Crypto-infections conference

Dublin

31st May-1st June 2019
Malaria in numbers

- **3 billion** people live in malaria infested areas - ie. half of the global population.
- **219 million cases in 2017** according to WHO figures (91% of cases in Africa)
- Primary cause of death from infectious diseases: **435 000 deaths each year** 70% of deaths in children under 5 years
- Appearance of **resistance** to insecticides and medicines
- Responsible for 30% of absenteeism at school and in the workplace
- Estimated annual cost for Africa: **1.7 growth points**

Current solutions

- **Medicines**: Quinine, ACT, Malarone, ...
  - Resistance and significant side effects
  - Cannot be taken continuously
  - Difficult to access, high price
  - On average, **23% of medicines in circulation are counterfeit**
  - Not suitable for extreme temperatures

- **Insecticide-treated nets**
  - Resistance to insecticides (pyrethroid insecticides)
  - Can cause health problems
Artemisia - an additional solution

Artemisia annua L. & Artemisia afra Jacq.: 

- Plants in the Asteraceae family (Wormwood and african Wormwood)
- Used for centuries in China and Africa as antihelmintics, antimalarial (“fevers”)
- Infusions and oral consumption of the dried leaves and stems of the plant have prophylactic and therapeutic efficacy
- Hundreds of active ingredients: including phytosterols, flavonoids, essential oils, terpenes like artemisinin (in A.annua only), polysaccharids...
- No toxicity and no side effects noted to date (the use of medicinal plants along history is normally considered to be an evidence for their efficacity and non-toxicity, GRAS in USA)
- No resistance developed despite centuries of use
Who will finance research on a therapeutic solution that does not earn money?

An NGO!
Genesis of La Maison de l’Artemisia

- In Ethiopia, spectacular recovery of Alexandre Poussin from malaria falciparum with *Artemisia annua* herbal tea
- La Maison de l’Artemisia association founded in 2012 to work towards an effective, accessible and local solution available for affected populations.
  - Demonstrate the clinical effectiveness of *Artemisia annua* and *afra* herbal teas
  - Set up specialist centers bringing together medical and agronomic skills according to a charter of best practices: *Les Maisons de l’Artemisia*
Fundings of La Maison de l’Artemisia

✓ Foundations

✓ Corporate Sponsorship
Philanthropic donations by our own Foundation under the aegis of the Caritas Foundation:

Foundation Terra Artemisia allows donations of real estate wealth tax IFI.
Fundings of La Maison de l’Artemisia

✓ Hundreds of individual donors interested in this cause:

✓ La Maison de l’Artemisia NGO is an officially recognised charitable organisation.

✓ In France, the tax authorities encourage donations by individuals and companies by enabling them to claim a tax deduction on donations to charitable organisations and foundations.

✓ According to French tax law, individual donors can enjoy a tax reduction of 66% of the amount they donate, up to a maximum of 20% of their taxable income.
In 2015: we funded 2 randomized, double blinded large clinical trials in DCR

1. Effect of *Artemisia annua* and *Artemisia afra* tea infusions versus Prazicantel on schistosomiasis on 800 patients.

2. *Artemisia annua* and *Artemisia afra* tea infusions vs. artesunate-amodiaquine (ASAQ) in treating *Plasmodium falciparum* malaria on 957 patients.
An international team

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Effect of *Artemisia annua* and *Artemisia afra* tea infusions on schistosomiasis in a large clinical trial

Jérôme Munyangi¹, Lucile Cornet-Vernet²*, Michel Idumbo³, Chen Lu⁴, Pierre Lutgen⁵, Christian Perronne⁶, Nadège Ngombe⁷, Jacques Bianga⁸, Bavon Mupenda⁹, Paula Lalukala¹⁰, Guy Mergeai¹¹, Dieudonné Mumba¹², Melissa Towler¹³, Pamela Weathers¹³

Phytomedicine 51 (2018) 233–240
✓ Superiority trial
✓ Patients were randomly assigned to one of 3 arms
✓ Written consent to participate in the trial; parents signed for minor patients.
✓ Sample size determined

Methods
Study design

800 Patients enrolled
Kakutya: 163
Kamundala: 159
Kinkingwa: 157
Lubile: 162
Kakozwa: 159

Arm Artemisia: 400
Artemisia afra: 200
Lost contact: 1
Protocol violation: 3
Withdraw consent: 1
Full follow-up
Arm Artemisia: 390

