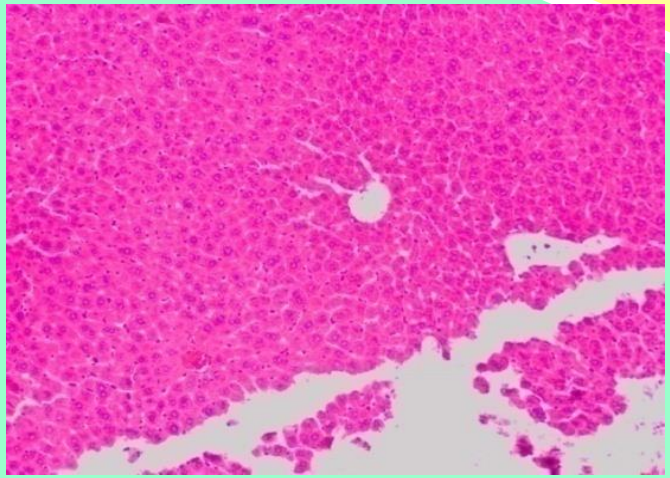
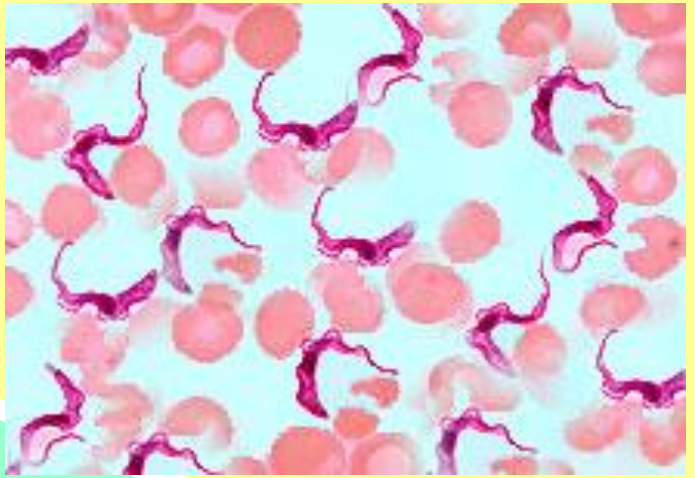
الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يونيورسيتي إسلام انترابغسا ملسيا

IN-VIVO ANTIPARASITIC ASSESSMENT OF ALLICIN AGAINST THE GROWTH AND SURVIVAL OF HAEMOFLAGELLATE, *Trypanosoma evansi*





Asst. Prof. Dr. Mohd Shukri Bin Baba

Department of Biomedical Science, Kulliyyah of Allied Health Sciences,
International Islamic University Malaysia

INTRODUCTION

INTRODUCTION

Trypanosoma evansi

- Species of *Trypanosoma* → Trypanosomiasis clusters
- Trypanosomiasis = vector-borne parasitic diseases (sleeping sickness, chagas disease and surra disease)
- *T. evansi* : haemoflagellated protozoa in mammals → surra disease (human zoonotic disease)
- 1st discovered (1880) by Griffith Evans in Punjab, India
- Malaysia : 1903 → cow & sheep due to livestock migration

INTRODUCTION

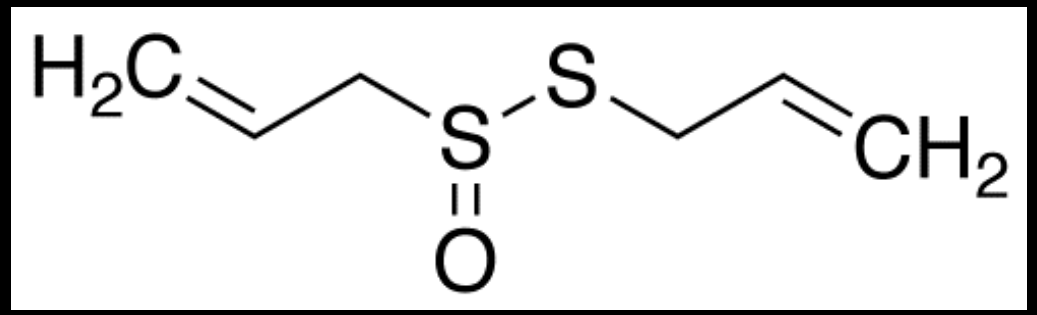
VECTORS OF TRYPANOSOMIASIS

INTRODUCTION

ALLICIN

- Chemically : 2-profenil-1-dialil tiosulfinat ($C_6H_{10}OS_2$)
- Naturally, not part of garlic compound (*Allium sativum*)
- Crushed/damaged garlic → AA (allin) interacted with catalytic enzyme (allinase) → allicin

$$(SCH_2CH{=}CH_2)_2 + R\text{-}CO_3H \rightarrow C_6H_{10}OS_2 + R\text{-}COOH$$



INTRODUCTION

ALLICIN : THE TESTIMONIAL

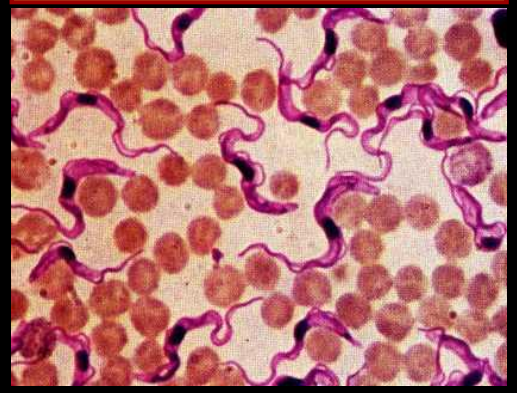
- As anti-inflammatory and antibacterial agents in China since more than a century (Cavalitto et al. 1944)
- Natural insecticide & fungicide (Cavalitto et al. 1944)
- *In-vivo* & *in-vitro* anti-leishmanial activity against *Leishmania donovani* in mice (Nok et al. 1996)
- Inhibit the growth of *Candida albicans*, *Giardia lamblia* and *Entamoeba histolytica* (Ankry et al. 1997)
- Antibacterial property with very low IC_{50} against *E. coli* EPEC strain (Ankry & Mirelman, 1999)
- Promising anti-hypertensive and anti-asthmatic effects on infants and young ages hosts (Rabinkov et al. 1998)

MATERIALS & METHODS

METHODOLOGY

FLOW CHART

T. evansi stock



T. evansi administered i.p.
(5 × 10^3 *T. evansi* / mice)

0.1 mL 15 µg/mL allisin-dH_2O solution administered orally

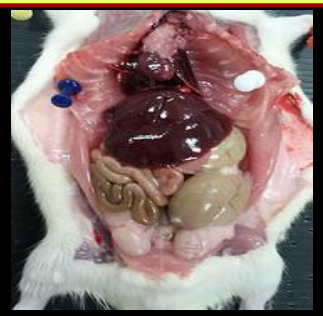
Giemsa blood slide for inhibition rate evaluation

Blood slide for electron microscopic observation

Physical observation of symptoms and mice survival

Blood biochemistry and renal function tests

Vital organ histology for toxicity assessment



METHODOLOGY

EXPERIMENTAL DESIGN

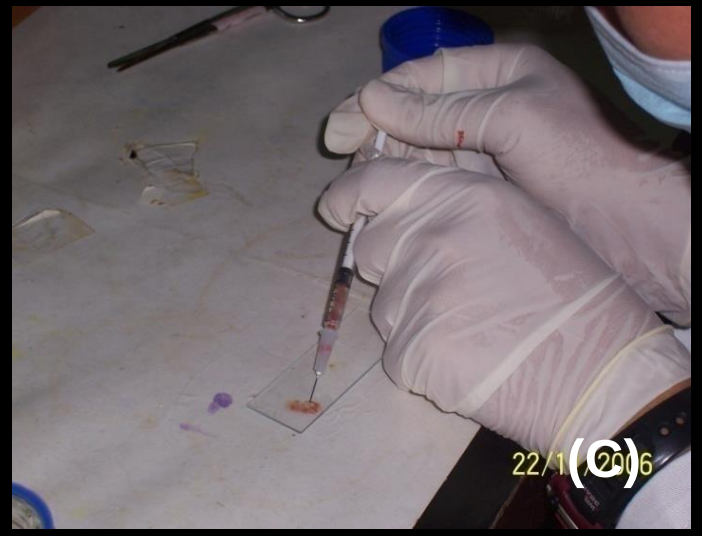
GROUP	REGIME	DESCRIPTION	DOSAGE
TREATMENT	PREVENTIVE	7 days pre-infection	0.1 mL 15 µg/mL allisin-dH_2O
		5 days pre-infection	0.1 mL 15 µg/mL allisin-dH_2O
		3 days pre-infection	0.1 mL 15 µg/mL allisin-dH_2O
	CONCURRENT	2 hours post-infection	0.1 mL 15 µg/mL allisin-dH_2O
	CURATIVE	3 days post-infection	0.1 mL 15 µg/mL allisin-dH_2O
		5 days post-infection	0.1 mL 15 µg/mL allisin-dH_2O
		7 days post-infection	0.1 mL 15 µg/mL allisin-dH_2O
CONTROL	POSITIVE	Berenil (Sigma-Aldrich KL)	0.01 mL 3.5 mg/kg bw
	NEGATIVE	0.9 % Normal Saline	0.1 mL 0.9 normal saline
	LETHAL INFECTION	Infection without treatment	5×10^3 parasites / mice (i.p.)

METHODOLOGY

PARASITE ADMINISTRATION & ANIMAL TAGGING


(A)


(B)


(C)


(D)

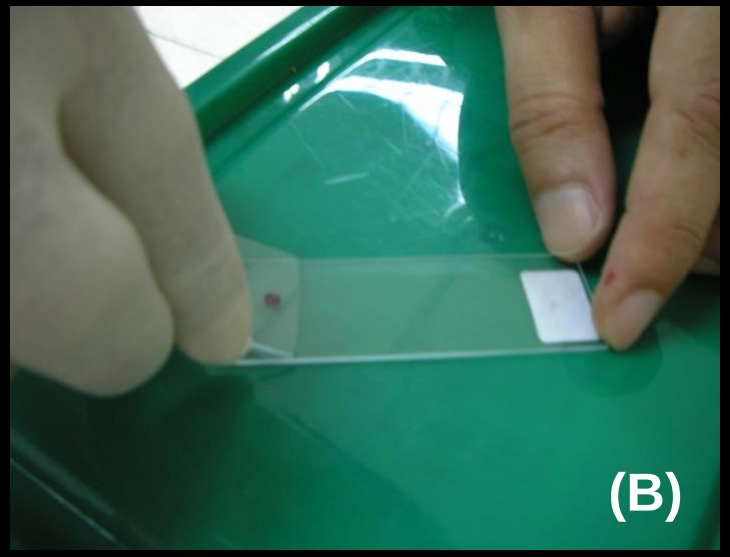

(E)

