Suppression effects of *Tinospora crispa* extract on microneme protein inhibit host cell invasion in *Toxoplasma gondii* infection *in vitro*

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INTRODUCTION

Infection with *Toxoplasma gondii* still remains of public health significance. Host cell invasion is actively coordinated by calcium-dependent protein kinases (CDPK). The chemotherapeutic agents used against toxoplasmosis are mainly antibiotics and anti-malaria. However, treatment failures with these agents, attributed to host drug intolerance with severe side effects as well as development of resistance have been reported. This study aims to evaluate the effect of the ethanolic extract of *T. crispa* (EETC) on CDPK genes of *T. gondii* that facilitate host cell invasion during infection.

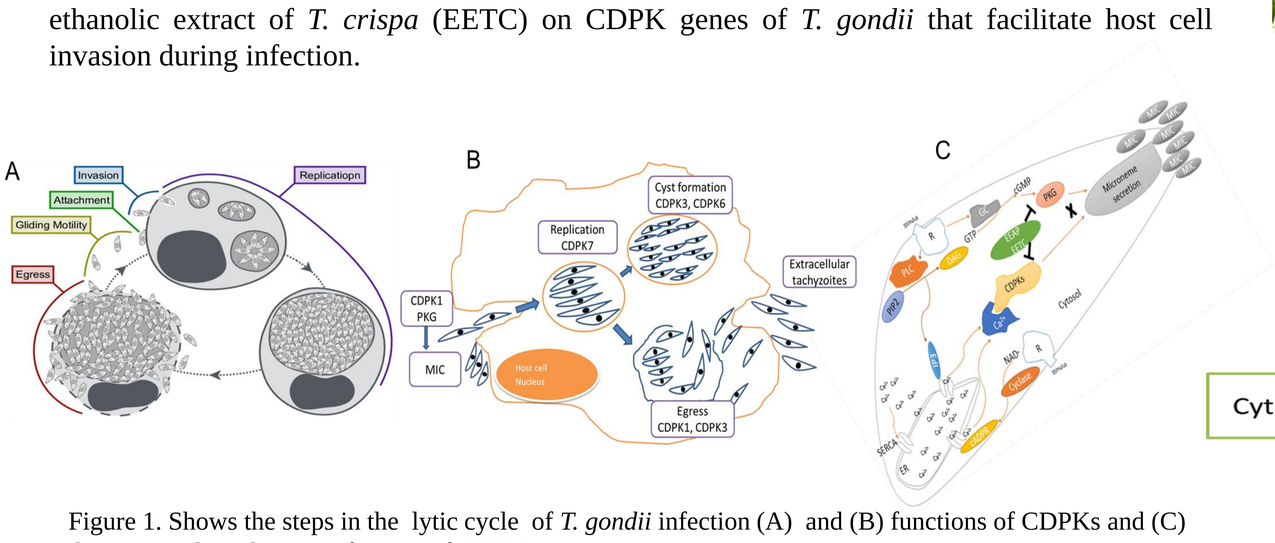

Figure 1. Shows the steps in the lytic cycle of *T. gondii* infection (A) and (B) functions of CDPKs and (C) the proposed mechanism of action of EETC.