Artemisia annua: 200
Lost contact: 2
Protocol violation: 2
Withdraw consent: 1
Full follow-up
Arm PZQ: 390

Arm PZQ: 400

Lost contact: 10
Methods

Criteria for inclusion of patients

- Based on *S. mansoni* positive stool examinations, 800 patients were selected from five sites.
- They had not taken any medication for treatment of *S. mansoni* infection,
- Medical treatment was maintained for patients already receiving treatment for another known disease
- Excluded:
  - ✓ children under the age of six,
  - ✓ elderly patients (over 60),
  - ✓ pregnant women,
  - ✓ patients with concomitant acute or severe chronic diseases.
  - ✓ Patients with the hepatosplenic form of the disease
Methods

Phytochemical analysis of Plant Material

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Partial phytochemical composition of <em>Artemisia</em> cultivars used in this clinical trial (mg/g DW).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytochemical</td>
<td>A. afru PAR</td>
</tr>
<tr>
<td>Voucher id</td>
<td>L00019529 Université de Liège</td>
</tr>
<tr>
<td>Total terpenoids and flavonoids</td>
<td>27.92a</td>
</tr>
<tr>
<td>Total terpenoids</td>
<td>3.74a</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.045</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>nd</td>
</tr>
<tr>
<td>Deoxyartemisinin</td>
<td>nd</td>
</tr>
<tr>
<td>Artemisinic acid</td>
<td>nd</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.07a</td>
</tr>
<tr>
<td>Luteolin</td>
<td>0.07a</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>0.45a</td>
</tr>
<tr>
<td>Cholesterenic acid</td>
<td>nd</td>
</tr>
<tr>
<td>Camphor</td>
<td>0.10a</td>
</tr>
<tr>
<td>Essential oils</td>
<td>0.32a</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>nd</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>nd</td>
</tr>
<tr>
<td>β-pinene</td>
<td>nd</td>
</tr>
<tr>
<td>L8 class E (eucalyptol)</td>
<td>0.47a</td>
</tr>
<tr>
<td>Bornol</td>
<td>0.57a</td>
</tr>
<tr>
<td>Spinalol</td>
<td>0.12</td>
</tr>
<tr>
<td>α-cyclocedrol</td>
<td>0.51a</td>
</tr>
<tr>
<td>Phyllol</td>
<td>nd</td>
</tr>
<tr>
<td>Thujone</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Plant cultivar origins (BUR, Burundi; LUX, Luxembourg; PAR, Paris; SEN, Senegal) had 3-4. Significance at p ≤ 0.05; a,b letters compare A. afru PAR and SEN; x,y letters compare A. annua LUX and BUR; nd, not detectable. Statistical analysis impossible when 1 of the 2 samples was nd.

- Expressed as amonitin equivalents.
- Expressed as quercetin equivalents.
- Expressed as camphor equivalents.
Methods

Drug administration

- **In the Artemisia treatment arms**, patients drank 0.33 l of *A. annua* or *A. afra* infusion 3 times daily for 7 days. Infusion was prepared as follows: 5 g dried leaves and twigs of *A. annua* or *A. afra* were added to 1 l of boiling water, infused for 10 min, and filtered through a sterilized 1mm mesh. To double-blind the trial they receive PZQ placebo (pill-shaped saccharose/glucose candies) from D0-7.

- **For the PZQ group**, patients were treated with PZQ tablets (40 mg/kg in 1 day), followed by PZQ placebo for 6 days in order to follow the 7 days treatment schedule for the *Artemisias*. They also received *Artemisia* placebo (tea was prepared with 0.2 g/l of plant material) from D0-7.
Results

Egg elimination from fecal samples

- Eggs disappeared faster in fecal samples and within 14 days for both *A. annua* and *A. afra*-treated patients compared to those treated with PZQ.
- No significant differences between *Artemisia* sp. from D3 through D28.
- Significant difference in egg count between *Artemisia* sp. and PZQ from D3 through D21.
Results

Adverse effects

- In PZQ-treated patients: Vomiting 26.5%, abdominal pain 18.5%, and headache 15.5% were the most common adverse events observed.

- In the two *Artemisia* arms, no patients suffered any of those or other undesirable effects.
Conclusion

▪ This trial showed that both *A. annua* and *A. afra* tea infusions were at least 30% faster acting against bilharzia than PZQ, but with equal efficacy at D28 for both genders.