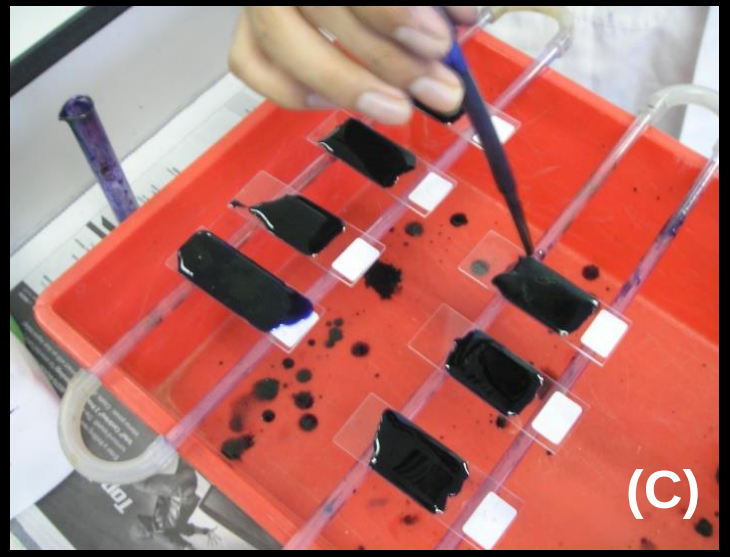

(F)

METHODOLOGY

GIEMSA STAINING & MICROSCOPIC OBSERVATION

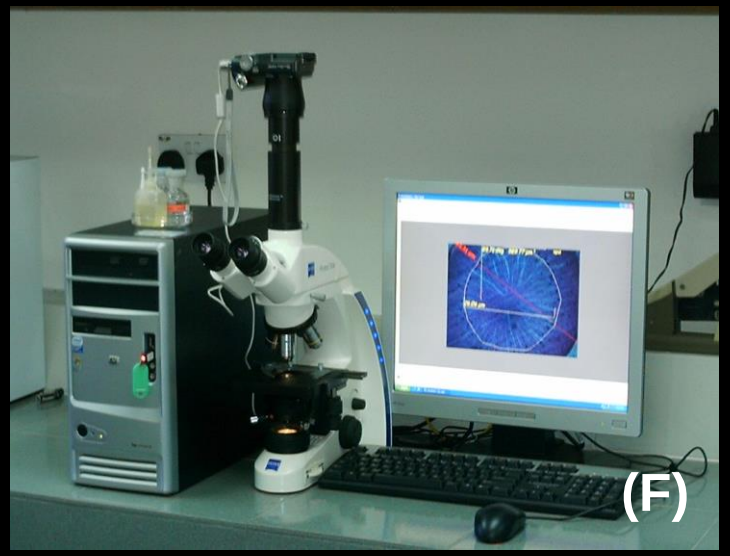

(A)


(B)


(C)

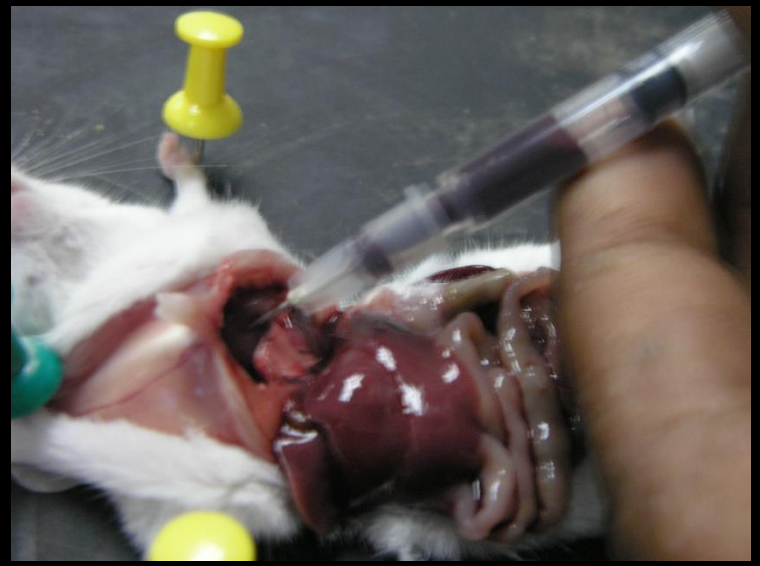

(D)

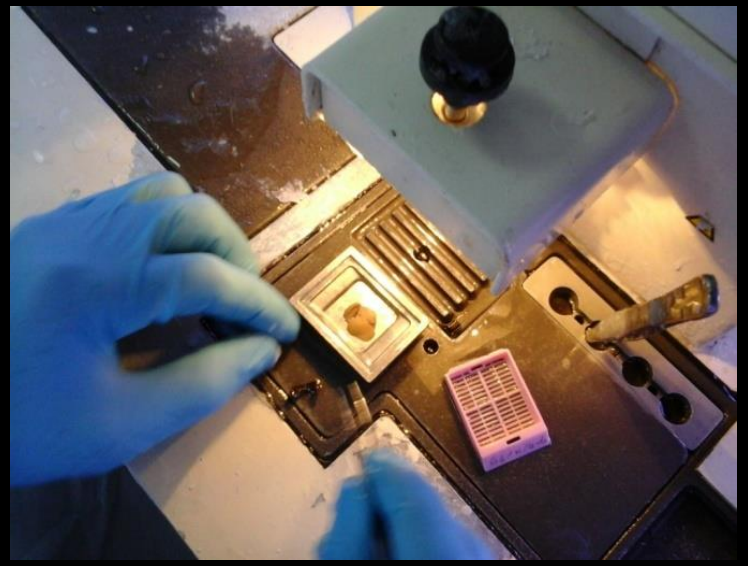

(E)


(F)

