

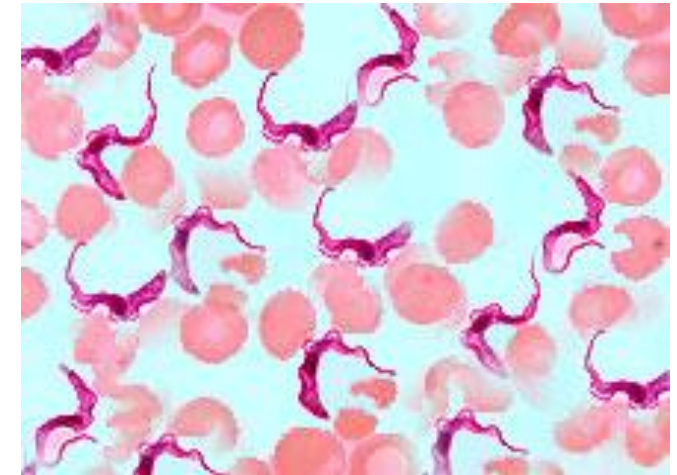


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INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يونسيفيتي إسلامية ابتكاراً بجسداً مليئاً
Garden of Knowledge and Virtue

ONE HEALTH CONCEPT ON THE IN-VIVO ANTIPARASITIC ACTIVITY AND TOXICITY EVALUATION OF *Elettaria cardamomum* AGAINST THE GROWTH AND SURVIVAL OF ZONOTIC HAEMOFLAGELLATE, *Trypanosoma evansi*



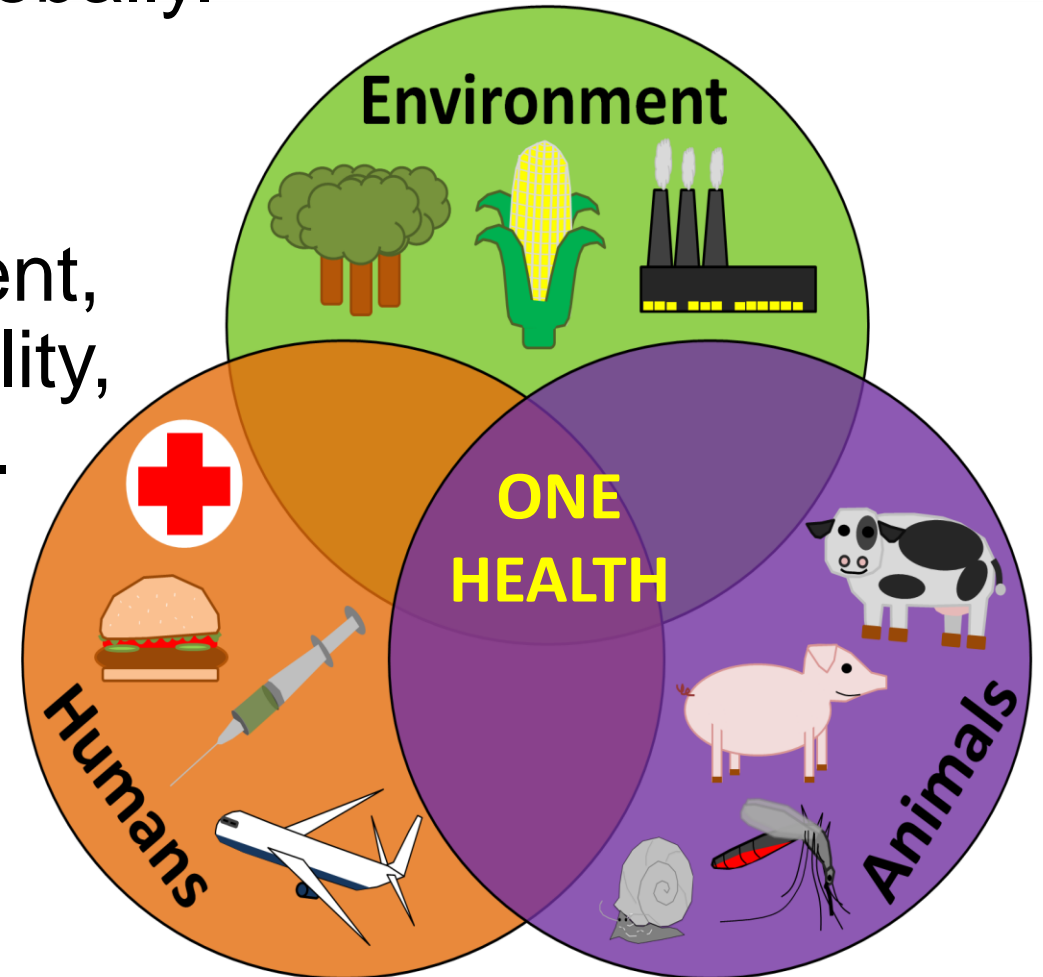
MOHD SHUKRI BIN BABA
International Islamic University Malaysia

INTRODUCTION



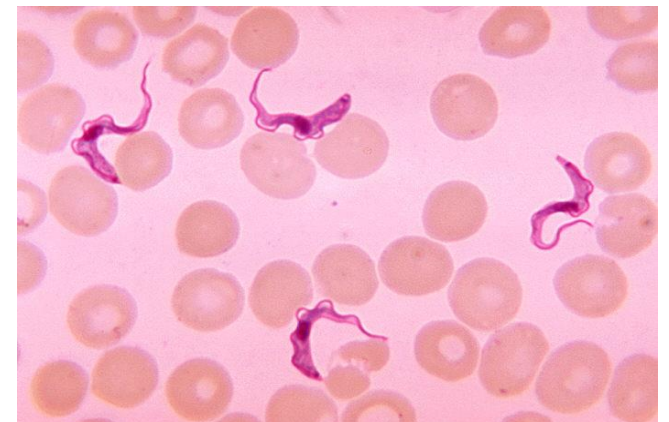
One Health (OH) Concept

- Recognizes interrelationship between animal, human and environmental health, locally and globally.
- Holistically embraces a very broad scope: biodiversity, rural development, food security, ecosystem sustainability, policy issues, wildlife diseases, etc..
- Initiate worldwide strategies for interdisciplinary collaboration & communication in all aspect of healthcare and services



Trypanosoma evansi

- First discovered by Sir Griffith Evans in Punjab India (1880)
- Haemoflagellated protozoa in both human and animals → zoonotic vector-borne disease
- Caused atypical human trypanosomiasis (AHT) in human and Surra disease in livestock
- Wide variety of distributed vectors → easily spread worldwide
- Drug resistant in some regions → suramin, pentamidine, berenil



Vectors of *Trypanosoma evansi*



Horsefly / *Tabanus striatus*



Tsetse fly / *Glossina morsitans*



Horn fly / *Haematobia* spp.



Muscidae fly / *Stomoxys* spp.

Another Possible Vectors of *Trypanosoma evansi*



Triatominae bug / *Triatoma* spp.



Argasidae tick / *Ornithodoros* spp.



Buffalo leech / *Hiruninaria manillensis*



Vampire bat / *Desmodus rotundus*

Documented AHT (Philippe *et al.* 2013)

No	Location	Species / Subspecies	Date	Identification Method	Fever	Treatment	Outcomes
1	Ghana	<i>T. vivax</i>	1917	Morphology	ND	ND	ND
2	Pasteur Inst.	<i>T. b. brucei</i>	1930	Morphology	ND	ND	ND
3	Congo	<i>T. b. brucei</i>	1947	Morphology	Present	None	Self-cured
4	Ethiopia	<i>T. b. brucei</i>	1987	Morphology	ND	ND	Cured
5	Ghana	<i>T. b. brucei</i>	2003	PCR	Present	None	Self-cured
6	Ivory Coast	<i>T. congolense</i>	1998	PCR	Present	Pentamidine	Cured
7	India	<i>T. evansi</i>	1977	Morphology	Present	Atoxyl	Cured
8	Sri Lanka	<i>T. evansi</i>	1999	Morphology	Present	None	Self-cured
9	India	<i>T. evansi</i>	2004	PCR	Present	Suramin	Cured
10	India	<i>T. evansi</i>	2005	Morphology	Present	None	Death
11	Egypt	<i>T. evansi</i>	2010	Morphology	Present	ND	Cured
12	Malaysia	<i>T. lewisi</i>	1933	Morphology	Present	None	Self-cured
13	India	<i>T. lewisi</i>	1974	Morphology	Present	None	Self-cured
14	India	<i>T. lewisi</i>	1974	Morphology	Present	None	Self-cured
15	Gambia	<i>T. lewisi</i> -like	2003	PCR	Present	Melarsoprol	Cured
16	Thailand	<i>T. lewisi</i> -like	2003	PCR	Present	Antibiotic	Cured
17	India	<i>T. lewisi</i>	2006	Morphology	Present	None	Self-cured
18	India	<i>T. lewisi</i>	2007	PCR	Present	Suramin	Death
19	India	<i>T. lewisi</i>	2010	PCR	Present	Pentamidin	Cured