▪ There also were fewer adverse effects on patients treated with an *Artemisia* tea infusion than with PZQ.
Artemisia annua and Artemisia afra tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial

Jérôme Munyangi¹, Lucile Cornet-Vernet²*, Michel Idumbo³, Chen Lu⁴, Pierre Lutgen⁵, Christian Perronne⁶, Nadège Ngombe⁷, Jacques Bianga⁸, Bavon Mupenda⁹, Paula Lalukala¹⁰, Guy Mergeai¹¹, Dieudonné Mumba¹², Melissa Towler¹³, Pamela Weathers¹³

Phytomedicine 57 (2019) 49–56
DRC has one of the highest malaria rates, in 2016 > 50,000 people died, mainly children (WHO 2017).

Maniema province is a holo-endemic zone with >80% of the population infected by *P. falciparum* (PNLP Kinshasa 2015).

In 2005, Artemisinin Combination Therapy (ACT) became the first line treatment for all in the DRC.

Medicinal plants represent the main, if not the only treatment accessible to much of humanity (WHO 2014).

For traditional medicine, WHO provided guidelines (WHO 2000) and encouraged studies and clinical trials on herbal medicines (WHO, 2014).
WHO Traditional Medicine Strategy
2014-2023
**Background**

- *Artemisia annua* L. and *Artemisia afra* Jacq. ex Willd., of Chinese and South African origin, respectively, were used for centuries medicinally as infusions or powders to treat malaria (Willcox et al., 2004; Weathers et al., 2014; Liu et al., 2010).

- Both showed *in vitro* efficacy against *Plasmodium falciparum* (Kraft et al., 2003; Moyo et al., 2016; Gathirwa et al., 2007; Liu et al., 2010).

- A large-scale clinical trial aimed at confirming encouraging results from smaller-scale trials in Africa and South America with *A. annua* and *A. afra*. In those small trials cure rates of >95% were reported (Mueller et al., 2000; Gebeyaw et al., 2010; Chougouo et al., 2012; Zime-Diawara et al., 2015).
Methods

Plant material, handling and phytochemical analysis

Same as Schistosomiasis trial

*A. annua* had 1.34-1.70 mg artemisinin/g dry weight;
*A. afr* had 0-0.036 mg artemisinin/g dry weight.
Methods

Study design

✓ Superiority trial
✓ Sample size determined
✓ Patients with uncomplicated malaria were randomly assigned to one of three arms
✓ Written consent to participate in the trial; parents signed for minor patients.
✓ Drugs were distributed in numbered opaque envelopes selected randomly by each patient.
Methods

Criteria for inclusion of patients

▪ Adults or children ≥5 y with axillary temperature of ≥37.5 °C and parasitemia of 2000 – 200 000 trophozoites/μl.

▪ Medical treatment that did not violate trial exclusion criteria was maintained for patients having a known disease other than malaria at the beginning of the trial.

▪ Excluded, patients with:
  ✓ severe malaria,
  ✓ undernourishment,
  ✓ repetitive vomiting±diarrhea,
  ✓ known allergy to ACTs,
  ✓ pregnancy or breast feeding,
  ✓ concomitant infectious diseases.
  ✓ cardiac, hepatic, or renal deficiencies
  ✓ patients treated with antimalarial drugs within 7 days preceding the trial or with antibiotics that might have antimalarial effects.
Methods

Clinical and laboratory analyses

- Thick and thin blood smears were microscopically analyzed for trophozoites and gametocytes, respectively.
- Trophozoites were measured per μl of blood.
- Gametocytes were noted as present or absent to determine number of carriers.
- Slides were scrutinized by two independent laboratories.
- In case of dubious or conflicting results, a PNLP expert was consulted.
- A random 10% of slides collected at different stages of the trial were quality-control checked by a third party.
- Patient follow-up including thick and thin blood smears was at D 1, 2, 3, 4, 7, 14, 21, and 28 post trial enrollment.
- From D0–D7, patients were treated in hospital to insure therapeutic compliance.
- Hemoglobin, ALAT and ASAT also were measured.
Methods

Drug administration

In the ASAQ arm:

- Artesunate-amodiaquine (ASAQ Winthrop, Sanofi–Aventis) was administered as tablets 25 mg/67.5 mg, 50 mg/135 mg, and 100 mg/270 mg.