METHODOLOGY

BIOCHEMICAL TEST AND ORGAN HISTOLOGY

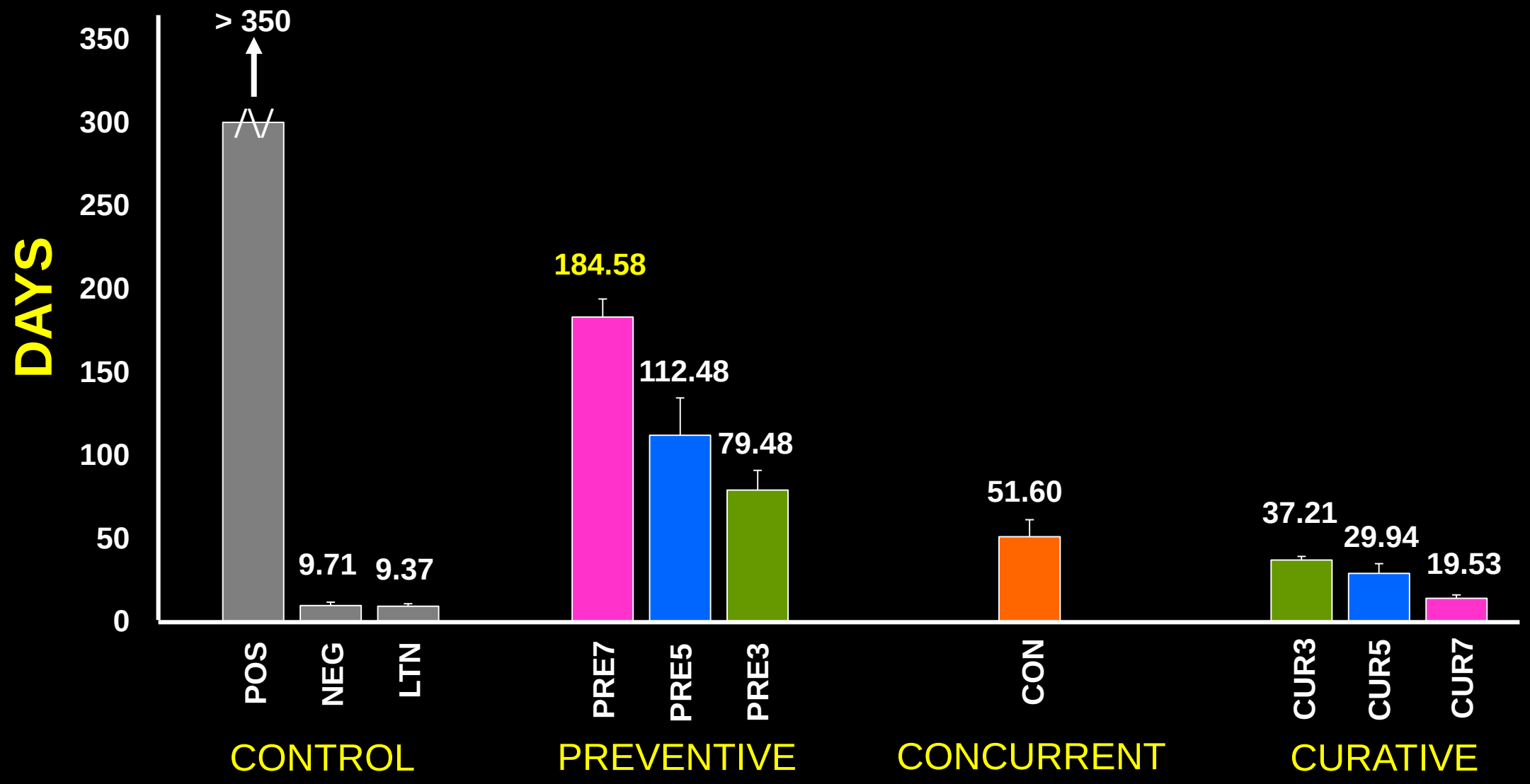




RESULTS & DISCUSSIONS

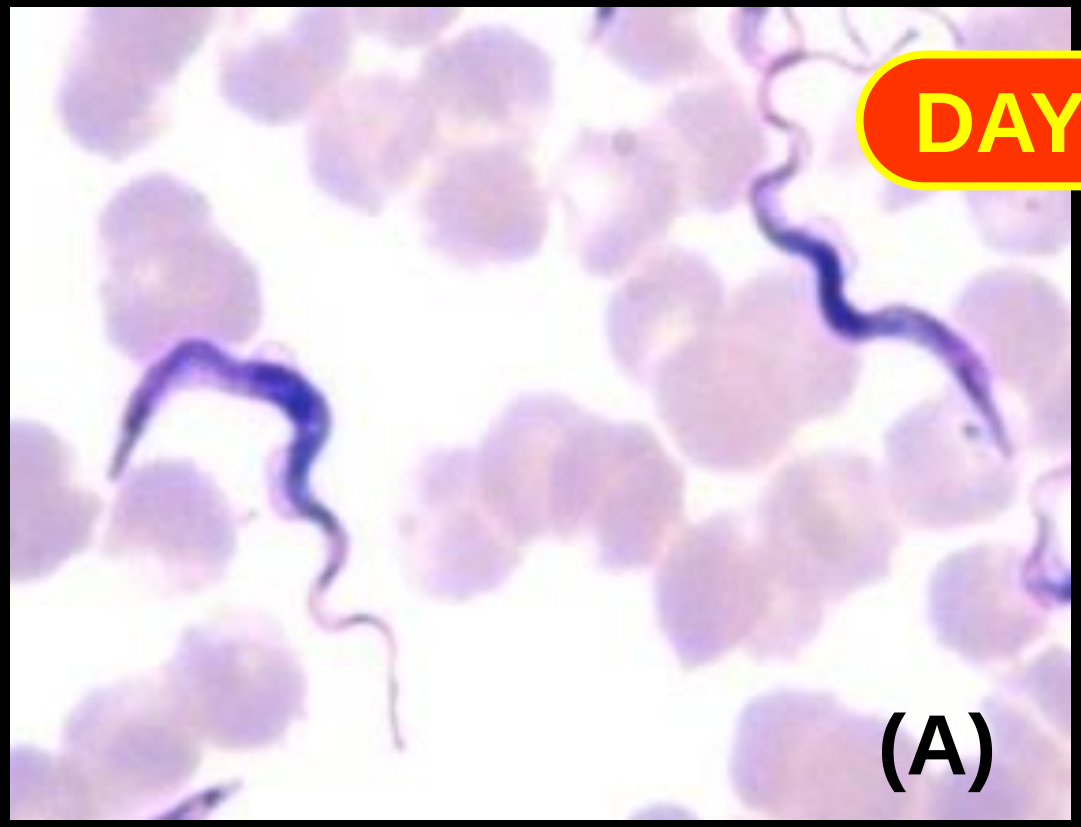
RESULT & DISCUSSION

MICE SURVIVAL TIME



RESULT & DISCUSSION

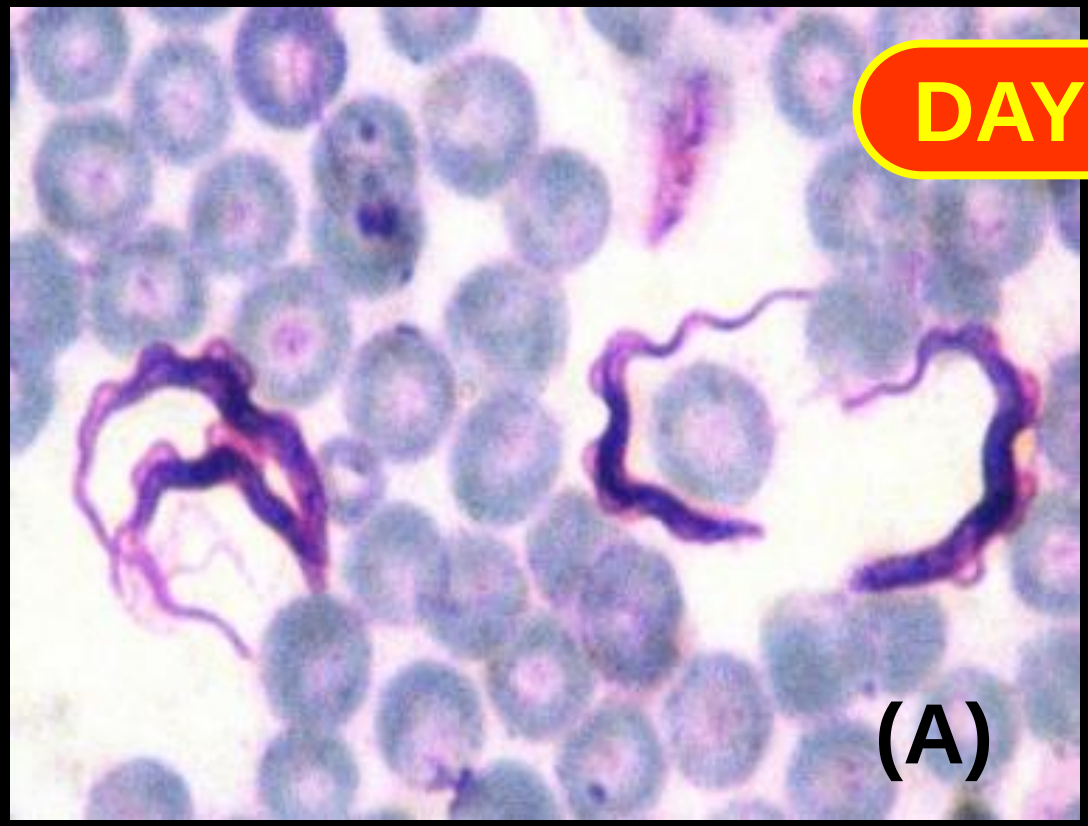
PARASITE SURVIVAL IN PRO1 GROUP: ON 100th DAY

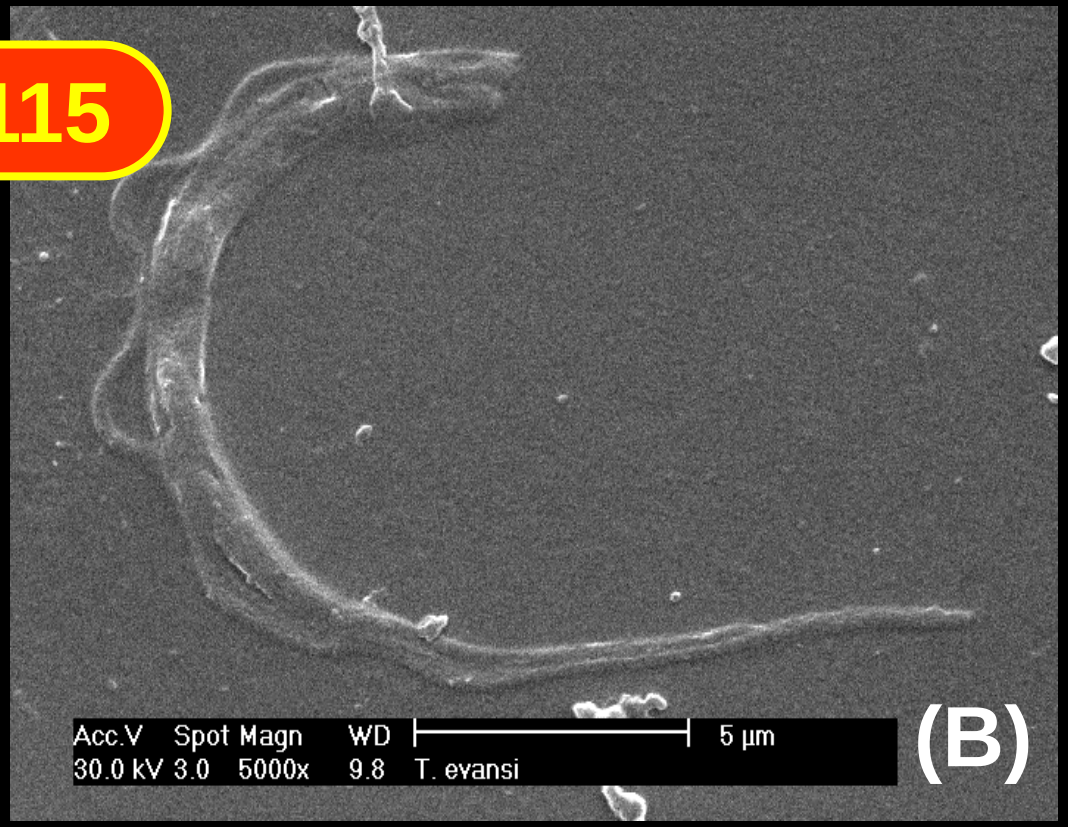


Giemsa thin blood smear of the mice from PRE7 group taken on day 100 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30) (B)

RESULT & DISCUSSION

PARASITE SURVIVAL IN PRO1 GROUP: ON 115th DAY

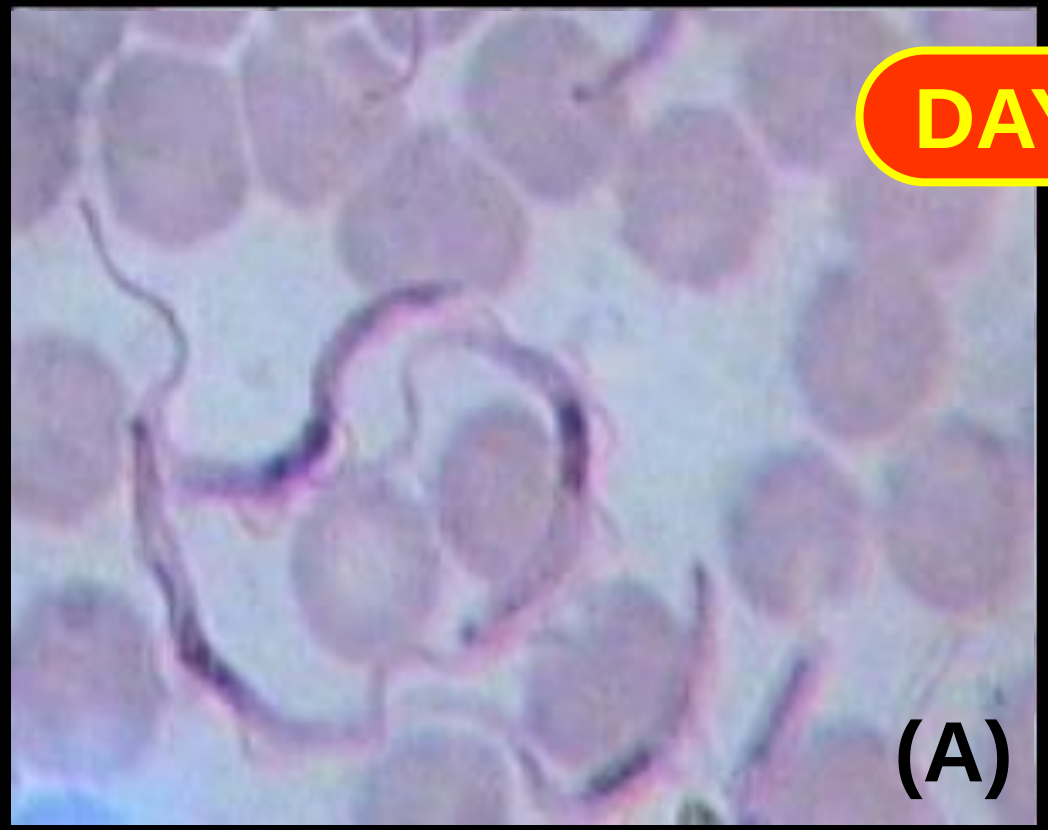


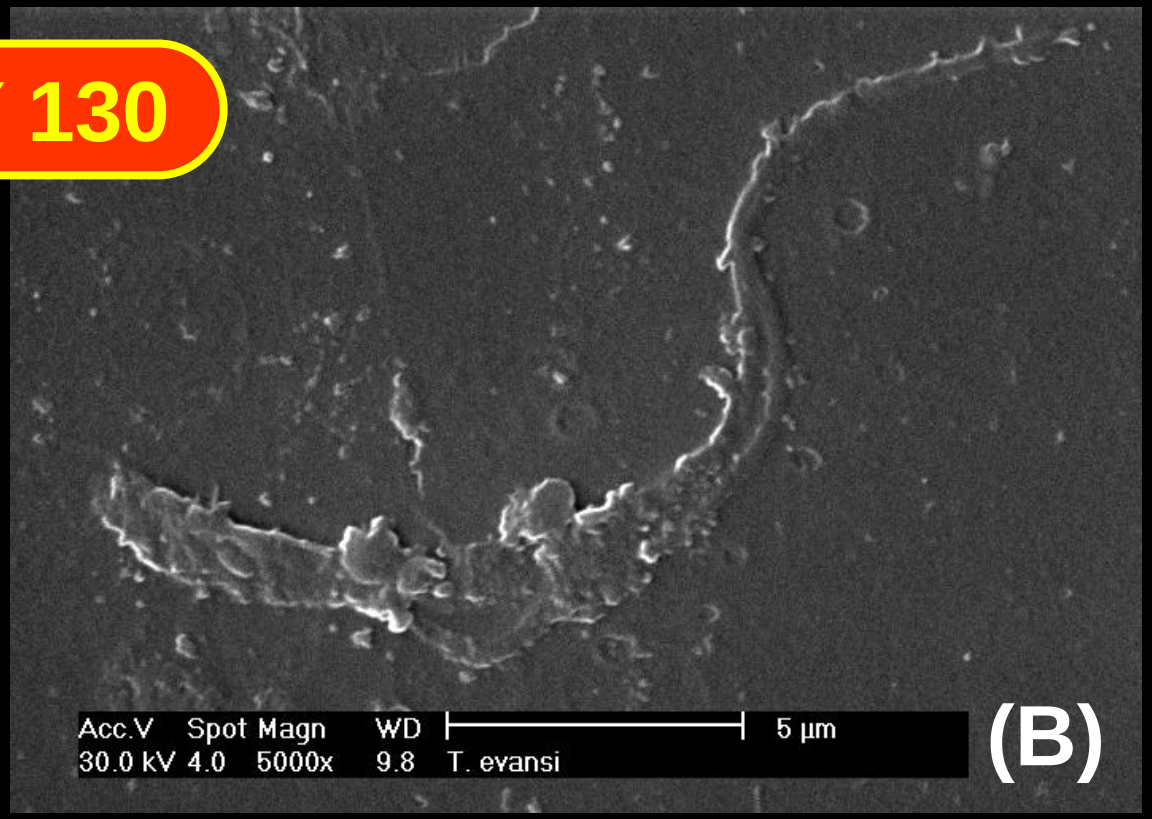


Giemsa thin blood smear of the mice from PRE7 group taken on day 115 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30) (B)