Elettaria cardamomum

- “*Queen of Spice*” → South Asia, SEA, Middle East, Africa and Europe (Ravindran 2010)
- In 100gm → 300kcal, 68g CHO, 15g protein, 28g fibers & no cholesterol
- Vitamin A & C, Na, K, Ca, Fe, Mn, P, Cu, Mg & Zn (Cox et al. 2000)
- Cardamom powder → therapeutic effects & significant biological activities (Gao et al. 2008)



E. cardamomum: The Testimonies

In-vitro inhibits 87% - 95% of *Leishmania amazonensis* & *L. braziliensis* promastigotes growth (Denise et al. 2010)



Cardamom oil exhibits anti-gonorrhoea and anti-nephritis property in male rabbit's urethra (Turi et al. 2011)

Inhibits the synthesis of peptidoglycan molecules in bilayer lipid structure of organism plasma membrane (Ogunlana et al. 2013)



Curing asthma and bronchitis symptoms by increasing blood circulation to the lungs (Berhe et al. 2009)

Cardamom powder → good therapeutic preventive effect and significant biological activities (Gao et al. 2008)

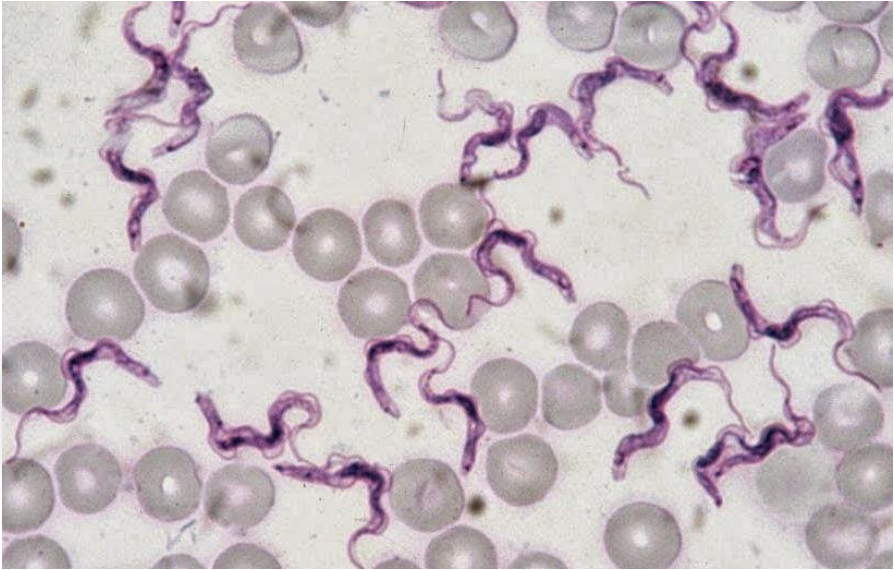


Anti-colorectal tumour with 68% inhibition rate on human colon adenocarcinoma cells HCA-2 and HCA-7 (Gayathri et al. 2010)

MATERIALS & METHODS

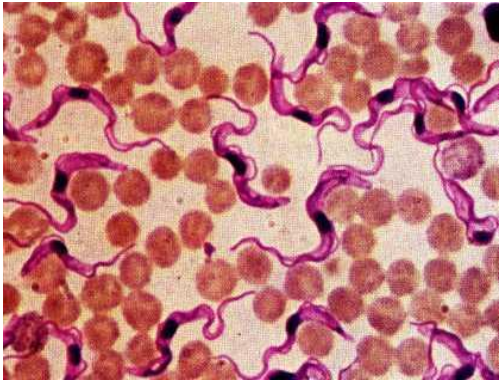


Experimental Materials



Flow Chart

T. evansi stock



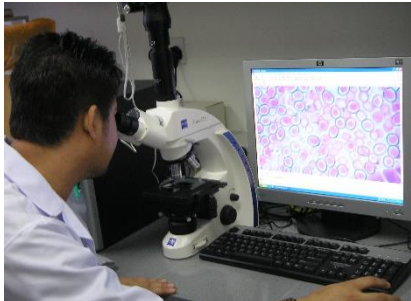
T. evansi administered *i.p.*
(5×10^3 *T. evansi* / mice)



Oral administered of 0.1 mL/mouse of
10 μ g/mL of *E. cardamomum*-dH₂O extract



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



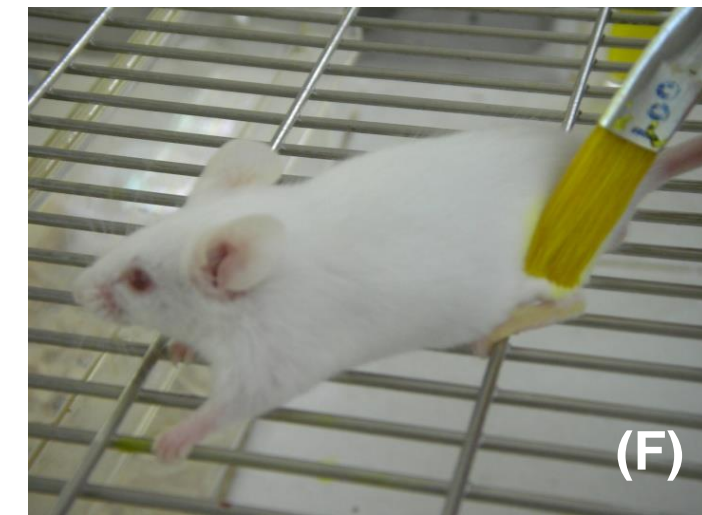
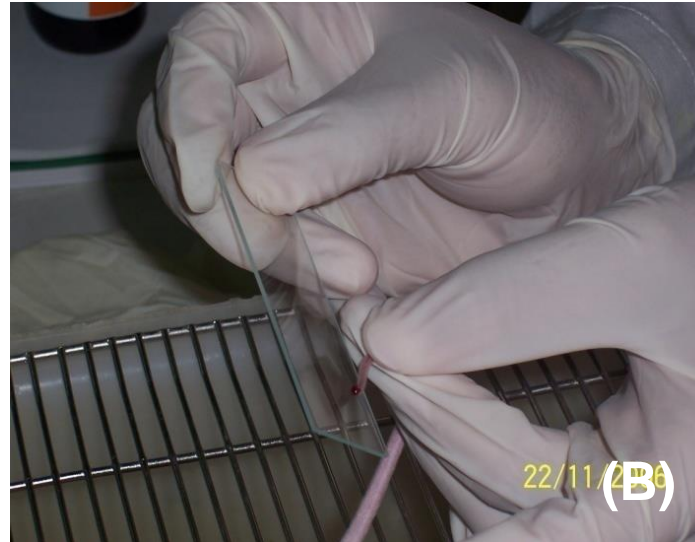
Vital organ
histology for
toxicity
assessment



