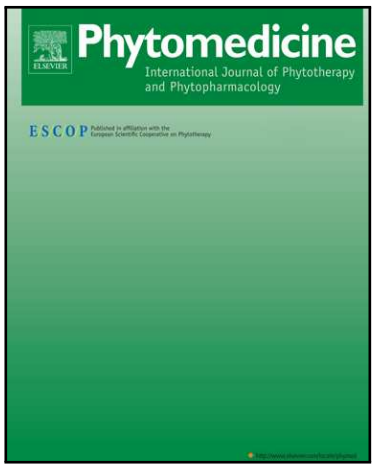

RESPONSE to Argemi et al. 2019

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RESPONSE to Argemi et al. 2019

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Introduction:

We thank the authors for their in-depth analysis of our study and wish we had received many of their excellent comments prior to publication. To begin, this is not a registration study and further trials, integrating the conclusions and perspectives highlighted by the published study, are anticipated. The perfectly understandable operational difficulties concerning the implementation of this type of clinical trial and the collection of samples and clinical parasitological data led us to make methodological choices that may diverge from international standards for pharmaceutical drug development in order to obtain the maximum amount of pertinent information using as few therapeutic trials, human, and financial resources as possible.

First, we wish to emphasize that use of *Artemisia* is not an artemisinin monotherapy, nor an ACT. Rather it is a potential antimicrobial polytherapy by virtue of its plethora of antimicrobial phytochemicals (see as examples, Efferth 2009; Suberu et al. 2013; Weathers et al. 2014). This study was justified on the basis of chance observations during our *Artemisia*/malaria trial (Munyangi et al. 2019), that *Artemisia* herbal teas seemed to eliminate schistosomiasis eggs from tea-treated patients. Those observations led us to envisage this trial, which was then authorized by the Kinshasa ethics committee and to include comparison to the standard treatment drug, praziquantel (PZQ).

Schistosomiasis is not a life-threatening disease, at least not in the short or medium term. Phases I-II were not considered prerequisites as PZQ has been authorized worldwide for decades and *Artemisia annua* and *A. afra* have been used widely by millions of consumers for centuries, especially in China, where pharmacovigilance has indicated no concerning adverse effects with the herbal tea. Furthermore, the Kinshasa ethics committee did not issue any comments or restrictions on this approach.

The conclusions of the trial clearly point to tangible activity elements. This indication is the first stage for initiating a more targeted project.

Below we address specific issues noted by Argemi et al.

Study protocol:

PZQ dosage was indeed 40 mg/kg per patient taken in one dose: we acknowledge this important typographical error and by this reply we have provided an importnat *erratum* to rectify. Concerning children: the tablets were crushed and added to a small ball of "fufu", a cassava flour paste, to facilitate drug delivery. For *Artemisia* infusion, they received the same tea infusion volume as adults. Adults over 60 were excluded from the study in deference to concerns about possible frailty and mental acuity during this first assessment of *Artemisia* for this use.

Egg analyses: For egg analysis, two stool samples were collected per patient. All samples declared positive for *S. mansoni* infection were re-examined the following day in such a way that two filters and slides were examined per patient. Quality control on results focused on rereading 10% of the total number of filters and slides, randomly selected by an experienced reader from another unit or another laboratory. If the difference (10-egg difference) between the number of eggs counted by the two readers was below 20% (except for cases of subjects with hyperparasitism), the average parasitaemia was used for analysis. If the difference between the two readers was equal or above 20% of cases, a third reader reread all filters and slides. We regret use of the term "viable" with reference to eggs; we did not measure viability, but rather egg presence.

Issues in the field and difficulties encountered in monitoring the study led to some deviations in long-term monitoring. We agree that long-term assessment is important, but we were not able to extend this study to 3 months follow-up. Nevertheless, our study provided strong data indicating the anti-schistosomal activity of *Artemisia* teas thereby providing the data that had previously been missing for investigating use of this traditional medicine. This information is very important for the next stage of study.

