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Short communication

The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects

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Abstract

The plant *Artemisia annua* L. (Asteraceae) is listed in the Chinese pharmacopoeia as a remedy for various fevers including malaria, and contains the well-established antimalarial compound artemisinin. In this study, a hybrid form of *A. annua* was successfully cultivated in Central Africa. The aerial parts of the plant contained 0.63–0.70% artemisinin per dry weight, and approximately 40% of this artemisinin could be extracted by simple tea preparation methods. Five malaria patients who were treated with *A. annua* tea showed a rapid disappearance of parasitaemia within 2–4 days. An additional trial with 48 malaria patients showed a disappearance of parasitaemia in 44 patients (92%) within 4 days. Both trials showed a marked improvement of symptoms. In our opinion, these results justify further examinations of the antimalarial effect of *A. annua* preparations.

Keywords: Antimalarials; *Artemisia annua*; Medicinal plant

1. Introduction

In terms of morbidity and mortality, malaria is still the most important parasitic disease of mankind and has a very serious impact upon health and economic welfare in the tropical world. The majority of malaria-related deaths occur in sub-Saharan Africa (Trigg and Kondrachine, 1998), where a large part of the population has no access to antimalarial drugs. War and the resulting destruction of the infrastructure leave more and more people in abandoned areas with no access to modern medicines or medical services.

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The plant *Artemisia annua* L. (Asteraceae) has been used in China for more than 2000 years to treat fevers. The current pharmacopoeia of the People's Republic of China (The People's Republic of China, 1985) describes the dried herb of *A. annua* as a remedy for fevers including malaria; for therapeutic use, the herb is extracted with hot water according to the rules of traditional Chinese medicine (Stöger, 1991). *A. annua* contains artemisinin, which was isolated in 1972, and since that time its efficacy against malaria has been amply demonstrated (Hien and White, 1993; de Vries and Dien, 1996). Clinical evaluations have focussed on pure, isolated artemisinin and its semisynthetic derivatives, artesunate, artemether and arteether. The content of the natural compound artemisinin in different genotypes of *A. annua* varies from 0.01 to 0.5% (Hien and White, 1993). For the present study, we used *A. annua* cv. *Artemis*, a hybrid from Chinese and Vietnamese genotypes which is suitable for cultivation and has been described as containing 0.5–0.75% artemisinin in the dried plant material, after professional cultivation in Europe (Delabays, 1997).

The present study was carried out to investigate the following questions.

1. Can *A. annua* cv. *Artemis* be cultivated in tropical Africa, and what artemisinin content is obtained after cultivation, drying and storage under local conditions?
2. How much of the poorly water-soluble compound artemisinin is contained in a tea prepared by local methods from the plant material?
3. Do teas prepared from *A. annua* plant material show a positive effect in the treatment of malaria in endemic areas, as suggested by the Chinese pharmacopoeia?

2. Methods

2.1. Plant material

A. annua cv. *Artemis* is a hybrid described by Delabays (1997) and was obtained from Centre de Recherche des Plantes Médicinales et Aromatiques, Conthey, Switzerland. Reference specimens of the seeds and the leaves are available from the authors.

2.2. Cultivation and artemisinin content

Cultivation was carried out between December 1997 and July 1998 at three locations in Eastern Democratic Republic of the Congo, at Nebobongo Hospital, 64 km south of the town of Isiro on the northern border of the Ituri rainforest (altitude 650 m), and at two locations near the Lwiro Research Centre near Bukavu, on the eastern border of Democratic Republic of the Congo (altitude 1600 and 2000 m). The seeds of *A. annua* cv. *Artemis* were provided by Anamed in Germany. The seeds are very small (about 0.08 mg each) and were preserved in kaolin in sealed plastic envelopes. Seeds were sowed on soft, deep soil (depth 60 cm) enriched with compost. They were not covered with soil, but protected against rainwater, wind and excessive sun in a shady nursery, and watered carefully by spraying twice a day. Six weeks after germination the young plants were transplanted into plastic bags filled with fertile soil and protected from strong sunlight. After 6 weeks, they were transplanted into an open, unshaded field, with one plant per m^2. The fields were fertilized with compost, and weeds were removed at regular intervals. Just before flowering, the leaves were harvested and dried in the air at temperatures below 40°C.

2.3. Tea preparation

2.3.1. Method A (the recommended method)

Boiling water (1 l) was added to 5 g dried leaves. After brief stirring, the mixture was left to cool for 15 min, and filtered.

2.3.2. Method B

Dried leaves (5 g) were added to 1 l of water, heated to boiling point, kept boiling for 5 min, and filtered.