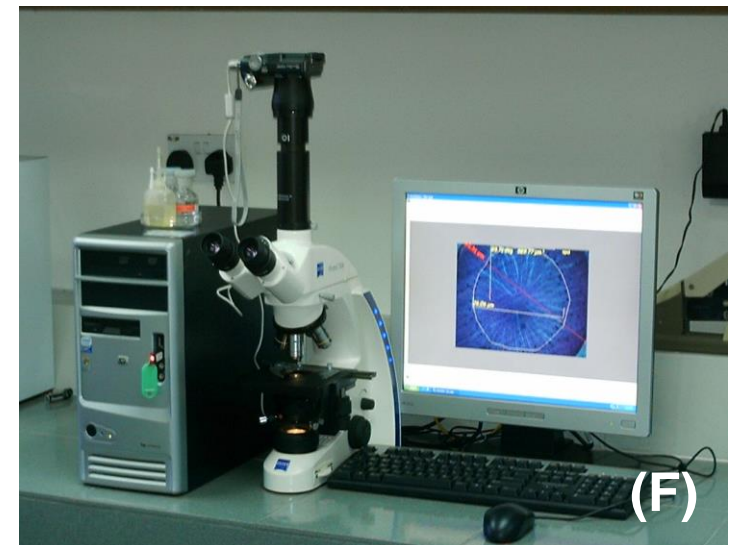
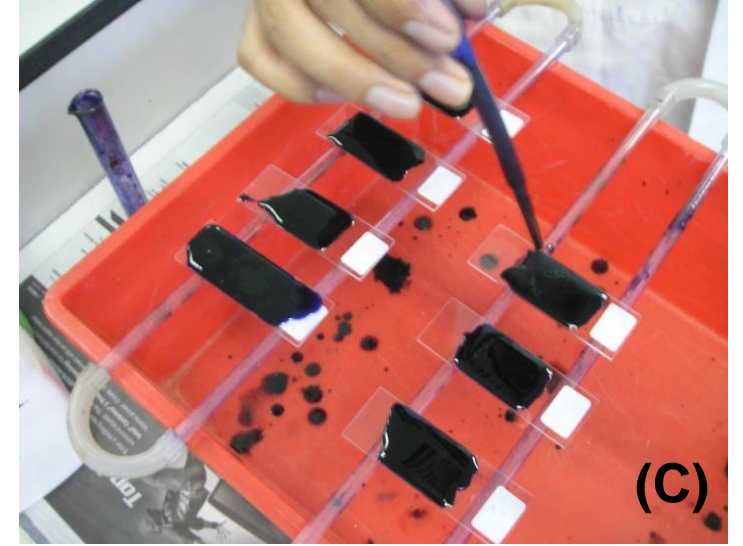
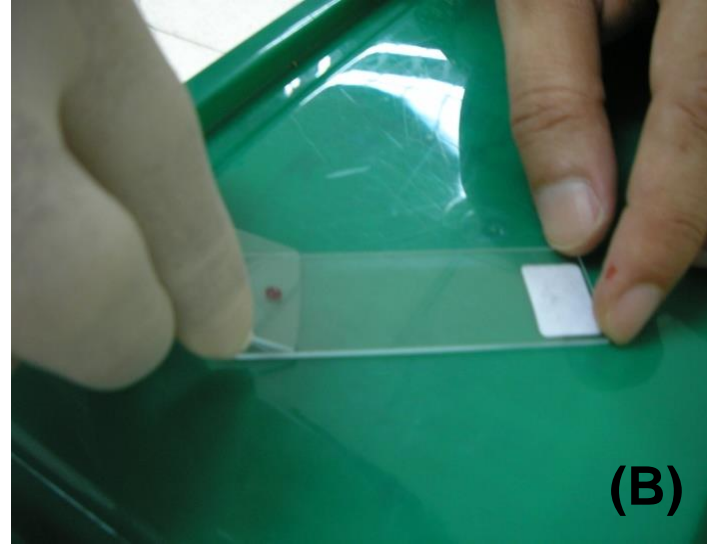
Experimental Design

GROUP	REGIME	DESCRIPTION	<i>E. cardamomum</i> DOSAGE
TREATMENT	PREVENTIVE	PRE14 : 14 days pre-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
		PRE07 : 7 days pre-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
		PRE03 : 3 days pre-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
	CURATIVE	CUR03 : 3 days post-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
		CUR05 : 5 days post-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
		CUR07 : 7 days post-infection	0.1 mL of 10 µg/mL of dH ₂ O-extract
GROUP	REGIME	DESCRIPTION	CONTROL DOSAGE
CONTROL	POSITIVE	POS : Berenil (Sigma-Aldrich KL)	0.01 mL 3.5 mg/kg bw
	NEGATIVE	NEG : 0.9 % Normal Saline	0.1 mL 0.9 normal saline
	LETHAL	LTN : Infection without treatment	5 × 10 ³ <i>T. evansi</i> / mice (i.p.)

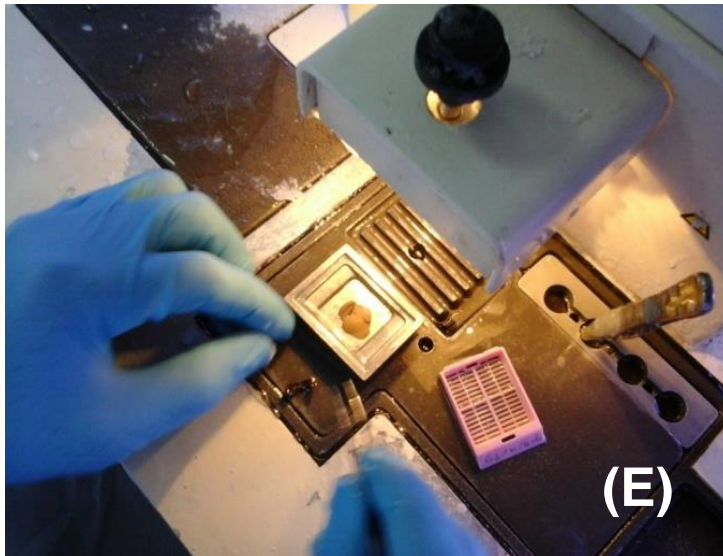
Parasite Administration And Animal Tagging



Giemsa Staining And Microscopic Observation



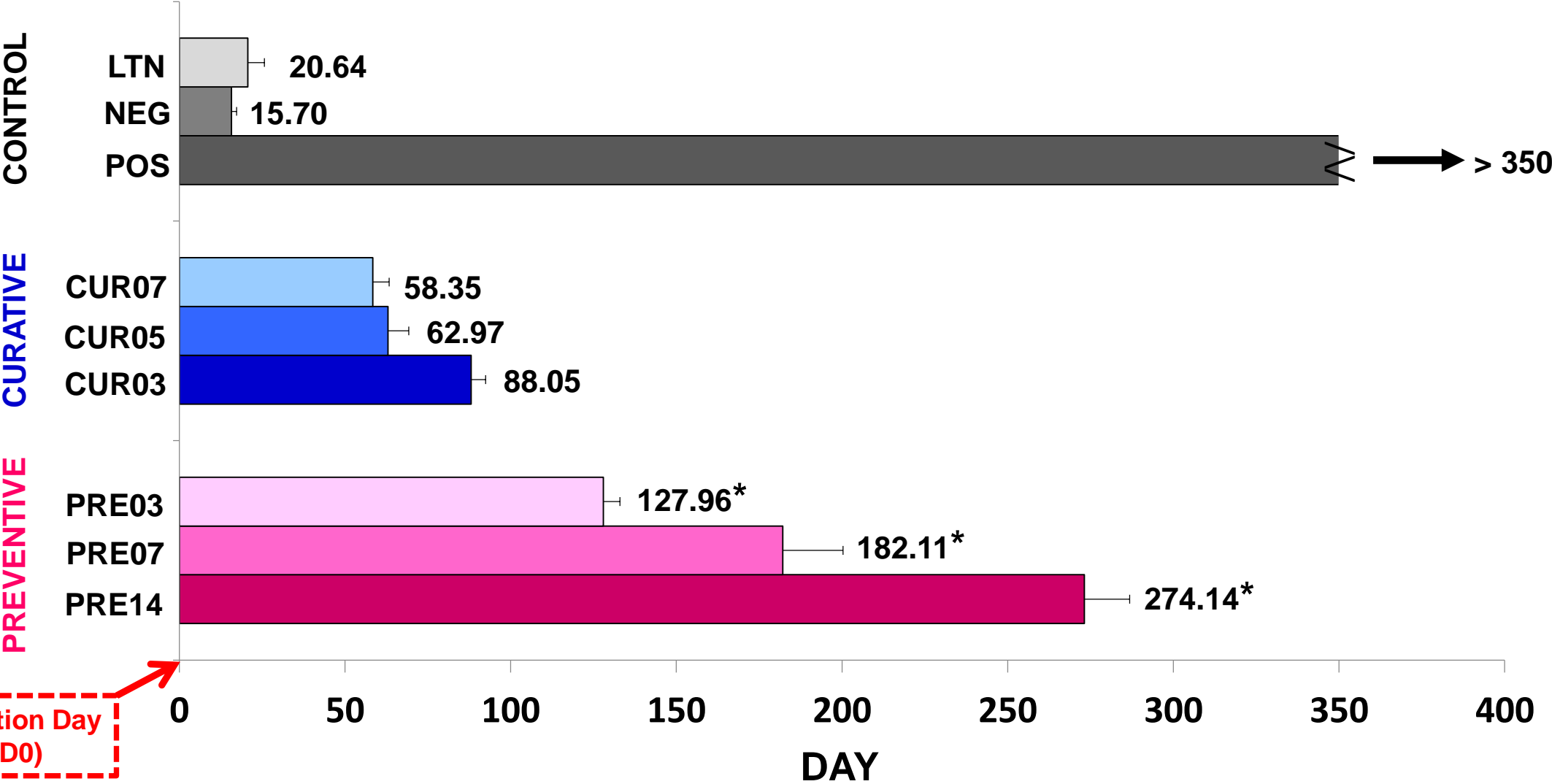
Biochemical Test And Histology Of Liver & Kidney