METHODS

T. crispa

Figure 2. The research flowchart

RESULTS

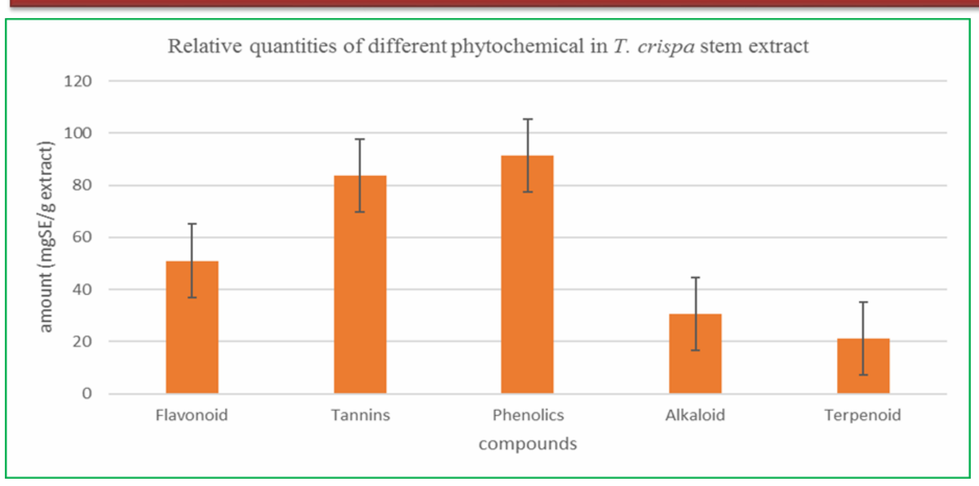

Figure 3. Quantification of major phytochemicals in EETC

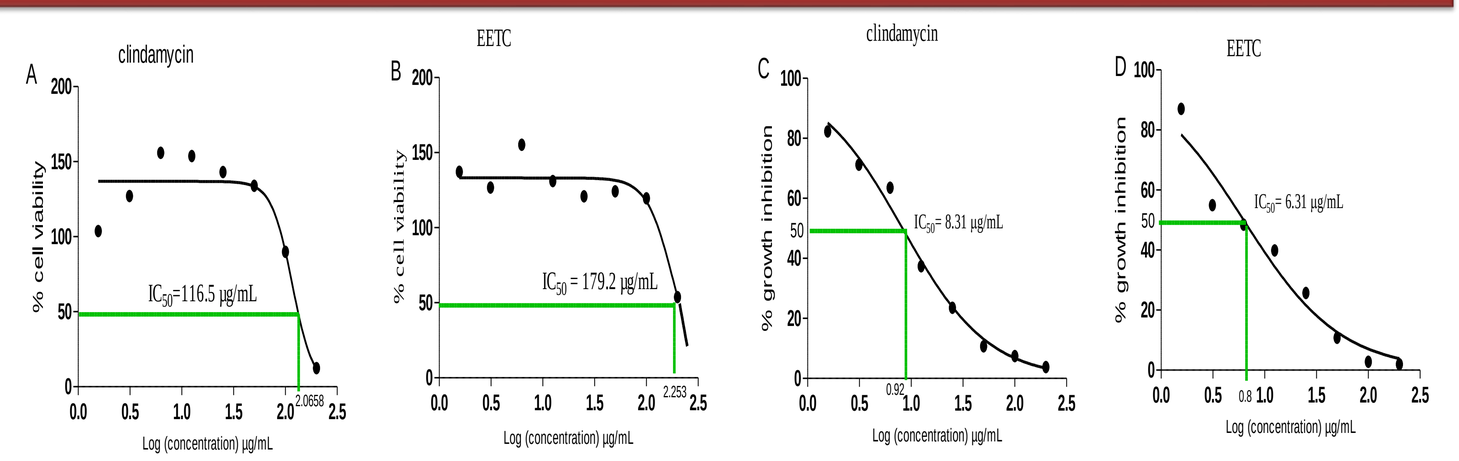

Figure 4. Shows cytotoxicity assay (A & B) and anti-parasitic assays (C & D) of clindamycin and EETC with estimated 50% inhibitory concentration

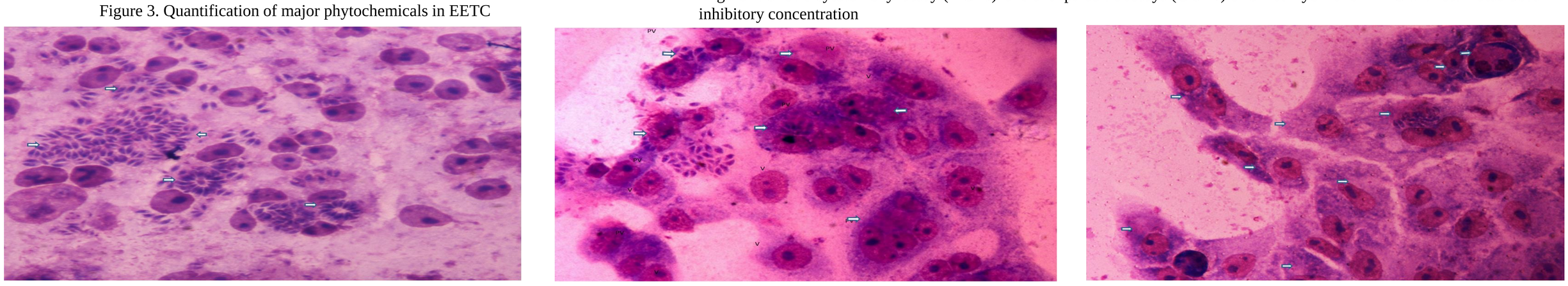

Figure 5. infection index and intracellular proliferation of *T. gondii*. More parasites are seen in the nontreated control (A) than (B) treated with clindamycin. There is marked reduction of the parasite in extracts treated group (C).