2.4. Chemical analysis

The artemisinin content in the leaves was determined by high performance liquid chromatogra-

phy (HPLC), following the procedure described by Zhao and Zeng (1985). An authentic reference sample of artemisinin (Sigma, Deisenhofen, Germany) was used for comparison. The artemisinin content in the tea was determined, after extraction of 500 ml tea with 2×250 ml of petrol ether. Control experiments showed that $>94\%$ of the artemisinin in the tea was extracted from the aqueous phase by this procedure.

2.5. Patients and clinical methods

Two uncontrolled pilot studies were carried out with the consent of the regional health authorities at the Lwiro Research Centre and at the Nebobongo Hospital. Informed consent was obtained from the patients according to the requirements of the committee responsible for ethical matters in each of the two centres. All patients treated had to stay either in hospital or in their homes nearby the hospital. They were closely supervised during the study, and conventional malaria treatment was kept immediately available in case the condition of a patient deteriorated. Participants were recruited from patients who reported to the centres with complaints of malaria. Inclusion criteria were, (1) the presence of malaria parasites in blood films; (2) subjective symptoms of malaria (see Table 2). In the Lwiro trial, a body temperature of $>38°C$ was an additional inclusion criterion. Patients were included on a voluntary basis, and only if they understood that the outcome of this therapy would be uncertain and that they had to report any inconvenience they experienced during or after the study.

Exclusion criteria were pregnancy, breastfeeding, an age of less than 11 years, and severe diseases besides malaria. Twenty-two patients were included at Lwiro, and 48 at Nebobongo.

Patients were treated with 1 l of *A. annua* tea per day. The medication was prepared and dispensed daily by the hospital staff and taken in four equal portions of 250 ml in the course of the day. In the Lwiro trial, tea was prepared by Section 2.3.1 (see above), and treatment was carried out for 5 consecutive days. In the Nebobongo trial, tea was prepared by Section 2.3.2 (see above), and treatment was carried out for 4 consecutive days. No other antimalarial drugs were given.

Thick blood films were examined after Fields staining, and trophozoites were counted in 100 highpower fields (approximately 0.38 μl total) as described by Petersen et al. (1996). In the Lwiro trial, five patients were examined by blood films on each treatment day. In the Nebobongo trial, blood films were taken from every patient at the beginning and at the end of the treatment. The laboratory staff did not know whether the patient was included in the study or not. In both trials, patients were interviewed daily for their symptoms.

3. Results

3.1. Cultivation

A. annua grew rapidly into large plants, especially at higher altitudes (2000 m), where they reached an average height of 2–2.5 m, 7–8 months after germination. A yield of 100–200 g dried leaves could be obtained per plant (= per m^2) under these conditions, compared with 250–300 g/m^2 reported for the professional cultivation in Europe (6). The leaves were dried locally in the air, and sent to Europe for chemical analysis.

Leaves produced at altitudes of 1650 and 2000 m showed 0.63 and 0.70% artemisinin per dry mass. In comparison, the leaf material of plants cultivated and dried by professional methods in Europe showed a content of 0.58% in our analysis, and this is in accordance with previous reports on such material (Delabays, 1997). Cultivation was also successful at Nebobongo (650 m altitude) in the Democratic Republic of the Congo, but results of a chemical analysis are not yet available.

3.2. Artemisinin content in tea preparations of *A. annua* leaves

Artemisinin is a poorly water-soluble compound, and we investigated whether it could be extracted by conventional tea preparation methods. Table 1 shows that chemically pure

Table 1
Artemisinin content in tea preparations from *A. annua*[a]

Amount of material per l water	Method of tea preparation	Artemisinin concentration in the tea (mg/l)	Extraction efficiency (%)
Pure artemisinin (250 mg)	A	10.6	–
Leaves (5 g)	A	12.0	41.4
Leaves (10 g)	A	24.5	42.2
Leaves (20 g)	A	32.8	28.3
Leaves (40 g)	A	64.4	27.8
Leaves (5 g)	B	7.2	24.8

[a] Dried leaf material containing 0.58% artemisinin was used. Tea preparation is described in Section 2.

artemisinin is dissolved to a low extent by hot water, but that tea preparations from the leaves of *A. annua* contain higher amounts of artemisinin. This is a well-known phenomenon, indicating that other constituents of the plant (e.g. flavonoid glucosides or saponins) can help to keep a lipophilic plant compound in aqueous solution.