Power analysis and statistics: Although as stated we did not have disease specific *Artemisia* data to conduct a power analysis, one could construe sample size as you suggested and herein described. In the Kalima health zone, the frequency of *S. haematobium* and *S. mansoni* infection in the population is 70.8%. We therefore estimated that by examining stools and urine from 1,150 subjects, we would detect 814 infected subjects. To calculate the sample size, we estimated a cure rate of 75% (60-95%) with PZQ and +10% with herbal teas. With 90% power and alpha type error equal to 5%, 354 patients were required for each treatment arm. An additional 46 subjects were recruited in each treatment arm to compensate for immeasurable observations (loss of data rather than non-observance of treatment by the patients). The total number of subjects required for the study was therefore fixed at 800 (100 blocks, 8 patients per block) using the closed envelope system. This was considered the most appropriate and easiest approach for logistical reasons. As previously stated, the PZQ group was justified, in particular, to characterize the profile of the population based on clinical and parasitological results noted with the product at an expected response rate fixed at 75%.

Regarding statistical analysis and mathematical models, the *p*-value for all three overall tests (likelihood, Wald, and score) is significant, indicating that the model is significant. In the multivariate Cox analysis, the covariates age and treatment remain significant ($p<0.05$). However, the covariate sex fails to be significant ($p=0.78$, which is greater than 0.05). The *p*-value for age is < 2e-16, with a hazard ratio HR = exp (coef) = 5.5180 (>1), indicating a strong relationship between the treatment groups and decreased number of eggs. Holding the other covariates constant, a lower value of treatment (PZQ = 1, Annua = 2, Afra = 3; where 1 is worst) is associated with a poorer clearance of the number of eggs. We used a post-hoc test (Bonferroni correction) for multiple comparisons. We used one-way ANOVA and post hoc test (Bonferroni) to test the significance of the reduction of the number of eggs among all three treatments. The results show that the reductions are statistically significant between PZQ and Annua, and between PZQ and Afra ($p<0.001$). The reductions, however, are not significant between Annua and Afra ($p=0.273$). The results of mixed ANOVA show that the number of eggs were significantly reduced through time ($F(1.103, 856.8) = 21419.655$, $p<0.001$). For the mixed ANOVA, time is a with-in subject with 6 levels (0 day, 3 day, 7 day, 14 day, 21 day, and 28 day), and treatment (PZQ, Annua and Afra) is a between subject. Bonferroni post-hoc tests comparing the number of eggs between adjacent time points revealed a significant difference ($p<0.001$). The interaction between time and treatments is also significant ($F(2.206, 856.8) = 194.49$, $p<0.001$). Bonferroni post-hoc tests comparing between treatments revealed a significant different ($p<0.001$) in the number of eggs between PZQ and Annua, and between PZQ and Afra. The comparison between Annua and Afra was not significant (*p=0.199*). We performed survival analysis and generated a Kaplan-Meier survival plot. A log rank test determined if there were differences in the survival distribution of number of eggs for the different treatments: PZQ, *A. annua*, and *A. afra*. The survival distributions for the three treatments were statistically significantly different, $\chi2(2) = 779.00$, $p<0.0005$. The mean for survival time of PZQ is 27.9 (95%CI: (27.914, 28)) and median is 28. The means and medians for survival time of *A. annua* and *A. afra* are both 14 (95% CI: 14), and earlier than that for PZQ. The survival curves of both *Artemisia* sp. fall at day 14 rather than day 7.

We are confused by the assertion that there was no primary outcome specified, as the primary and secondary outcomes were indeed specified in the first paragraph of the Results.

In our double blind protocol, the use of the conventional medicine placebo (sugar pills for PZQ) did not cause any significant problems. The herbal tea placebo was conducted using procedures suggested by WHO for testing traditional medicines such as a tea infusion (see: Guidelines for Methodologies on Research and Evaluation of Traditional Medicine http://apps.who.int/iris/bitstream/handle/10665/66783/WHO_EDM_TRM_2000.1.pdf;jsessionid=58426E99AFB3D5CEDC7DF3E3686E4D87?sequence=1). The population selected for the study was already familiar with *A. annua* and *A. afra* herbal teas that have a strong taste and smell. Finding a placebo with the same taste, smell, and even color as *Artemisia* was challenging; consequently, we instead used a very weak dose of *Artemisia* in the control group (0.2 g of dried leaves and stems in 1 liter of water, not “a brief infusion of plants” as stated) compared to 5 g dried leaves and stems in 1 liter of water for the experimental group.