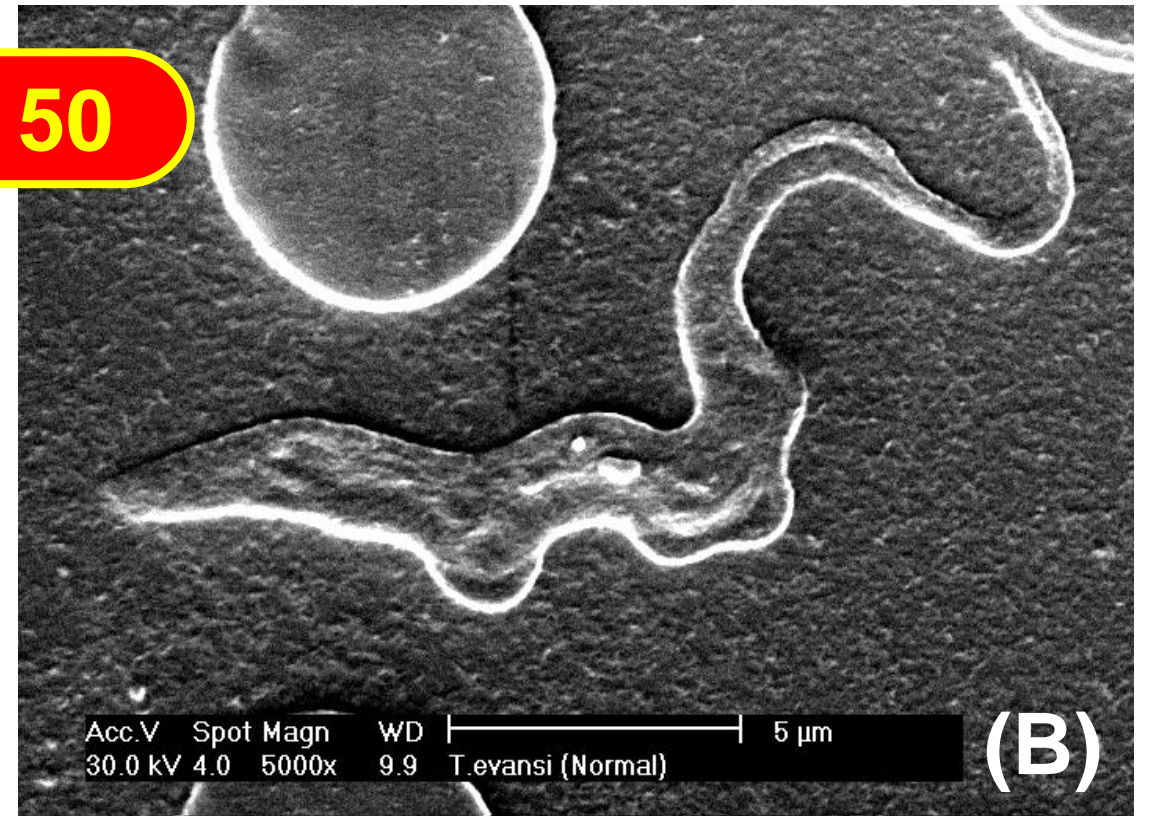
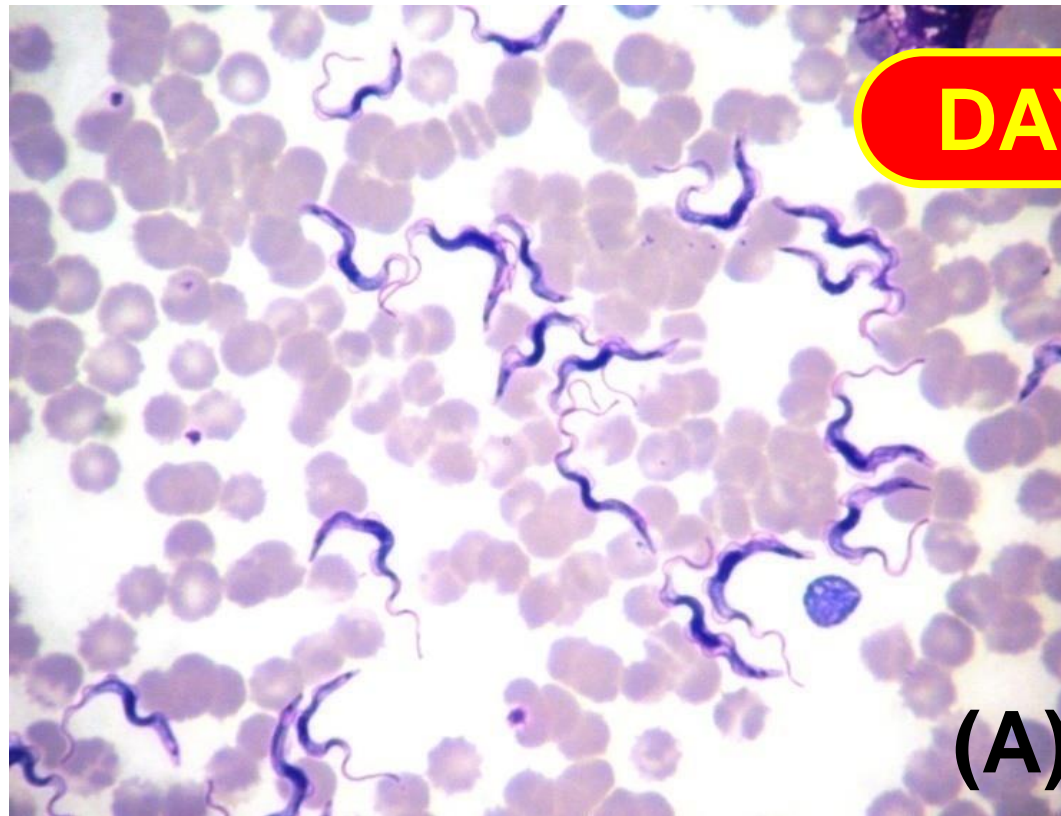
RESULTS & DISCUSSIONS



Mice Survival Time (Day)