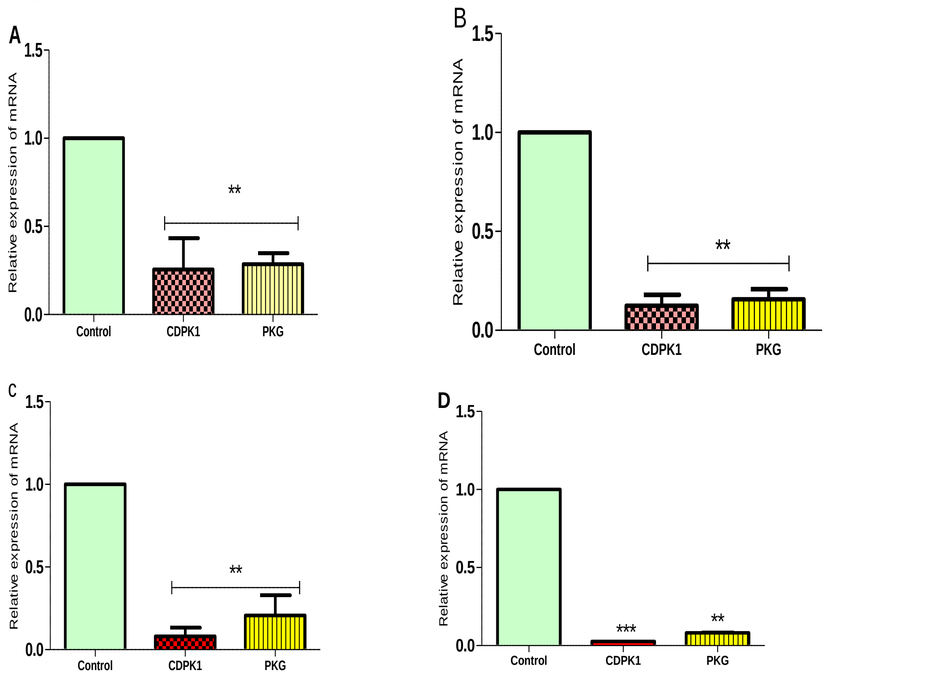

Figure 6. Relative expression of protein kinase genes after exposure to the extract in 4 h (A and B) and 24 h (C and D) post-infection treatment. It is shown that there is downregulation of all the PK genes.

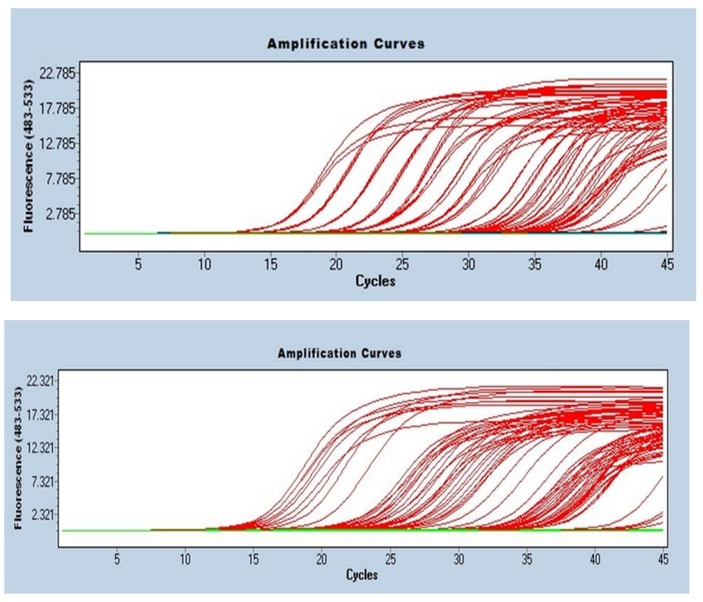

Figure 7. Microneme protein expression after treatment with EETC. Expression of MIC is significantly low in EETC treatment in both treatment models.

CONCLUSION

This study shows that EETC modulates expression of protein kinase genes and significantly decreased expression of microneme proteins thereby inhibiting host cell invasion in *T. gondii* infection. The EETC therefore contains phytochemicals that are effective against *T. gondii* that can be developed into effective drugs for treatment of toxoplasmosis

REFERENCES

1. Lebrun, M., Carruthers, V. B, and Cesbron-Delauw, M. F. (2014). *Toxoplasma* secretory proteins and their roles in cell invasion and intracellular survival. In *Toxoplasma gondii* (pp. 389-453). Academic Press.
2. Blader, I. J., Coleman, B. I., Chen, C. T, and Gubbels, M. J. (2015). Lytic cycle of *Toxoplasma gondii*: 15 years later. *Annual review of microbiology*; 69:463-485.

ACKNOWLEGEMENT

The authors would like to thank the Ministry of Higher Education, Malaysia by supporting this work through the Fundamental Research Grant Scheme (FRGS) from the Malaysian government [grant number FRGS/1/2015/SKK12/UPM/03/01]