RESULT & DISCUSSION

PARASITE SURVIVAL IN PRO1 GROUP: ON 130th DAY

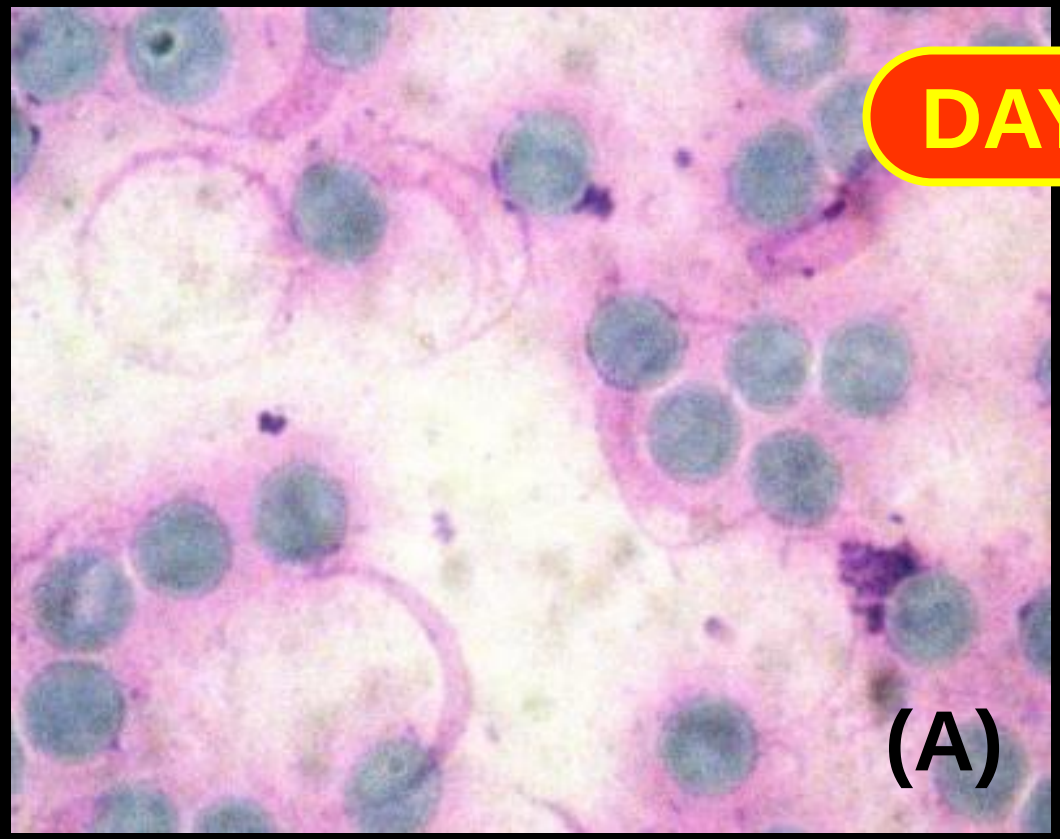


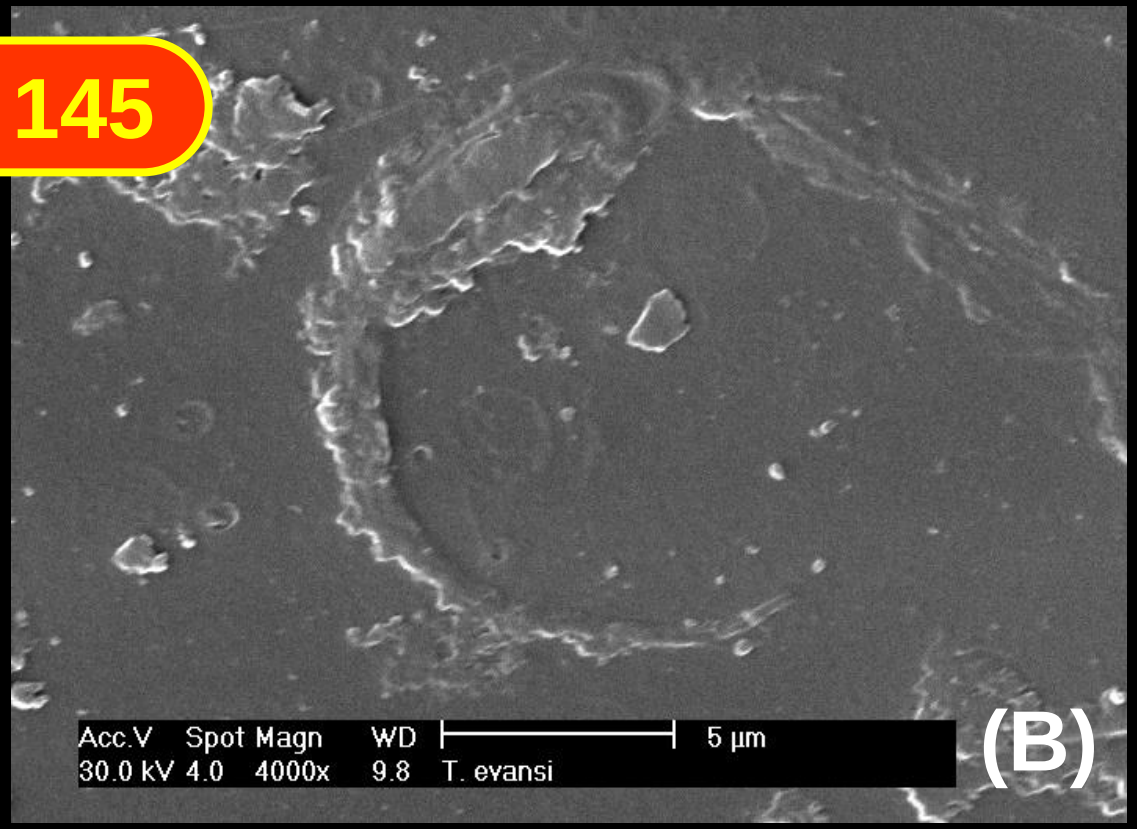


Giemsa thin blood smear of the mice from PRE7 group taken on day 130 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30) (B)

RESULT & DISCUSSION

PARASITE SURVIVAL IN PRO1 GROUP: ON 145th DAY

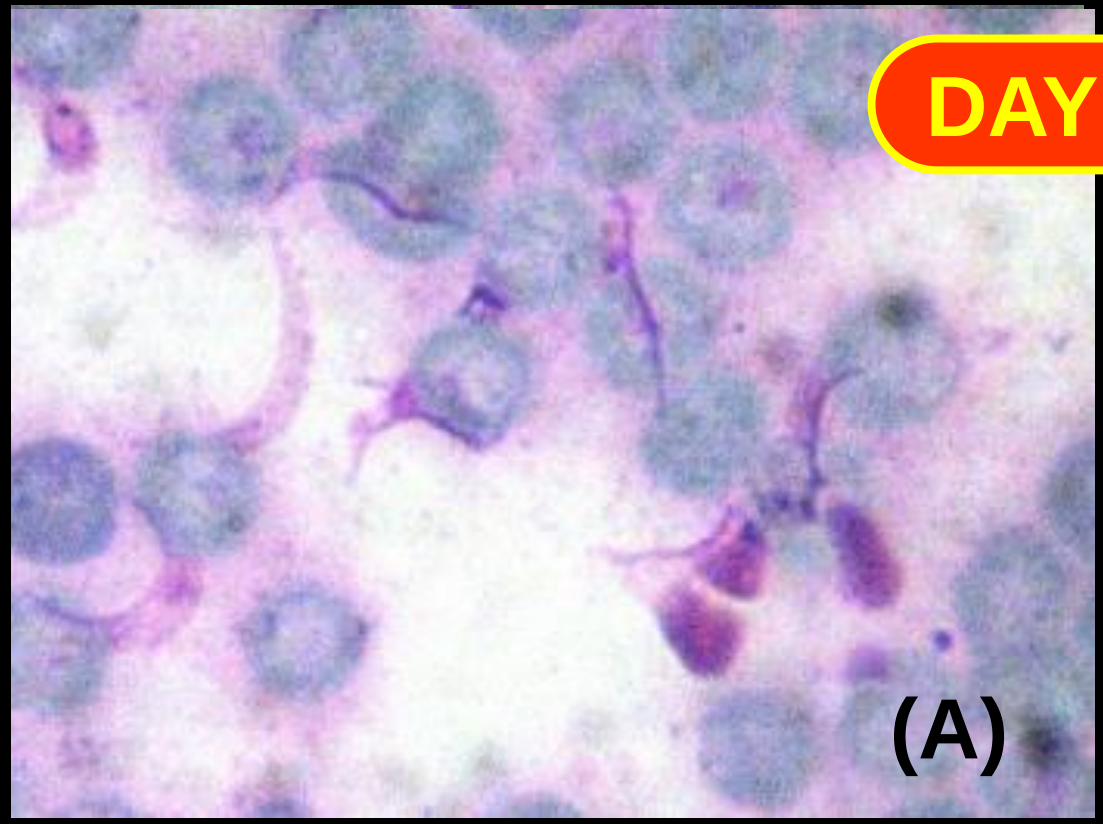


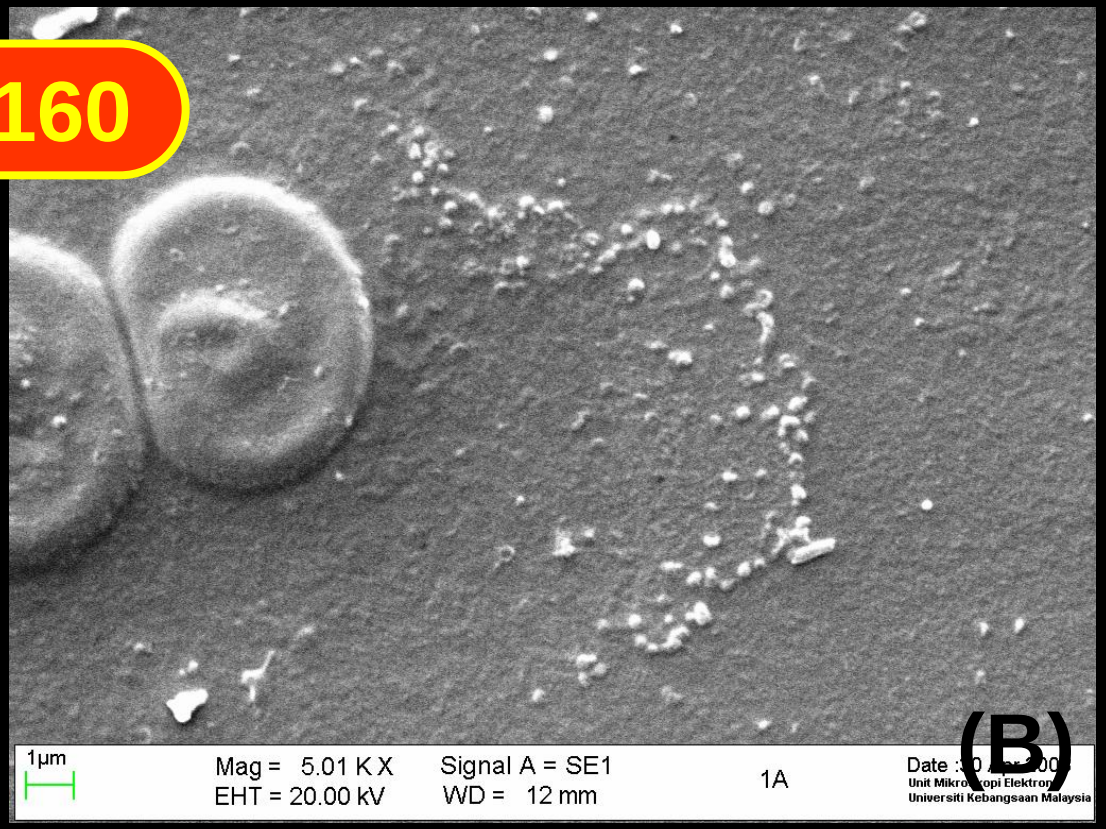


Giemsa thin blood smear of the mice from PRE7 group taken on day 145 as observed under x100 magnification of light microscope (A) and x4000 magnification of SEM (Phillips XL30) (B)