While the precise amount of artemisinin was not measured *per se* in the prepared and consumed tea, it was analyzed in the dried leaves used in the study and there was at most 8.5 mg of artemisinin delivered daily to the *A. annua*-treated patients. We did note in the Discussion that there was a trace amount of artemisinin in the placebo. The placebo tea leaves used for the PZQ-treated patients delivered at most 0.34 mg artemisinin, a significant dilution of that singular compound. Tea preparation does not extract all available artemisinin, but according to van der Kooy and Verpoorte (2011), when precisely prepared "efficiencies of above 90% can be reached" with artemisinin "stable for at least 24 hours" at ambient temperature. Future studies should also analyze the tea preparations *per se*. As previously noted, dosage was the same for all patients, regardless of age or body weight, and yes, children also drank the 3 daily aliquots of tea. In developing countries, use of herbal tea infusions is embedded in the culture and thus less of a problem than for western more developed cultures. While it is impossible to measure or calculate precise dosage of every phytochemical delivered, analysis of the dried leaves (Table 1) detailed some key analytics, e.g. the presence or near absence of artemisinin.

Since the comment about management of missing data is vague, we are unable to respond. The study design aimed for Afra, Annua, PZQ enrollment of 200+200+400, but actual numbers were slightly different as indicated in Fig. 1 and Table 2, where we identified the number of patients in each arm that were lost to the study. Data were analyzed under intent to treat (ITT), the definition of which is some matter of debate (Yelland et al. 2015); however, we did try to account for randomization errors as much as possible (see statistical discussion). When the data were received, we initially reviewed all as 3 treatment arms, but after noting there were no statically different results between the two *Artemisia* arms, we decided to discuss the data from those arms together vs. PZQ.

We regret the misstatement of adverse effects of the *Artemisia* groups and that sentence should have read as follows: "In the two *Artemisia* arms, few patients suffered any of those or other undesirable effects (Table S1)." It was stated as such in the Conclusions paragraph. Our percentages of melena at D0 are correct (see Table 3 column header: Patients with melena) if calculated on total enrollment as stated, but the baseline values stated by Argemi et al. are also correct in that they are relative to their respective treatment arms. Regarding inconsistencies between Fig. 2 and text at D0 and D3, yes, there were typos and the figure values are correct. Nevertheless the egg counts were very high in all patients and by D3 those values dropped by 90% to 66. Egg disappearance was essentially the same yielding those low, but identical values for both *Artemisia* sp. Regarding abnormal 5x values between *Artemisias* and PZQ data on adverse effects: first, we analyzed the data as received, second, results were similar to those for other *Artemisia* trials that were against malaria. In short, blindness was not broken and the consumption of mainly *A. annua* dried leaves and tea infusions has been demonstrated in other informal and more formal studies by us and others as benign to patients (Onimus et al. 2013; Daddy et al. 2017; ICIPE 2005; Munyangi et al. 2019). The long ethnobotanical tradition using these two *Artemisia* plant species also belies their safety.

Authors' conclusion that because PZQ is currently an efficient treatment for schistosomiasis, no one should conduct phase III clinical trials ignores the fundamental tenement of biology: resistance will eventually emerge against any single use drug. Studies of alternatives are always warranted. When there are medicinal herbs with GRAS and/or long-standing successful ethnobotanical history of safe and apparently efficacious use, a Phase III human trial is deemed acceptable to scientifically validate their efficacy. Whereas we may not have conducted a long-term (3 mon) confirmation of parasite disappearance, and there are a few typographical errors, the data are unchanged and the results still show that there is a strong antischistosomal effect of both *Artemisia* sp. in these patients.

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