THERAPEUTIC RESPONSE OF ARTEMISIA ANNUA TEA IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS: STUDIES IN VIVO

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INTRODUCTION

Cutaneous leishmaniasis (CL) is endemic in the tropics and neotropics. Most available drugs are expensive, require long treatment regimens and are increasingly ineffective. Therefore, the discovery of new compounds and the development of new alternatives for the treatment of CL is a global priority. *Artemisia annua* is a Chinese plant traditionally used to treat infectious and noninfectious diseases. The metabolite artemisinin is one of the most studied metabolites for antimalarial activity. However, *A. annua* has also in vitro activity against *Trypanosoma cruzi*, *T. b. brucei* and *T. b. rodhesiense*, *Schistosoma mansoni* and *S. caproni* and *Fasciola hepatica*. Additionally, this plant has also *in vitro* and *in vivo* activity against *Leishmania donovani*. In order to determine the potential of *A. annua* tea in the treatment of LC, the aim of this study was to evaluate the *in vitro* leishmanaicidal activity of the several products derived from *A. annua* (grass ground) cultivated in Luxembourg containing only 0.1% of artemisinin. The therapeutic response *in vivo* of the lyophilized acquous extract (LAE) of *A. annua* tea was also determined.

METHODOLOGY

*Cure: Complete re-epithelialization
Clinical improvement: reduction lesion size >30%
Failure: increase lesion size >50%
Relapse: Reactivation of lesion after cure

RESULTS

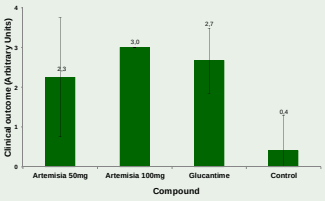
In vitro Cytotoxicity and leishmanicidal activity of *A. annua*

Product	CL_{50} µg/ml (X± DS)	CE_{50} µg/ml (X± DS)	
		Axenic amastigotes	Intracellular amastigotes
Crude	51.1 ± 2.8	162.8 ± 28.9	142.9 ± 49.3
Fraction 0	4.6 ± 0.7	**87.1 ± 17.2**	> 4.6
Fraction 1	106.7 ± 3.0	843.2 ± 154.9	**17.1 ± 1.8**
Fraction 2	48.0 ± 5.0	169.9 ± 36.5	> 48
Tea (infusión)	419.1 ± 1.3	1480.0 ± 309.7	> 419.1
LAE*	> 500	> 100	> 100
SbV (Glucantime)	495.9 ± 55.6	>200	6.3 ± 0.09

Degree of toxicity: Highly toxic: LC_{50} <10 mg/ml; Toxic: LC_{50} >10 to <50 mg/ml; Moderately Toxic: LC_{50} >50 to <200 mg/ml; Potentially Non Toxic: LC_{50}>200 mg/ml. Degree of activity: Highly Active: EC_{50} <10 µg/ml; Active: >10 to <50 mg/ml; Moderately Active: EC_{50} >50 to <100 µg/ml; No Active: EC_{50} >100 µg/ml. *LAE: Lyophilized acquous extract

Fraction 1 was the most active product against intracellular amastigotes. Fractions 0 and 2 were highly toxic. The tea infusion and LAE did not show leishmanicidal activity nor toxicity.

Clinical outcome at the end of study



The figure shows the clinical outcome in arbitrary units after 3 months of follow up. 0: Failure; 1: Relapse; 2: Clinical Improvement; 3: Cure.
Therapeutic response obtained with LAE 100 mg/kg was higher than SbV. No relapses were observed when hamsters were treated with LAE 50 or 100 mg/kg/day

Clinical outcome during follow up

Scheme (Mg/kg) (n)	EoT	1 month AEoT	2 month AEoT	3 month AEoT
LAE 50	0	0	50	75
LAE 100	0	0	40	100
SbV	85	100	100	71
Placebo (PBS)	0	0	0	0
Control	0	0	0	0

The table shows the clinical outcome in terms of % of cure at the end of treatment (EoT) and at 1, 2 and 3 months after the end of treatment (AEoT).

The therapeutic response obtained for LAE varied from 50% to 100% of cure after 3 months of the end of treatment, being the highest doses the most effective. No differences in the parasite load among LAE and SbV was observed.

No body weight lost or any histological change associated to treatment with *A. annua* was observed.

Representative figure of the clinical outcome in hamsters treated with LEA 50 mg/kg/d x 20d (upper left), LEA 100 mg/kg/d x 20d (lower left), SbV (upper right) and control w/o treatment (lower right).

CONCLUSIONS

- ✓ Lyophilized aqueous extract of *A. annua* (grass ground) cultivated in Luxembourg, contain only 0.1% of artemisinin.
- ✓ Fractionation of the *A. annua* extract may potentiate the leishmanicidal activity.
- ✓ Treatment with *A. annua* LAE showed no significant changes in the weight, histology and ALT, BUN, and Creatinine values compared with controls (Placebo, SbV and no treatment) suggesting no toxicity associated to the treatment.
- ✓ Unexpectedly, no correlation between *in vitro* and *in vivo* leishmanicidal activity of the *A. annua* LAE was observed. Although *A. annua* LAE did not show leishmanicidal activity *in vitro*, treatment of infected animals with 50 or 100 mg/kg/day produced 75% and 100% of cure after 3 months of the end of treatment, respectively. Complete cure began observed after one month of the end of treatment. No relapses were observed in hamsters treated with *A. annua* LAE whereas relapse was observed in 2/7 animals treated with SbV. The poor correlation between *in vitro* and *in vivo* assays could be due to differences in these two systems.
- ✓ Given the high effectiveness against *Leishmania*, we suggest that *A. annua* tea could be a promising candidate for the oral treatment of CL and therefore more studies to further optimization of the product are needed.

ACKNOWLEDGEMENTS: This work is funded by the University of Antioquia (CODI-CIIEs 2010).