurgence of malaria following an initial period of use (e.g. Trape et al., 2011; Hetzel et al., 2012).

The other strategy has involved prophylactic or curative use of anti-malarial drugs. Monotherapies involving single drug treatments with antimalarials have largely lost their effectiveness due to the development of drug resistance (e.g. Talisuna, et al. 2004). Many populations of the malaria parasite have become multi-drug resistant and malaria patients do not respond to pyrimethamine-sulfadoxine, mefloquine, and chloroquine (Ronn et al., 1996). These have recently been replaced by combination therapies which involve two drugs

acting complimentarily. The most effective combinations at present are based on derivatives of artemisinin, a constituent of the plant *Artemisia annua* with novel mechanism of action associated with the lactone-endoperoxide group and its relatively greater antimalarial potency compared to other compounds (Eckstein-Luwig et. al., 2003). These combinations, referred to as ACTs ('artemisinin-based combination therapy'), are effective against the most virulent form of malaria. Most countries worldwide have adopted ACTs as the first-line treatment for *P. falciparum* (WHO 2008). However, levels of use in sub-Saharan Africa have remained generally low largely because of poor access to ACTs in rural areas, affordability and availability of cheaper but ineffective drugs. In Kenya, for example, it is estimated that only 12% of children receive anti-malarials within 24 hours of malaria symptoms (Wasunna et al., 2008). Moreover, although ACTs are less likely to cause resistance than monotherapies, they are not immune to this threat. Indeed, resistance to one ACT was noted in an area of Thailand (Vijaykadga et al., 2006). More recently, resistance to artemisinin was reported in western Cambodia (Dondorp et al., 2009) and its spread to Africa could have serious consequences on the efficacy of ACTs in the continent.

One also needs to look into also the economic implications. Eradicating malaria in Africa with traditional means is estimated by WHO (WHO 2011 report) at a cost of USD 5 billion/year although the international community has so far encountered difficulties collecting USD 2 billion/year. These estimates do not include about USD 7 billion needed to pre-empt resistances that have appeared with the ACTs (Global Plan for Artemisinin Resistance Containment – WHO – 2011). Hence, a strategy that includes the use of cheaper, more accessible means of combatting malaria has been called for including by the African Regional Committee of WHO (Malaria Journal 2011, 10 (Suppl 1):S6)

2. Initial study with *Artemisia annua*

Initial exploratory study initiated with *A. annua* was motivated by concerns on frequent absenteeism among students and teaching staff in many schools located in malaria-prone areas of Kenya and on the resulting poor academic performance of the students. The study sought to explore the effects of combined use of *A. annua* plantations to repel mosquitoes from students' halls and *A. annua* tea for prophylactic control of malaria.

The use of herbal plants and herbal products to repel or kill mosquitoes and other insect pests is widespread in Africa (Seyoum et al., 2002). Methods of deployments of such plants include growing them around family households or in pots placed in bed rooms, placing branches at insect entry points, and burning leaves or bark. In China, volatiles derived from burning *Artemisia* spp. have been used to repel mosquitoes. A recent study has shown that hydro-distilled oil of *A. annua* leaves is repellent and toxic to post-harvest beetles (Tripathy et al., 2000). In the exploratory study, *A. annua* plantations were strategically grown upwind from students' residential halls to provide continuous flow of volatile emissions to repel mosquitoes downwind that sought to fly to their human hosts.

For traditional therapeutic use in China, *A. annua* has been extracted with freshly boiled water (4.5-9.0g of dried herb/1 liter of water) and the resulting tea taken on daily basis (The People's Republic of China, 1985). These extracts also contain high levels of flavonoids, which have shown a variety of biological activities and may also synergize the effects of artemisinin against malaria (Willcox, 2009) and, interestingly, also cancer (Ferreira et al., 2010). There is no evidence of toxic effects from the use of this plant. *A*. *annua* is included in the pharmacopoeia of the People's Republic of China, with recommendations for its dose and therapeutic use, and it is now regarded as a safe ethno-medicine globally (Mueller et al., 2000).

Previous clinical trials with *A. annua* tea showed a rapid reduction in parasitemia and clinical improvements, although followed by high recrudescence rates (>20 %) after therapy (WHO, 2001). A detailed pharmacokinetic study after oral intake of tea preparation showed rapid absorption of the artemisinin, with maximum plasma concentration occurring within ½ hour and then dropping in the next 4 hours (Räth et al., 2004). Such a pharmacokinetic pattern may allow rapid reduction of the bulk of parasites during the first 24 hours but may fail to reach late parasite developers located in the liver unless the intake of *A. annua*-tea is taken

for longer periods. Thus, in our exploratory study, each student and member of staff was provided with *A. annua* tea for 7 consecutive days during the first week of each month.

Results from a piloting phase (March 2010 to October 2010) and intervention phase (November 2010 to December 2011) were very encouraging (IDAY Conference Report, 2011) including positive therapeutic effects (reduction in malaria and typhus cases compared to pre-intervention years as well as in menorrhea among teenage girls) and associated reduction in absenteeism of students and teachers. These were reflected in improvement in the academic performance of the students and significant financial savings in school medical costs. Launched in 2 pilot schools in Nyanza Province in May 2010, the programme spread rapidly and about 70 schools and related institutions in 6 provinces of Kenya have since joined the programme.