Artemisinin is known to be heat-unstable, and Table 1 shows that tea prepared by the addition of boiling water to the leaves without further heating (Section 2.3.1) yields higher artemisinin concentrations than if the leaves are boiled for several minutes (Section 2.3.2). Obviously, the artemisinin concentration in the tea can be raised by increasing the amounts of leaf material per l water (Table 1). The (daily) dose of *A. annua* given in the Chinese pharmacopoeia for the treatment of various fevers including malaria is 4.5–9 g of dried plant material, extracted with water under heating (The People's Republic of China, 1985; Stöger, 1991).

3.3. Clinical observations

We had obtained case reports, which appeared to show that tea preparations of *A. annua* had a positive effect in the treatment of malaria patients. Proof for such an efficacy may potentially offer considerable benefits. Therefore we decided, with the consent of the local medical authorities, to conduct two pilot studies with *A. annua*, using a dosage within the range given in the Chinese pharmacopoeia. We tried to give the utmost consideration to the safety of the patients (see Section 2). In a first trial at the Centre de Recherche en Sciences Naturelles at Lwiro, 22 patients with fever (≥ 38°C) and parasitaemia (16 *Plasmodium falciparum*, six *Plasmodium malariae*) were included and treated daily with four equal doses of *A. annua* tea. Five of the patients (all *P. falciparum*) agreed to have a daily blood film taken. Fig. 1 shows that the parasite counts in the blood dropped rapidly to 0, similar to the clinical effect described for artemisinin derivatives (de Vries and Dien, 1996). On the last day of the treatment, all five patients were free of trophoizites and also free of malaria symptoms. Of the additional 17 patients, 15 reported complete disappearance of malaria symptoms within the course of the treatment.

In a second trial at Nebobongo Hospital, 48 patients with parasitaemia (all *P. falciparum*) and subjective symptoms of malaria were included (see Table 2). They were treated with *A. annua* tea for 4 days. Blood films taken at the end of the

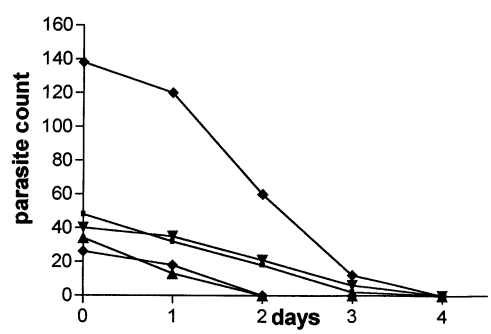

Fig. 1. Parasite counts in thick blood films of five malaria patients undergoing treatment with *A. annua* tea. Parasitaemia was determined as described in Section 2.

Table 2
Clinical data of 48 malaria patients before and after treatment with *A. annua* tea[a]

	Before treatment	After treatment
Parasitaemia (*P. falciparum*)	48 (100%)	4 (8%)
Subjective symptoms	48 (100%)	11 (23%)
Among these		
Headache	37	3
Fever	27	1
Chills	10	0
Arthralgia	14	4
Vertigo	6	1
Others	27	8

[a] Eighteen men, 30 women; mean age 38 years, range 18–67 years.

treatment showed that the parasites had disappeared in 44 (92%) of the patients (Table 2). Thirty-seven patients (77%) were free of any symptoms, 11 (23%) still had complaints (see Table 2), which might or might not have been related to malaria, two out of these 11 patients with symptoms still showed a positive blood film. Twelve patients (25%) complained about nausea during the treatment, which disappeared when the treatment course was finished. One patient reported vomiting. No other side effects were observed. Only three patients complained, within 1 week after the end of the treatment, about new symptoms possibly related to malaria; however, their blood films proved to be negative.

No control group was included in this study, but from the records of Nebobongo Hospital, we identified 12 patients who, (1) had been diagnosed for malaria by positive blood films and subjective symptoms; (2) did not receive antimalarial treatment (for various reasons, such as refusal of treatment); and (3) had a second blood film taken within approximately 1 week after the first. Three (25%) of these patients had been diagnosed as free of parasitaemia at the time of the second blood film, and this may serve as a rough estimate of the spontaneous reduction of parasitaemia below the detection limit in untreated patients in the examined semi-immune population, using our diagnostic facilities.

4. Discussion

Our study shows that plant material from *A. annua* with a high content of artemisinin can be produced in Central Africa. Malaria patients treated with a tea from *A. annua*, in a dose corresponding to the recommendations of the Chinese pharmacopoeia, showed a very rapid disappearance of malaria parasites from the blood, similar to the effect described for pure artemisinin (de Vries and Dien, 1996). This effect clearly exceeds the rates of parasite clearance expected from the natural course of the disease.