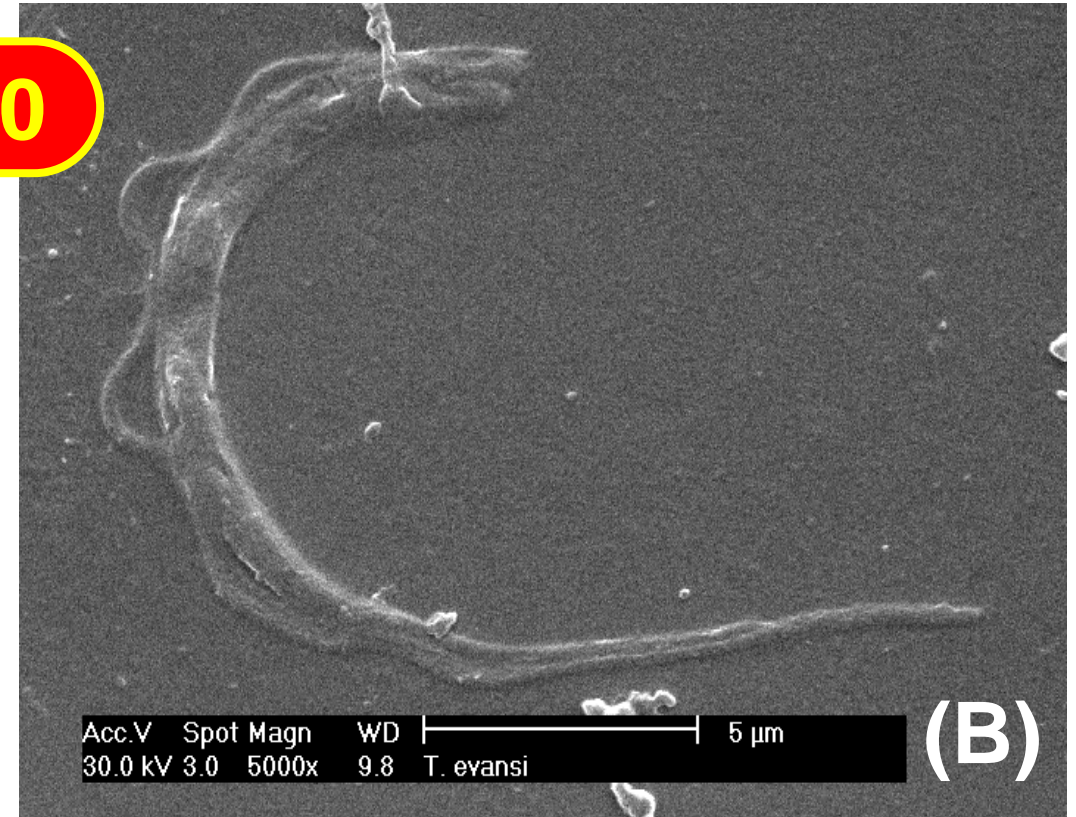
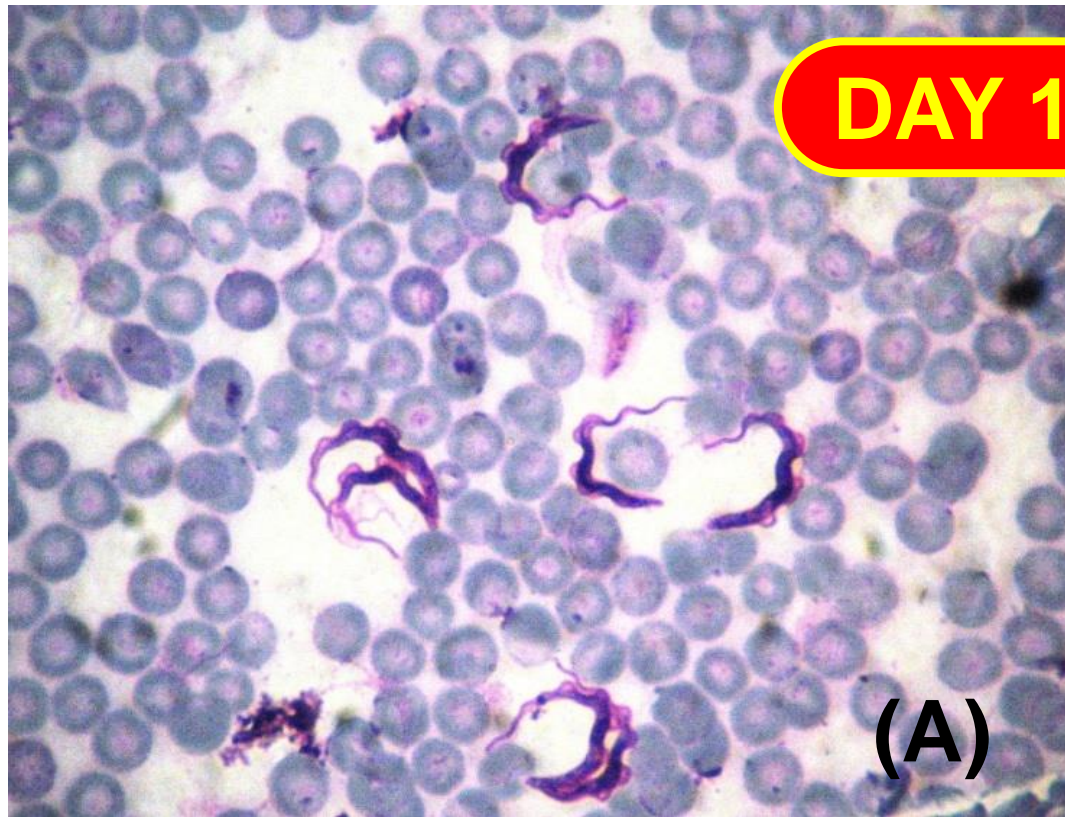


Parasite Survival In PRE14 Mice Group : 50th Day



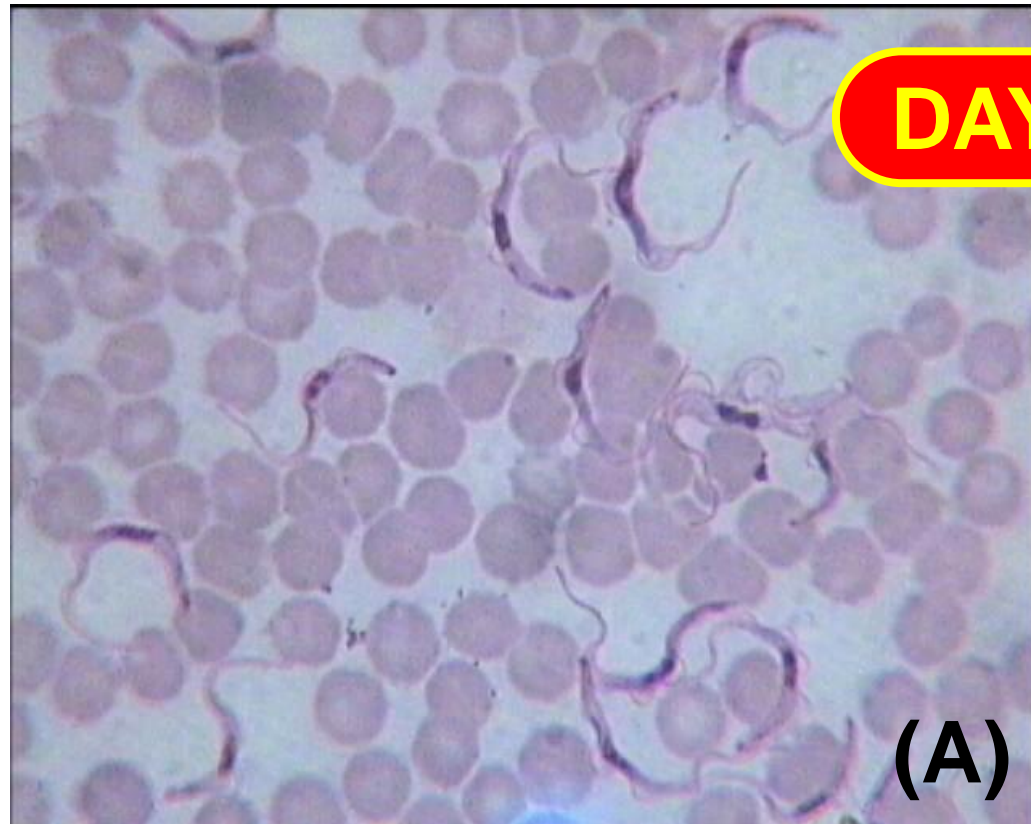
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 50 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 100th Day