However, the observations the exploratory study have raised a series of questions from different sources (specifically, WHO and Malaria Control Committee of Ministry of Public Health and Kenya), including: -

(i) The performance of the *A. annua*-based intervention strategy relative to other interventions that have been introduced in different parts of Kenya such as impregnated mosquito nets, residual spraying and use of ACT and Coartem in reducing mosquito numbers and/or malaria, and need for clear scientific evidence on the efficacy of the *A. annua*-based intervention;

(ii) Possibility of rapid development of resistance against *A. annua* tea with its restricted composition relative to the full phytochemical blend of the plant, and comparative evaluation of alternatives that provide the full blend of *A. annua*;

(iii) Quality-control measures and monitoring, together with improved agricultural practices that need to be put in place, to ensure efficacious and reproducible effects of the *A. annua* plant (and varieties) growing in different agro-ecological zones, both as *in vivo* source of repellent and in prophylactic or curative use;

(iv) Possible use of *A. annua* products (*A. annua* tea, whole-leaf tablets/capsules) by expectant mothers to establish their efficacy and safety on this group of malaria affected subjects; comprehensive clinical performance of the products in prophylaxis and/or for treatment needs to be documented.

A multi-disciplinary team of scientists from Kenyatta University School of Health (Medical Sciences, Pharmacy & Complementary/Alternative Medicine), School of Pure & Applied Sciences (Entomology, Chemical Ecology, Analytical Chemistry), School of Environmental Studies (Environment & Community Development) and School of Humanities and Social

Sciences (Sociology) was set up to address the above questions. Six complementary sub-projects have been proposed. These will be addressed in two phases as outlined below. The first phase seeks to address questions 1, 2 and 3 above. The second phase will address question 4 in two clinical trials, and is envisaged to follow up with a social science-led initiative to disseminate appropriate (depending upon the outcome of the other sub-projects) *A. annua*- based interventions widely in Kenya and beyond.

5. Project Management and Supervision

The Project will be coordinated by Dr Tobias Opiyo Arudo of Kenyatta University who has been actively involved in the initial phase of *A. annua* trials involving different schools in Western Province and Nyanza. He will be assisted by the multi-disciplinary committee that was set up to address the questions and concerns originating from WHO and Malaria Control Committee of Ministry of Public Health, Kenya.

The specific project components will be supervised by scientists with broad experience in the specific areas:

Phase 1 Sub-project 1 on clinical & entomological evaluation of *A. annua* in prophylactic use and mosquito repellence will be supervised jointly by Prof. Onesmo Ole Moi-Yoi formerly Director of Research, ICIPE, Dr. Rashid Juma of Kenya Medical Research Institute (both with very strong background in Health Science and clinical trials) and Prof. Elizabeth Kokwaro (an entomologist at Kenyatta University). Prof Ole Moi-Yoi and Dr Rashid Juma will also be involved Phase 2 clinical curative trials with *A. annua* products.

Phase 1 Sub-project 2 on cyclic exposure experiments will be undertaken by Ms Lucy Kangethe (a PhD candidate who is finalizing her work on related area under the supervision of Dr Sabah Omar of KEMRI and Prof Ahmed Hassanali of Kenyatta University).

Phase 1 Sub-project 3 on quality control measures in the performance of *A. annua* growing in different areas will be supervised jointly by Prof Ahmed Hassanali of Chemistry Department, Prof Nicholas Gikonyo of Pharmacy and Alternative Medicine Department, and Prof Hudson Nyambaka of Chemistry Department.

Phase 2 Sub-project on Social Science led dissemination initiative will be led by Dr Tobias Opiyo Arudo of Kenyatta University with assistance from scientists from the Multi-disciplinary Committee.

The Project will be conducted in close collaboration between Kenyatta University and IDAY-International, in connection with Iwerliewen.

6. Financing and dissemination of results

IDAY-International, which is part and parcel of this study and is managing several *Artemisia annua* projects in numerous schools in 18 African countries, will be responsible for raising the funds needed for the study, excluding the contribution of Kenyatta University. IDAY-International will supervise the funds allocation together with the appropriate authority within Kenyatta University. It will also be responsible for adapting its existing programmes according to first results obtained and developing new ones on the basis of the final results. IDAY-International will therefore recruit an *Artemisia annua* projet manager specifically responsible for the follow up of the financing of the study and the application of its results among its members. He or she will be posted in Kenya.

Kenyatta University will provide key personnel involved in the project, laboratory and office facilities, analytical instruments and all bulk reagents and chemicals used in the analyses without any incremental costs.

Total Indicative budget - Phase I

Research (Kenyatta University) …….USD 1001,420

Management support (IDAY-International) ……..USD 196,200

Contingencies (5 % on all but overheads) …….. USD 43,540

GRAND TOTAL PHASE I ……**USD 1,241,160**

Of which financed by Kenyatta University ……...USD 197,080

Outside financing required ……..**USD 1,044,080**

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