RESULT & DISCUSSION

PARASITE SURVIVAL IN PRO1 GROUP: ON 160th DAY

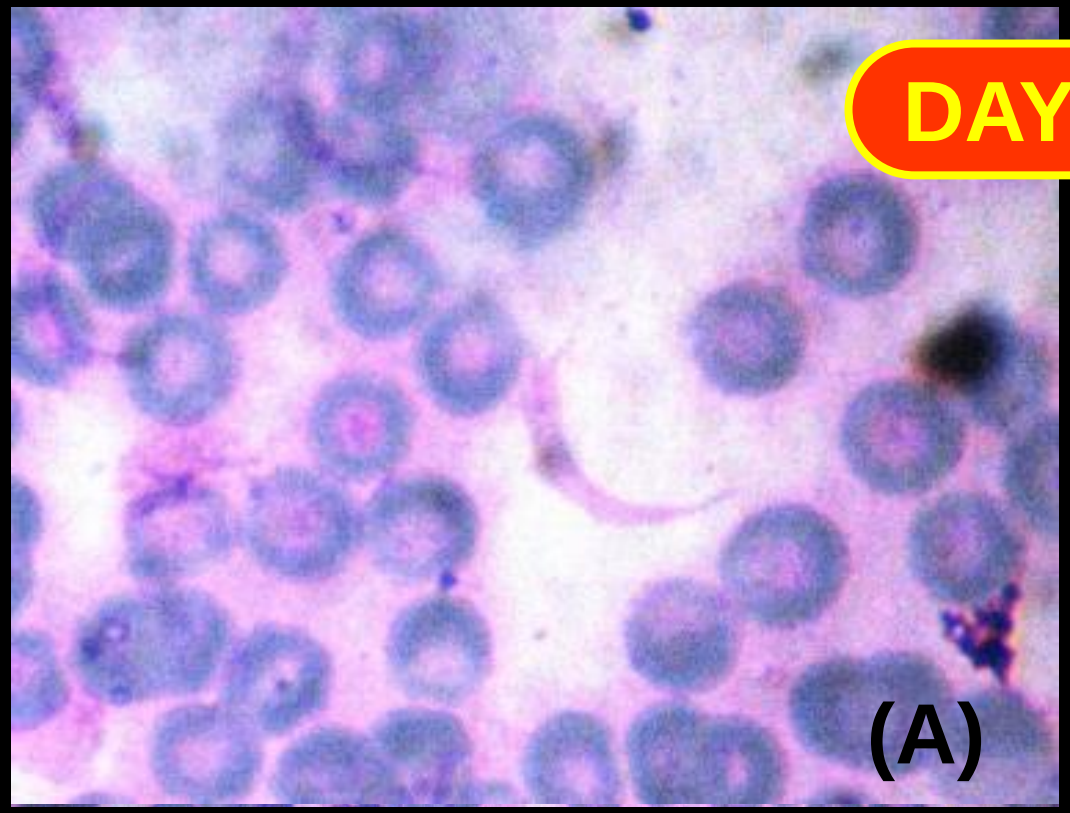


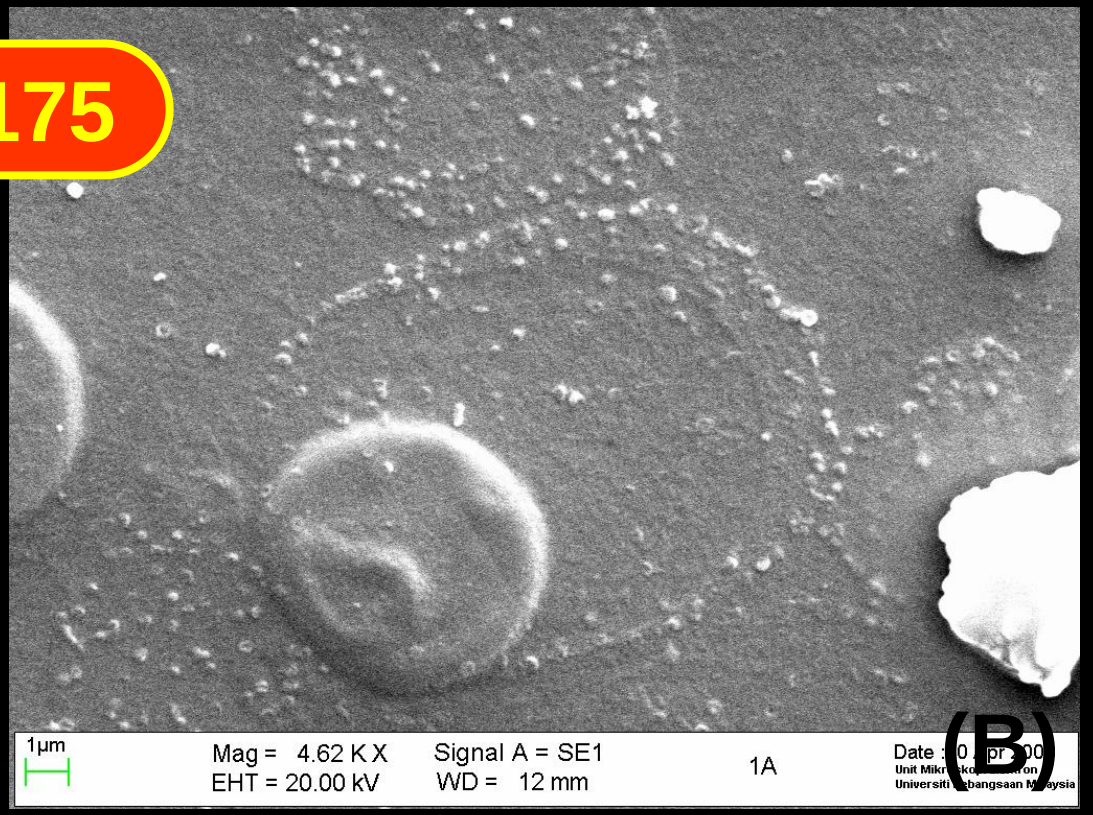


Giemsa thin blood smear of the mice from PRE7 group taken on day 160 as observed under x100 magnification of light microscope (A) and x5010 magnification of SEM (Leo 1450VP) (B)

RESULT & DISCUSSION

PARASITE SURVIVAL IN PRO1 GROUP: ON 175th DAY

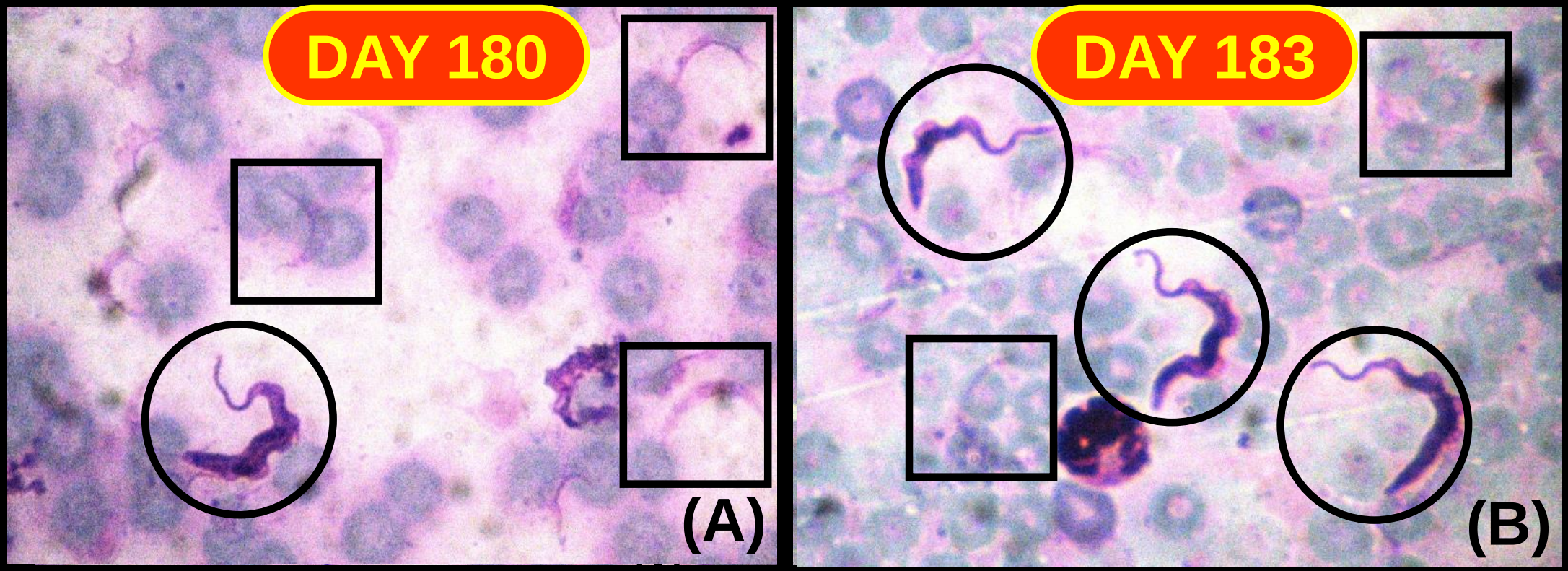




Giemsa thin blood smear of the mice from PRE7 group taken on day 175 as observed under x100 magnification of light microscope (A) and x4620 magnification of SEM (Leo 1450VP) (B)