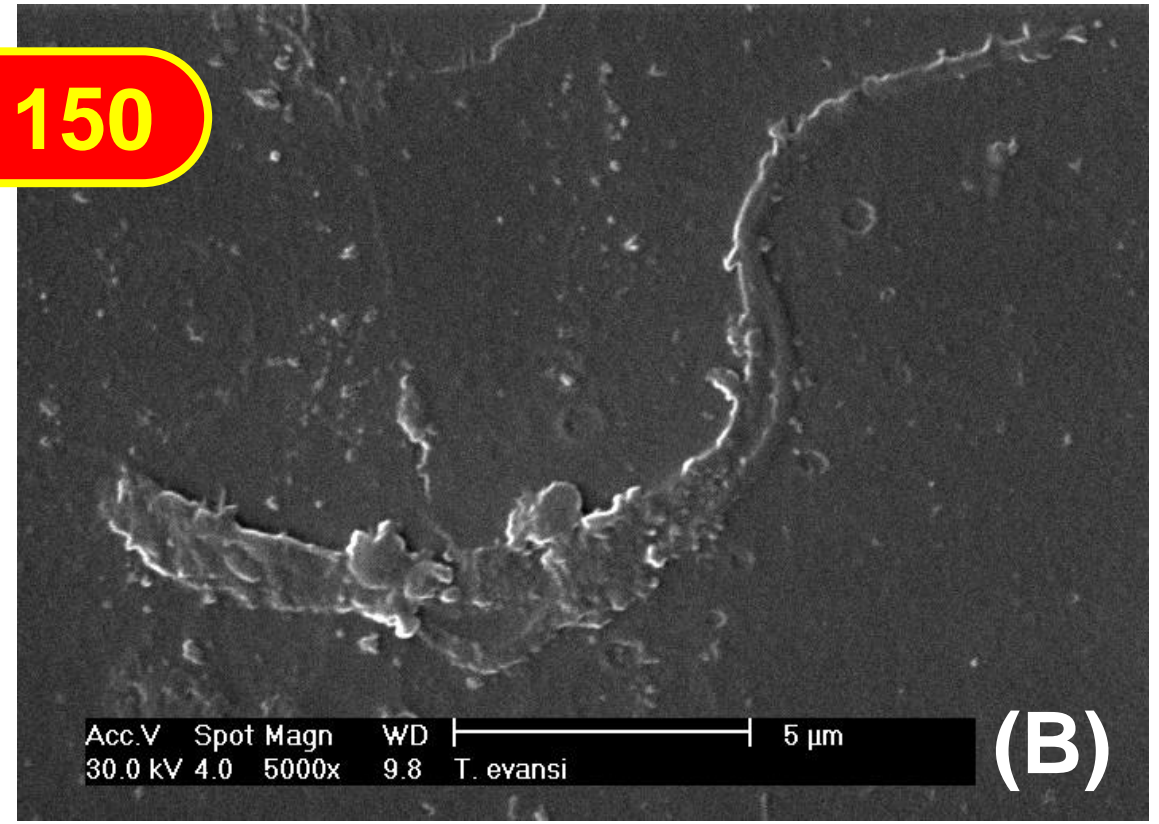


Giemsa thin blood smear of the mice from PRE14 mice group taken on day 100 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 150th Day

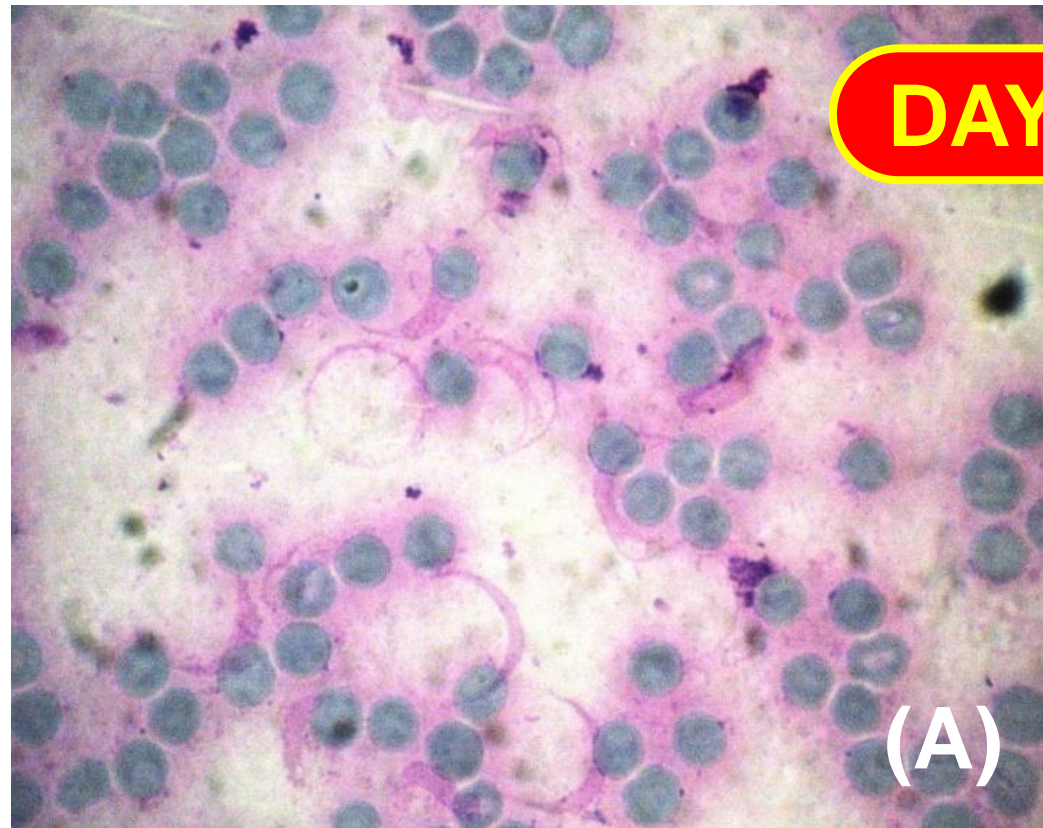


DAY 150

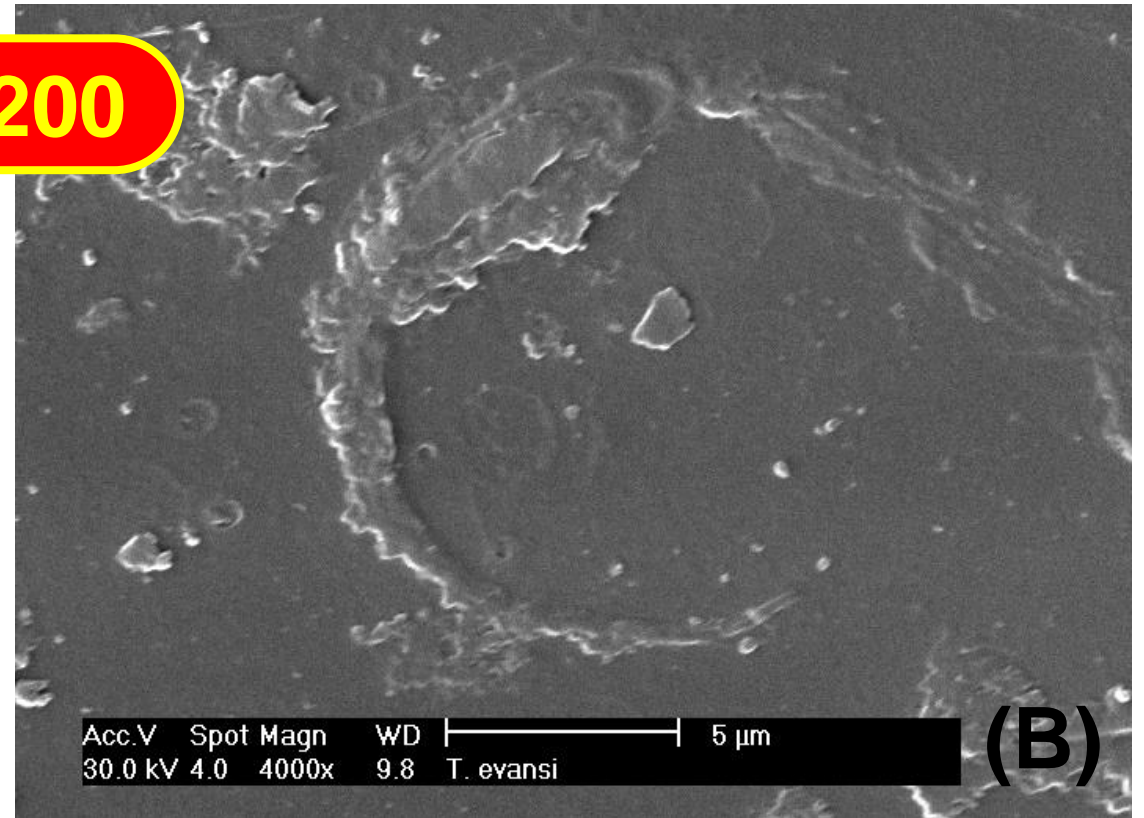


Giemsa thin blood smear of the mice from PRE14 mice group taken on day 150 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 200th Day