- Manufacturer posology was: 4 mg of artesunate and 10 mg of amodiaquine/kg, once daily for 3 days, followed by ASAQ placebo tablets for the remaining 4 days.

In the Artemisia arms:

- Patients drank 0.33 l of *A. annua* or *A. afra* infusion every 8 h for 7 days. Infusion preparation was:
  - 5 g of dried leaves and twigs of *A. annua* or *A. afra* to 1 l of boiling water, infuse for 10 min, and filter through sterilized 1mm mesh.

- Pediatrics and adults received the same amount of *Artemisia* tea infusion; there was no adjustment for body weight.
Methods

Drug administration: the double blind

In the ASAQ arm

As aq  As aq  As aq  Pl  Pl  Pl  Pl  Pl
Pl  Pl  Pl  Pl  Pl  Pl  Pl  Pl

In the Artemisia arms

Pl  Pl  Pl  Pl  Pl  Pl  Pl  Pl
Aa  Aa  Aa  Aa  Aa  Aa  Aa  Aa
Results and discussion

Parasitemia progression

Both *Artemisia* sp. cured malaria more effectively than ASAQ.
Results and discussion

Parasitemia progression

- There were 344 patients with parasites at D28:
  - 9 for *A. annua*,
  - 25 for *A. afra*,
  - 310 for ASAQ.

- Due to degradation of stored blood samples, we were unable to conduct a valid PCR analysis of the D28 patients with parasitemia.

- Considering the large number of D28 patients with parasitemia, a thorough PCR analysis is needed in future studies.
Results and discussion

Parasitemia progression

Cures rates were established by D28 parasitemia:

- ASAQ: 34.3%
- *A. afr*a: 88.8%
- *A. annua*: 96.4%
Results and discussion

Fever progression

Both *Artemisia* sp. cured malaria faster than ASAQ.
Results and discussion

Gametocytes carriage

At D28:

- ✓ in ASAQ treated patients 10 of 472 patients were detectable carriers

- ✓ In Artemisia treated patients no gametocyte carriers = no transmission

Fig. 4. Microscopically determined proportion of patients with gametocytes (carriers) throughout the trial period.
Results and discussion

**Side effects**

Distribution among patients of adverse effects from treatment.

<table>
<thead>
<tr>
<th>Observed adverse effects</th>
<th>Number of subjects in the <em>Artemisia</em> arms</th>
<th>Number of subjects in the ASAQ arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Fatty cough</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Nausea</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Pruritis</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Vertigo</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>210</td>
</tr>
<tr>
<td>% of total</td>
<td>5.0%</td>
<td>42.8%</td>
</tr>
</tbody>
</table>
Results and discussion

Biological tolerance

Hemoglobin levels:
This similarity between ASAQ and A. annua is likely the result of artemisinin, known to reduce hemoglobin during treatment (Kurth et al., 2016). A. afra has but a trace of artemisinin.

ALAT and ASAT were not altered: Although liver transaminases are often altered in malaria patients (Woodford et al., 2018), there were no significant differences observed in ALAT and ASAT among the three treatment.

Fig. 5. Hemoglobin levels during the first four days of treatment.
Results and discussion

Synergistic effects

- *A. annua* and *A. afr* contain hundreds of phytochemical molecules and minerals.

- Antimalarial activity has been demonstrated for some 20 of these as summarized in the review by Weathers et al. (2014).

- *A. afr* has only trace amounts of artemisinin and no detectable artemisinic parent molecules, patients treated by *A. afr* had almost the same therapeutic response as those treated with *A. annua*. 
Results and discussion

Artemisinin drug resistance

▪ Low artemisinin content of the plant elicited concerns that this might induce artemisinin drug resistance (Blanke et al., 2008; Mueller et al., 2004; WHO, 2012).

▪ Studies showed that the numerous phytochemicals in *A. annua* and *A. afra* provide synergies and multiple interactions, likely constituting a polytherapy against malaria (Elford et al., 1987; Li et al., 2018; Liu et al., 1992; Suberu et al., 2013).

▪ Together, those data and this study indicate that artemisinin is not the sole phytochemical demonstrating antimalarial efficacy by *Artemisia* sp.
Conclusion

- Artemisia plants can be grown and used by local populations, which could help counteract the problems caused by fake or obsolete antimalarial drugs.

- Further large-scale studies be launched to optimize use of these plants and posology for infant, pregnant women and others, in the case of uncomplicated as well as severe malaria.
MERCI !

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