RESULT & DISCUSSION

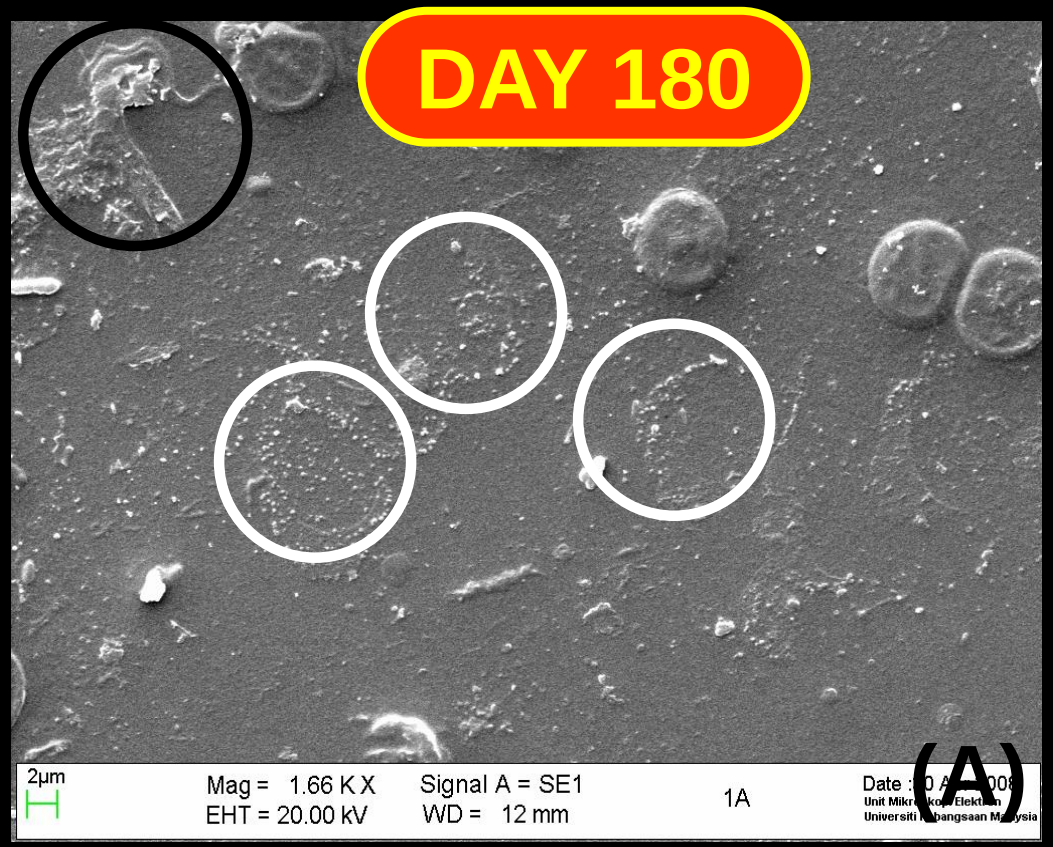
GIEMSA BLOOD FILMS

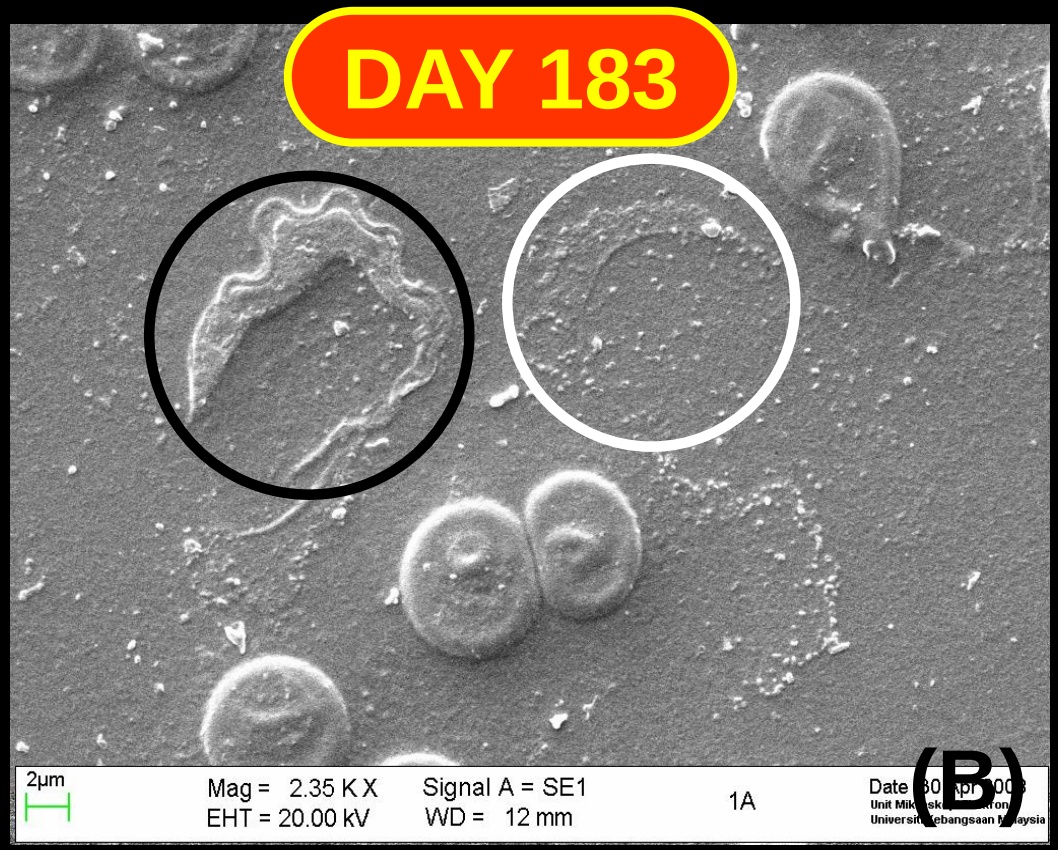


Reemerged of *T. evansi* which survived in PRE7 group mice on day 180 (A) and day 183 (B) due to the action of 'variable surface glycoprotein (VSG) stochastic genetic modification' as observed under x100 magnification of light microscope.

RESULT & DISCUSSION

ELECTRON MICROSCOPIC OBSERVATION

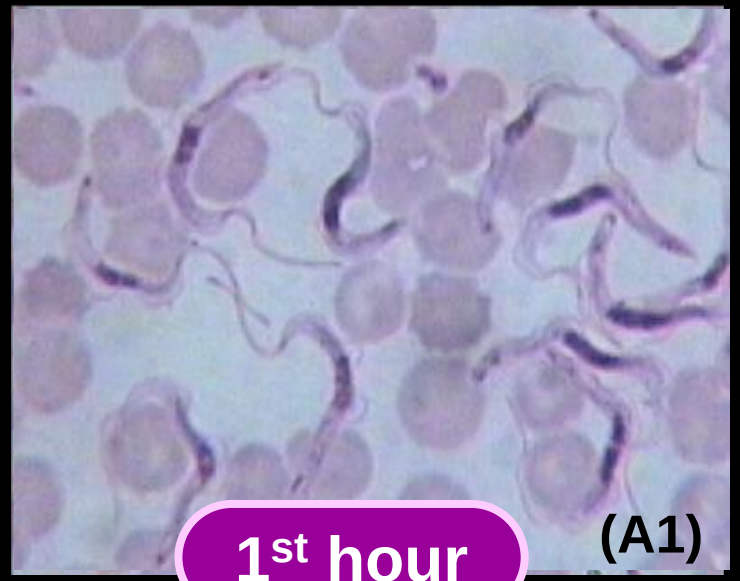




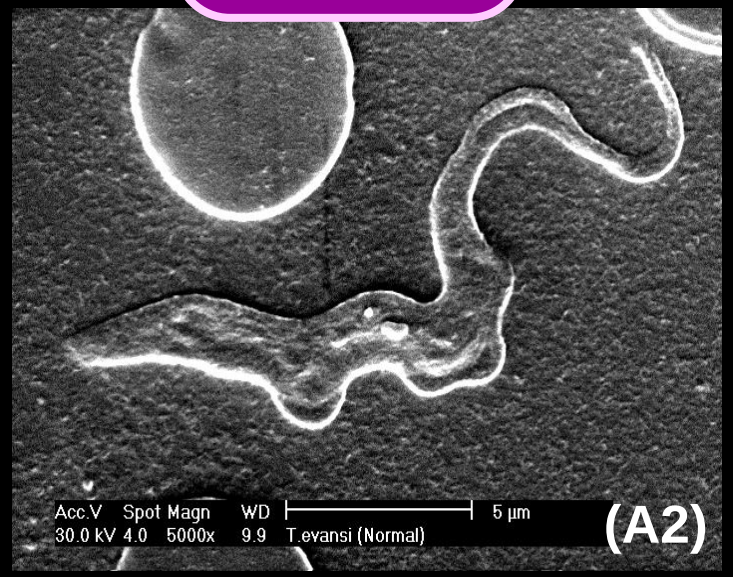
Reemerged of *T. evansi* which survived in PRE7 group mice on day 180 (A) and day 183 (B) due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as respectively observed under x1600 (A) and x2300 (B) magnification by SEM (Leo 1450VP).

RESULT & DISCUSSION

PARASITE GROWTH IN POSITIVE CONTROL GROUP



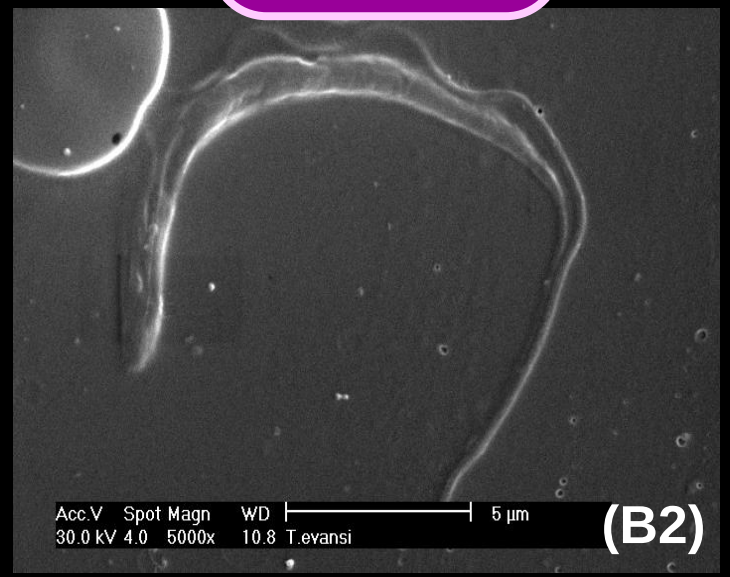
1st hour (A1)



(A2)

The growth of *T. evansi* in POS group mice taken on 1 hour (left) and 2 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and x5000 magnification of SEM electron microscope (Phillips XL30) (A2 & B2)

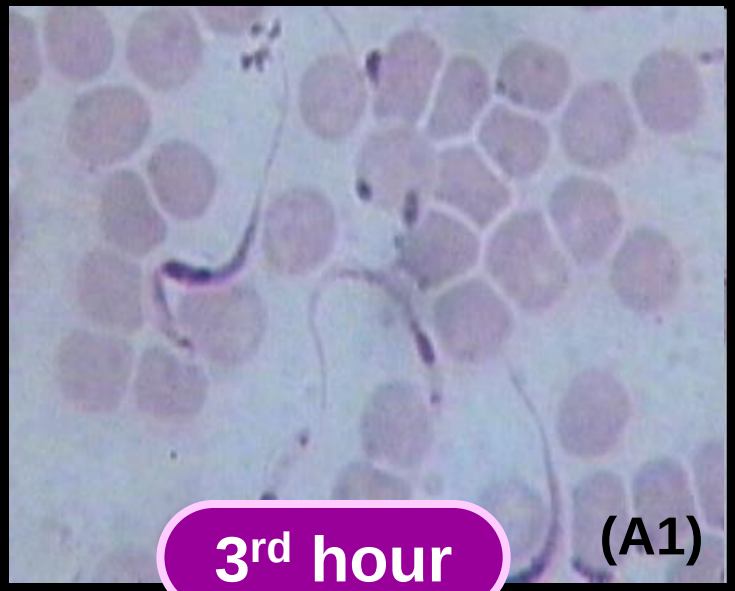
2nd hour (B1)