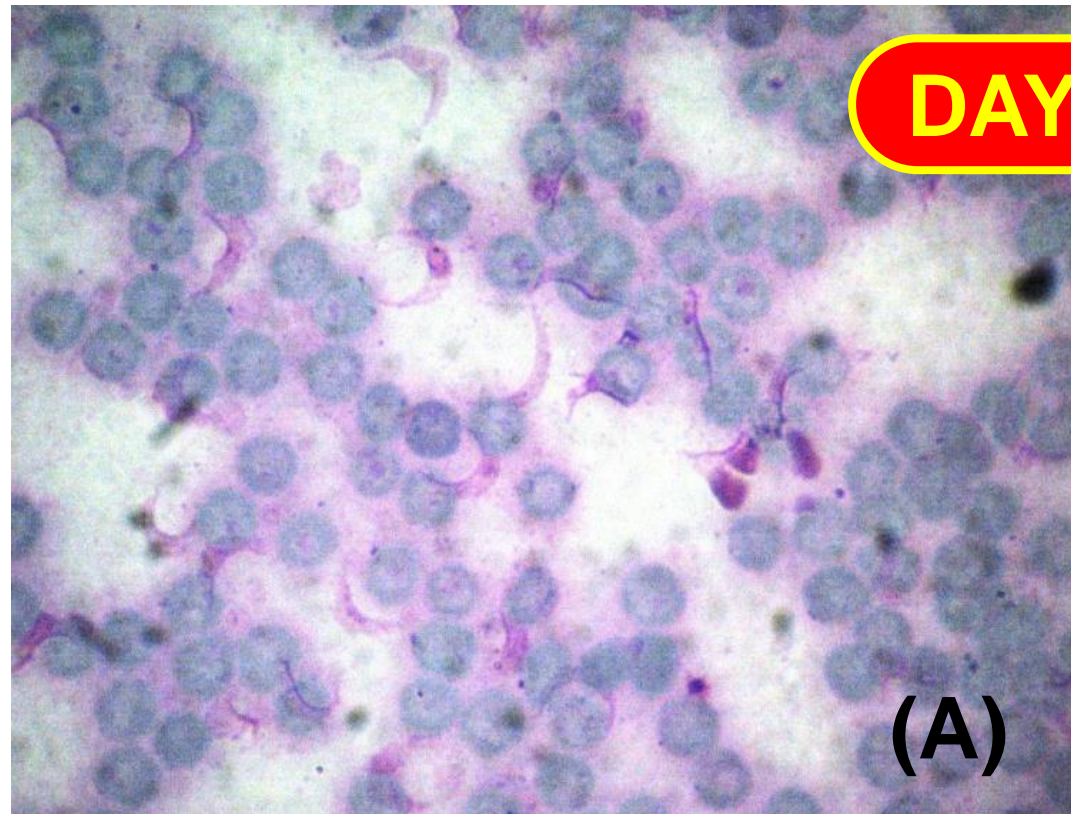


DAY 200

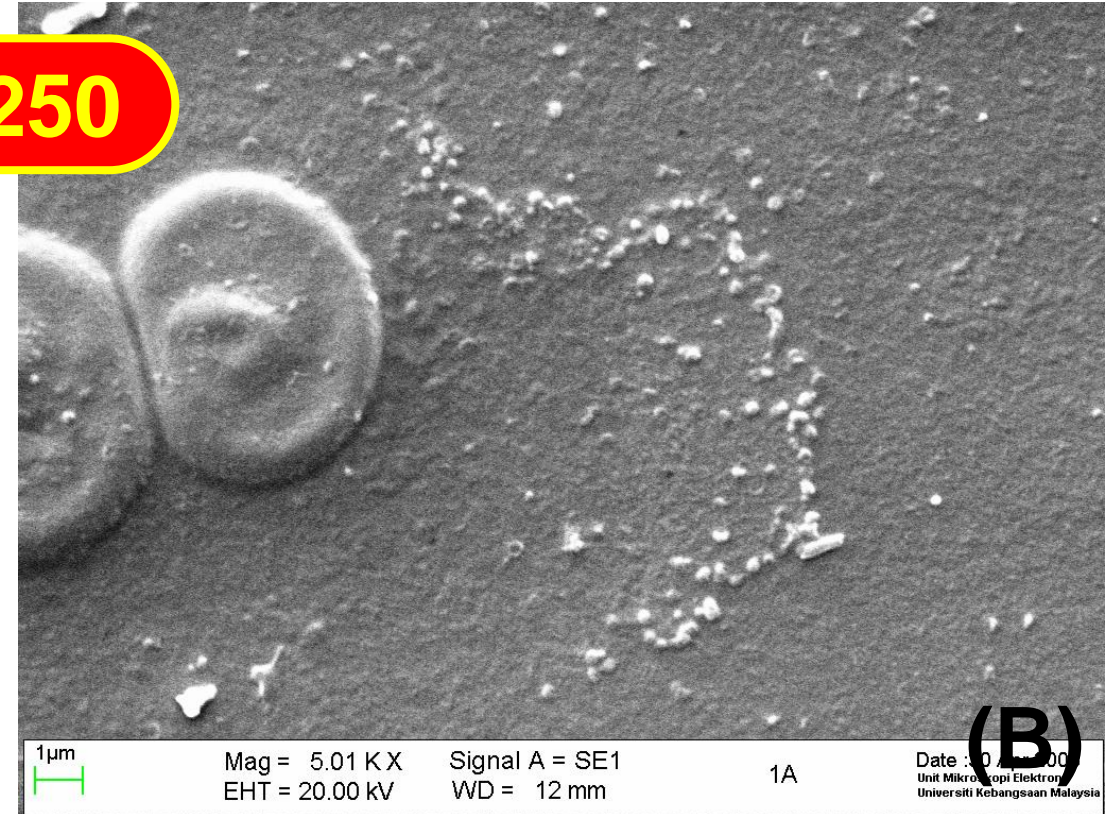


Giemsa thin blood smear of the mice from PRE14 mice group taken on day 200 post-infection as observed under x100 magnification of light microscope (A) and x4000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 250th Day