Table 1 indicates that a treatment course of 5 days × 5 g *A. annua* leaves, prepared in the form of a tea, may result in a total dose of approximately 60 mg of artemisinin, and 5 days × 40 g leaves would result in 320 mg artemisinin. In contrast, the usual clinical dose of the artemisinin derivatives (artesunate, artemether, arteether) is between 500 and 1000 mg total within 2–6 days of treatment (de Vries and Dien, 1996). Pure artemisinin, which is poorly water-soluble and has a bioavailability of less than 32% (Titulaer et al., 1990), is used in a dose of 5 days × 500 mg (Hien, 1994). All artemisinin derivatives probably act as prodrugs for the same biologically active intermediate, dihydroartemisinin.

The bioavailability of artemisinin from tea preparations may exceed that from pure artemisinin tablets, but nevertheless the intake of artemisia tea cannot produce the dihydroartemisinin blood levels, which have been proved to be effective against malaria in clinical trials of the pure artemisinin derivatives. However, it does appear feasible to exceed, by the use of *A. annua* tea preparations, the plasma concentration threshold of 10 μg/l above which a growth inhibition of the parasite is observed (Alin and Bjorkman, 1994). After absorption, the active metabolite dihydroartemisinin is concentrated in the erythrocytes 300-fold (Gu et al., 1984). Elimination half-life is approximately 4.3 h (Benakis et al., 1997), and it was suggested that a permanent

effective plasma concentration is to be preferred over intermittent concentrations (Titulaer et al., 1990; ter Kuile et al., 1993; de Vries and Dien, 1996), i.e. that several daily doses may be more effective than a single dose. In vitro investigations of the extract of the plant have shown that other constituents, notably flavonoids, enhance the antiplasmodic activity of artemisinin (Elford et al., 1987). The possibility of synergism between different components of antimalarial plants has recently been discussed (Kirby, 1997). In conclusion, it appears plausible that tea preparations from *A. annua* may show a certain clinical efficacy against malaria, as suggested by the Chinese pharmacopoeia and our clinical observations.

The risk of toxic effects from the use of this plant appears to be limited, since *A. annua* is included in the pharmacopoeia of the People's Republic of China, with recommendations for its dose and therapeutic use, and it may therefore be regarded as one of the established medicines in the present world. Adverse effects from pure artemisinin are minimal (de Vries and Dien, 1996). As with any experimental medication, however, close attention must be given to possible risks and side effects of *A. annua* preparations.

Additionally it must be underlined, that all the results presented in this study were obtained exclusively from adult patients with a significant degree of immunity to malaria. For these patients malaria is rarely a life-threatening disease.

This situation is, however, completely different for young children and people from areas in which malaria is not endemic. It must be emphasized that the results of this study are not applicable to these groups, for which malaria may be fatal.

Artemisinin and its derivatives have a 'reserve status' in the World Health Organization (WHO) essential drugs list; that means they should be reserved for use only in areas with multidrug-resistant malaria, such as southeast Asia. Obviously, associated with the use of locally prepared medications from *A. annua* plants in Africa is the extremely serious risk of inducing resistance against this important drug, especially when insufficient doses are used and when no complete cure is achieved. On the other hand, it may be argued that the use of *A. annua* preparations for the treatment of fevers has occurred in China for 2000 years, and continues to occur, apparently without the emergence of resistance.

The central question, therefore, before further studies are conducted, is whether or not the potential risks, namely of resistance development, outweigh the potential benefits of locally produced *A. annua* preparations, namely that malaria treatment could possibly be made available to a large number of so far untreated patients in this world. This question may be answered differently by people who presently do have access to modern antimalarial drugs as compared with people who do not.

In our opinion, a further principal investigation of the antimalarial potential of *A. annua* preparations is justified. Only additional studies, under inclusion of control groups, can determine the efficacy of *A. annua* tea, or other preparations from the herb, in the treatment of malaria. The potential risk of resistance development makes it necessary to look for regimens, which offer a radical cure, and therefore recrudescence rates must be closely monitored. As with monotherapy with artemisinin, a 5-day course should be the minimum; available clinical data for the artemisinin derivatives indicate that, given a dose sufficient to induce initial parasite clearance and clinical recovery, the duration of therapy is the determinant for recrudescence (de Vries and Dien, 1996). For reproducibility, studies have to use plant material from *A. annua* varieties, which have been clearly identified, with a known content of artemisinin. In any case, the consent of health authorities and medical experts has to be sought before such studies are initiated, and we do not recommend a widespread use of *A. annua* preparations before the benefits and risks of the use of such preparations have been assessed in more detail.

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