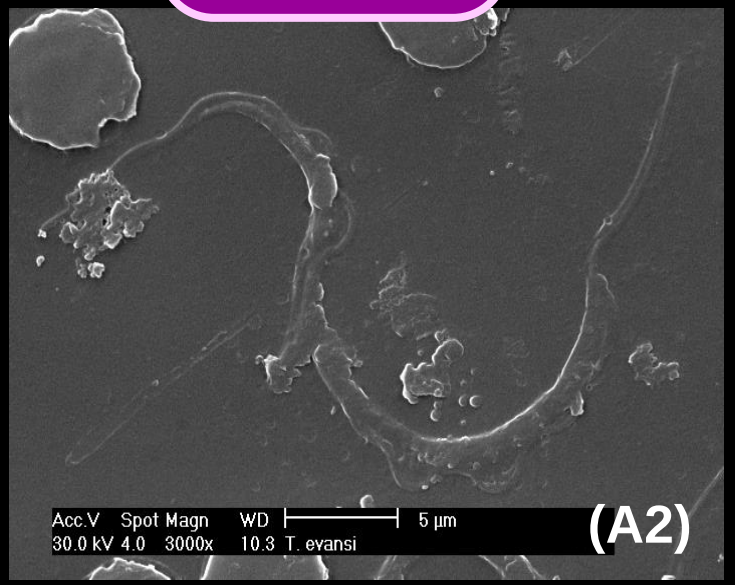
(B2)

RESULT & DISCUSSION

PARASITE GROWTH IN POSITIVE CONTROL GROUP

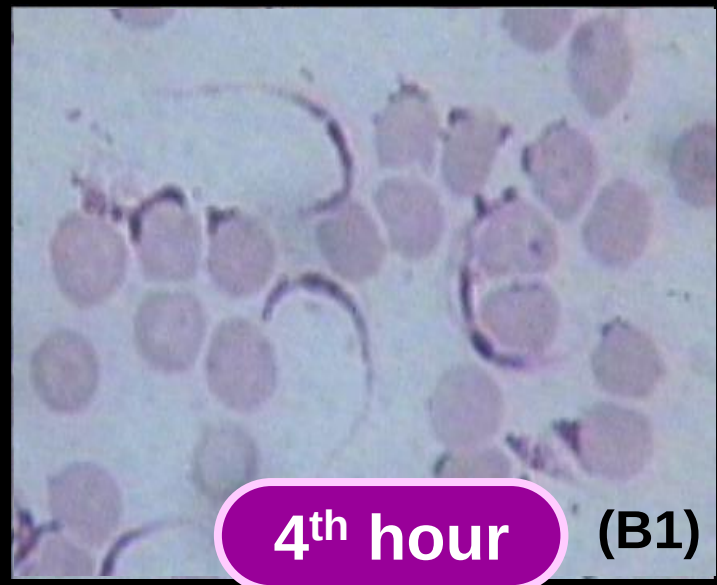


3rd hour (A1)



(A2)

The growth of *T. evansi* in POS group mice taken on 3 hours (left) and 4 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and respectively under x3000 (A2) and x2000 (B2) magnification of SEM electron microscope (Phillips XL30)



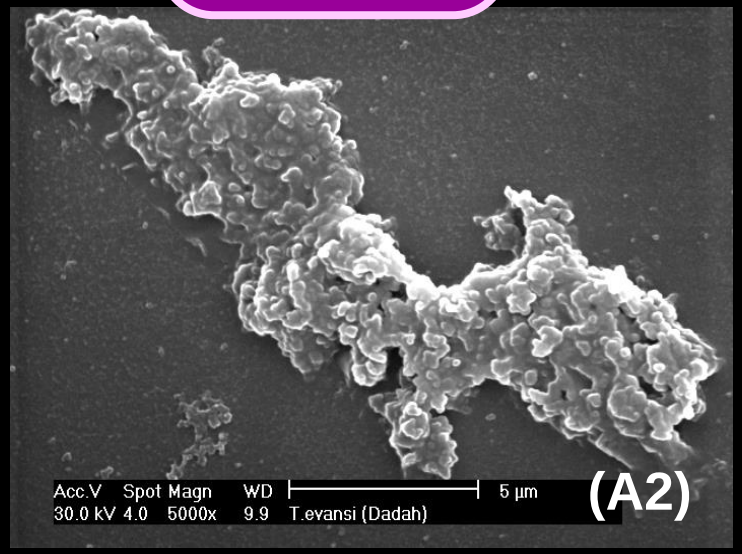
4th hour (B1)

(B2)

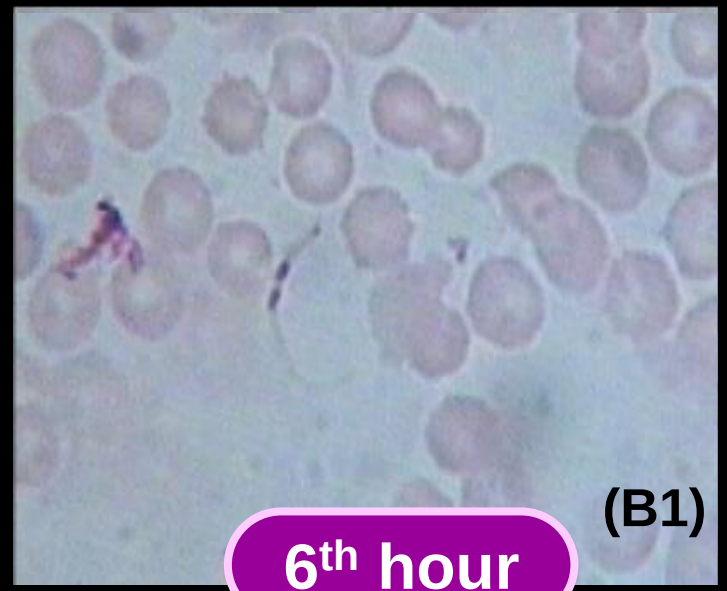
RESULT & DISCUSSION

PARASITE GROWTH IN POSITIVE CONTROL GROUP

5th hour



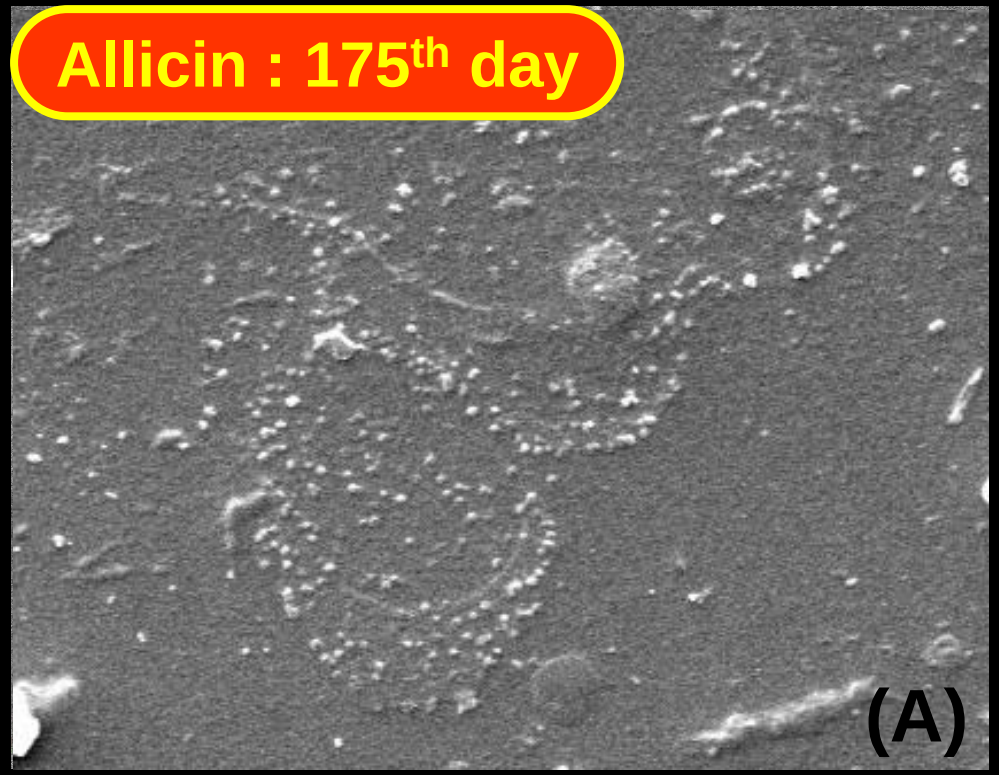
The growth of *T. evansi* in POS group mice taken on 5 hours (left) and 6 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and x5000 magnification of SEM electron microscope (Phillips XL30) (A2 & B2)

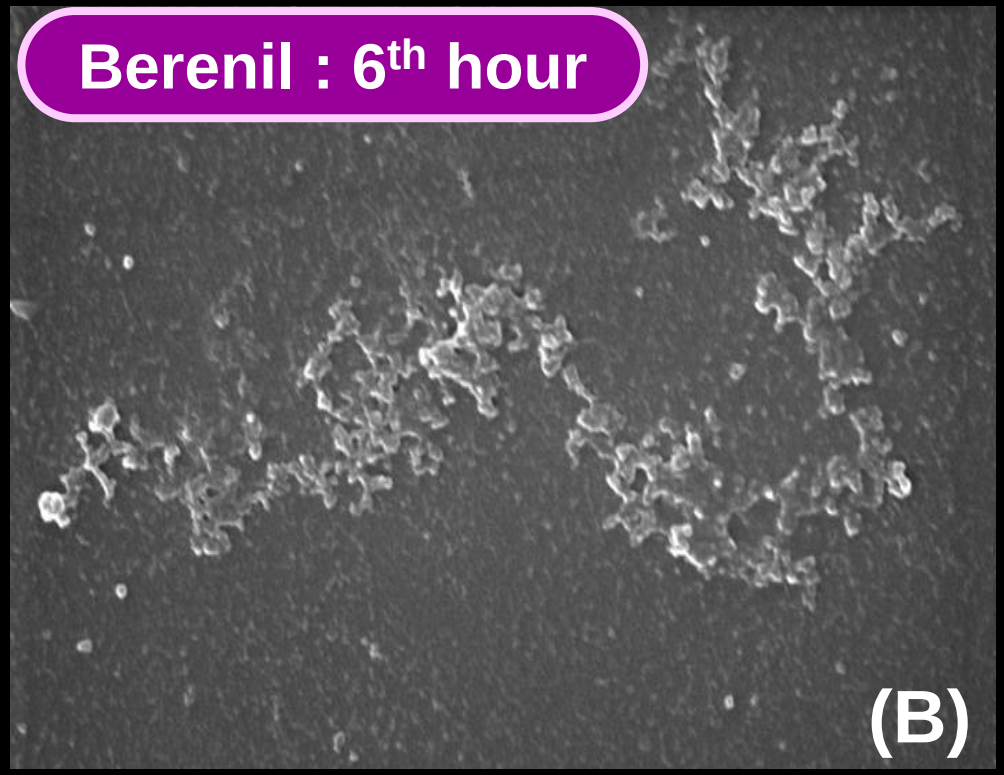


6th hour

RESULT & DISCUSSION

ALLICIN vs BERENIL

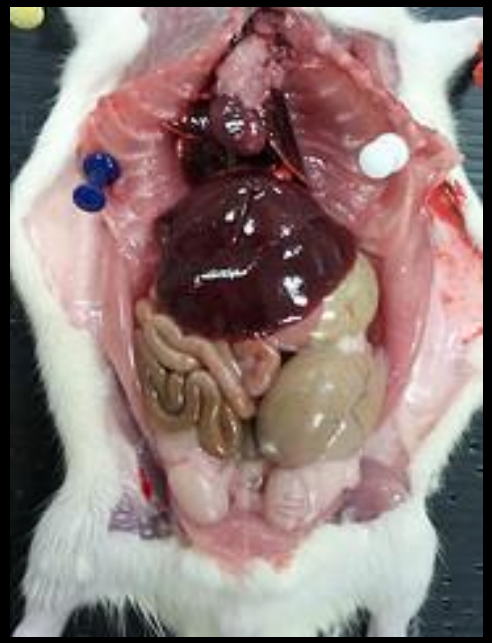




Scanning electron micrograph (SEM) showed the morphological changes of *T. evansi* in PRE7 mice on 175th day post infection (A) (Leo 1450VP) and in POS mice at 6th hours post treatment (0.01 mL 3.5 mg/kg bw Berenil) (B) (Phillips XL30)

RESULT & DISCUSSION

BIOCHEMICAL TESTS FOR TOXICITY ASSESSMENT



Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	**40 – 93**	IU/L
AST	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	**92 – 206**	IU/L
ALP	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	61.46 ± 2.46	58.32 ± 2.97	**54 – 115**	IU/L
STP	6.12 ± 2.32	7.21 ± 3.81	7.93 ± 2.01	8.83 ± 3.90	6.40 ± 1.01	6.80 ± 3.06	**5.8 – 9.5**	g/dL

TA : Sub-acute regime – Daily treatment (28 days)
TB : Sub-acute regime – Daily treatment (28 days) 2 hours post-infection
TC : Sub-chronic regime – Daily treatment (90 days)
TD : Sub-chronic regime – Daily treatment (90 days) 2 hours post-infection
CN : Control regime – Normal mice without infection and treatment
CI : Control regime – Infected mice on D0
ALT : Alanine aminotransferase
AST : Aspartate transaminase
ALP : Alkaline phosphatase
STP : Serum total protein

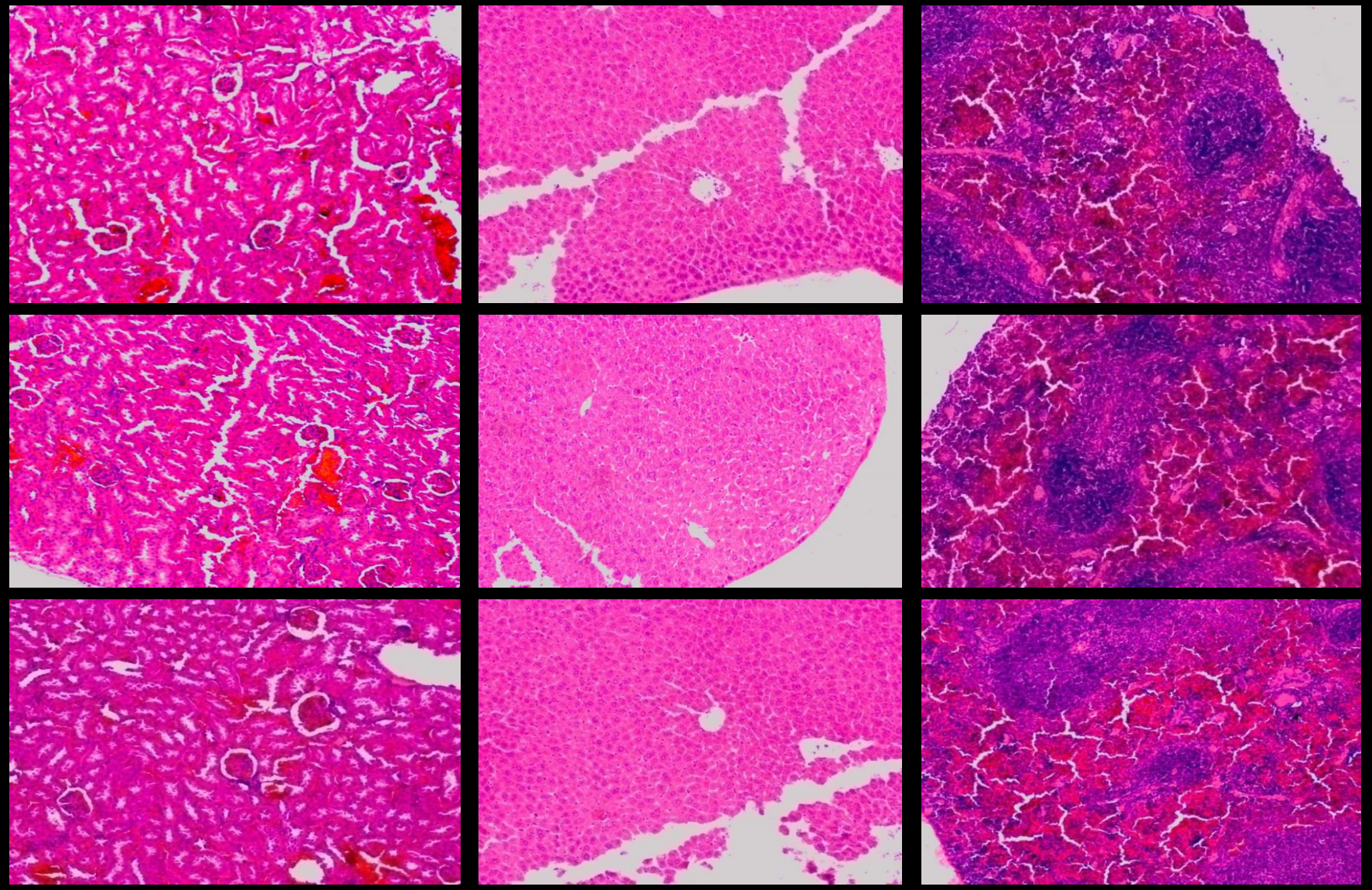
RESULT & DISCUSSION

ORGAN HISTOLOGY FOR TOXICITY ASSESSMENT

Treatment (Acute)

Treatment (Sub-acute)

Control



KIDNEY LIVER SPLEEN

DISCUSSIONS

DISCUSSIONS

DISCUSSION

- Allicin exhibited very significant and promising anti-parasitic activity against the infection of surra disease.
- Allicin could prolong the survival of infected host and leave no undesired toxicity effects towards the blood enzymes level and vital organs.
- New wave of infection → host is susceptible to infection and suffer with chronic infection (Cavalitto et al. 1944)
- Stochastic genetic modification of VSG is still the best weapon for trypanosome survival (Nok et al. 1996).
- The action of tiosulfinates group in allicin molecule towards – thiol group of parasite enzymes which was crucial for parasite proliferation (Ankry & Mirelman, 1999).

CONCLUSION

ABSOLUTE HYPOTHESIS

EAT GARLIC..!

NO HARM TO EAT AS MUCH AS YOU CAN

CONCLUSION

ABSOLUTE HYPOTHESIS

REFERRENCES

REFFERENCES

- Agarwal, M., Walia, S., Dhingra, S., Khambay B.P. 2001. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated from *Zingiber officinale* Roscoe (ginger) rhizomes. *Pest Manage. Sci*. 57: 289-300.

- Ankri, S. & Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. *Microbes Infect*. 1, 125-129.

- Ankri, S., Miron, T., Rabinikov, A., Wilchek, M. & Mirelman, D. 1997. Allicin from garlic strongly inhibits cysteine proteases and cytopathic effects of *Entamoeba histolytica*. *Antimicrob Agents Chemother.* 41, pp. 2286–2288.

- Chen, H.C., Chang, M.D. & Chang, T.J. 1985. Antibacterial properties of some spice plants before and after heat treatment. 18: 190-195.

- Cox, S.D., Mann, J.L., Bell, J.E. & Warmington, S.G. 2000. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). *Journal of Applied Microbiology* 88, 170–175.

- Denise, C.A., Fabio, D.A., Alejendro, M.K. & Silvia, R.U. 2004. Antileishmanial activity of the terene Nerolidol. Departmento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Brazil.

REFFERENCES

- Denyer, C.V., Jackson, P., Loakes, D.M., Ellis M.R. & Young, D.A. 1994. Isolation of antirhinoviral sequiterpenes from ginger (Zingiber officinale. *J. Nat. Prod*. 57: 658-662.

- Goto, C., Kasuya, S.,Koga, K., Ohtomo, H. & Kagei, N. 1990. Lethal efficacy of extract from Zingiber officinale (tradisional Chinease medicine) or shogaol and gingerol in Anisakis larvae *in vitro*. *Parasitol. Res*. 76: 653-656.

- Nok, A.J., William, S. & Onyenekwe, P.C. 1996. *Allium sativum* : induced death of African trypanosomes. *Parasitol. Res*. 82: 634-637.

- Ogunlana, E.O., Hoeglund, S., Onawunmi, G. & Skoeld, O. 1987. Effects of lemongrass oil on the morphological characteristics and peptidoglycan synthesis of *Escherichia coli* cells. *Microbios*, 50, 43–59.

- Rabinkov, A., Wilchek, M. & Mirelman, D. 1998. The mode of action of allicin: Trapping of radicals and interaction with tiol containing proteins. *Biochem. Biophys. Acta* 1379, 233-244.

- Zainal-Abidin, B.A.H. 1992. Infections of *Trypanosoma evansi* in Malaysia. *Malays. Applied Biology* 10: 1-8.

THANK YOU

INTRODUCTION

RATIONALE OF THE STUDY

Reliability of Berenil

- *T. evansi* strain from India & Indonesia are resistant
- Unaffordable → expensive in certain regions
- Wrong dosage & concentration → side effects

Economic Growth & Biotechnology Sector

- Biotechnology → Malaysian main focus in the next decade
- Garlic → consumable & easily manipulated herbal plant
- Surra disease → influenced productivity of the livestock

Current Issues of *T. evansi*

- Trans-host boundary : animal → human (Assam India 2008)
- Potential trans-continents zoonotic disease

‘Variable Surface Glycoprotein’ (VSG)

- Survival factor of *Trypanosoma* spp. in the infected host
- High density layer on the parasite cell membrane
- Contained 1 x 10^9 similar & uniformed glycoprotein molecules expressed by VSG-Trypanosome gene
- Protect the parasite from being identified/action of the host immune system
- Similar & uniformed glycoprotein molecule → only end region of `N-terminal loops’ structure (300-500 amino acid structures) can be identified by the host immune systems → specific antibody-antigen mechanisms

‘Variable Surface Glikoprotein’ (VSG) – cont.

- When the end region of `N-terminal loops’ structure being identified by the host immune systems → VSG-stochastic genetic modification’ of the parasite plays the role.
- VSG stochastic genetic modification = periodic changes of antigenic variation → the structures & characteristics of parasite cell membrane was modified whenever confronted with the host’s specific immune system which may varies.
- Periodic changes of antigenic variation → changes in parasitemia waves → longer survival time of the parasite → chronic infection on host

Survival Pattern of the Trypanosomiasis Infected-Host Due to VSG-Stochastic Genetic Modification Phenomenon