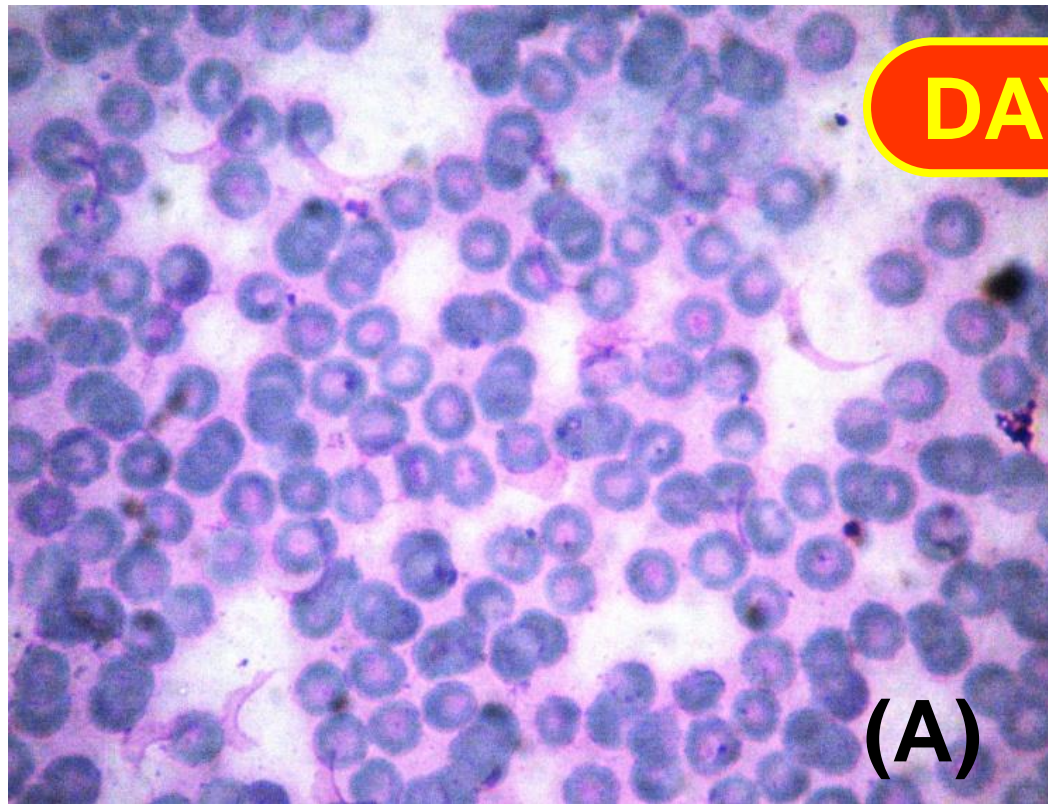


DAY 250

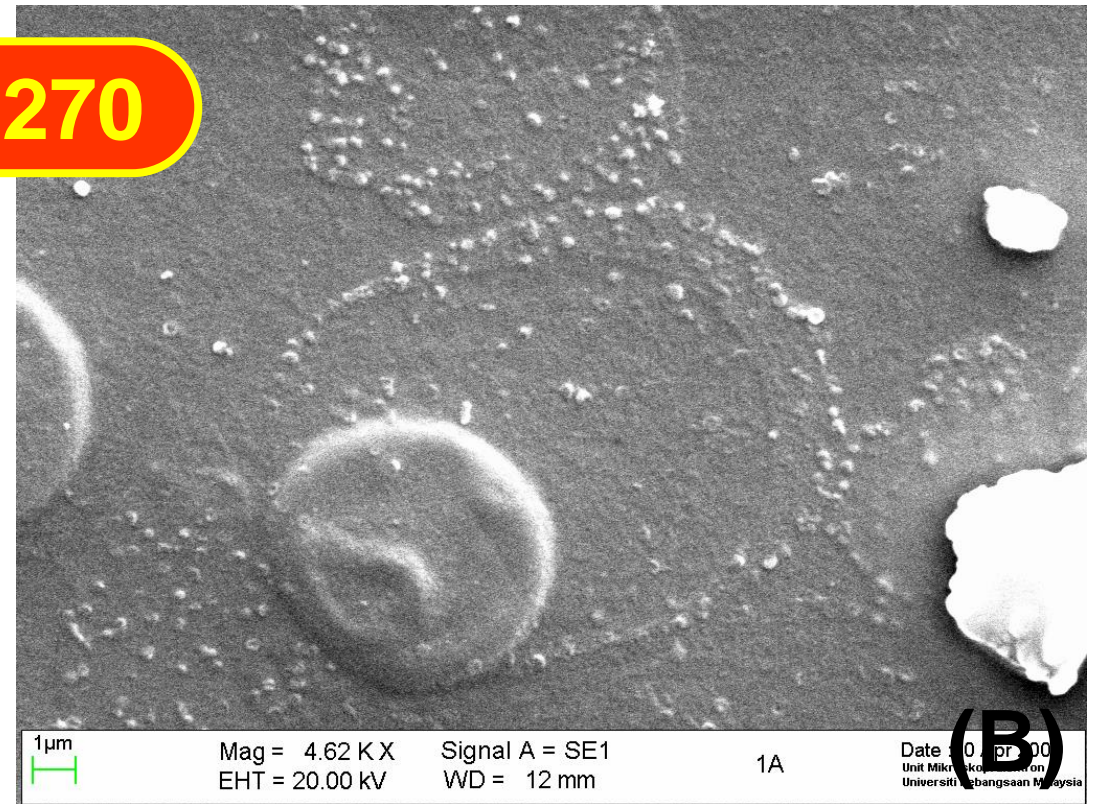


Giemsa thin blood smear of the mice from PRE14 mice group taken on day 250 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Survival In PRE14 Mice Group : 270th Day

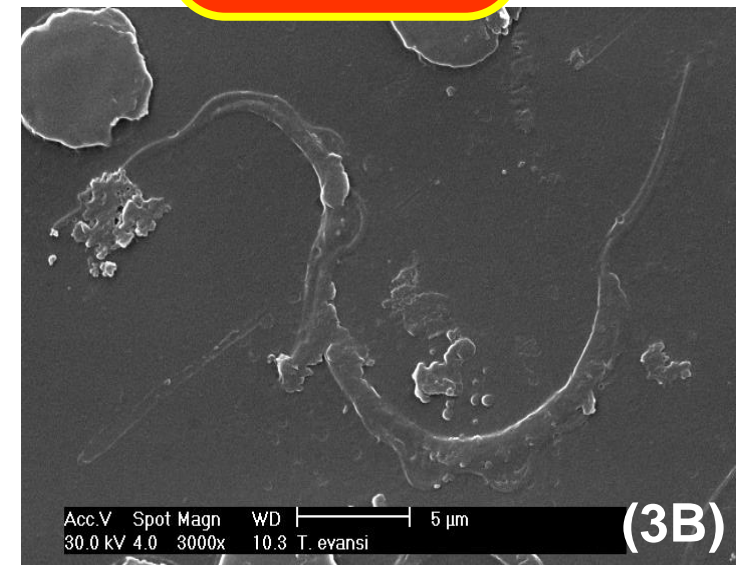
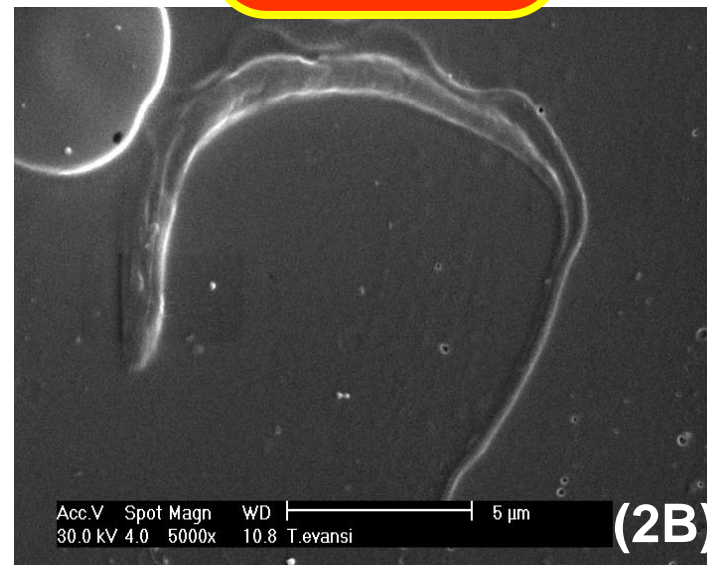
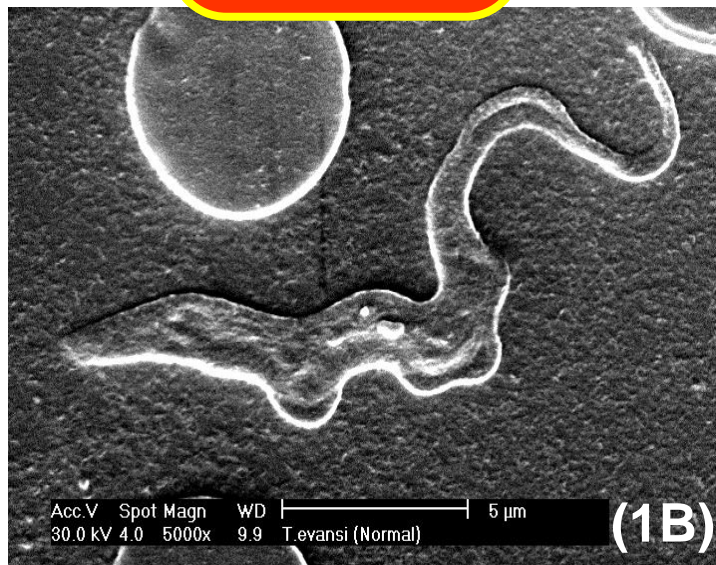
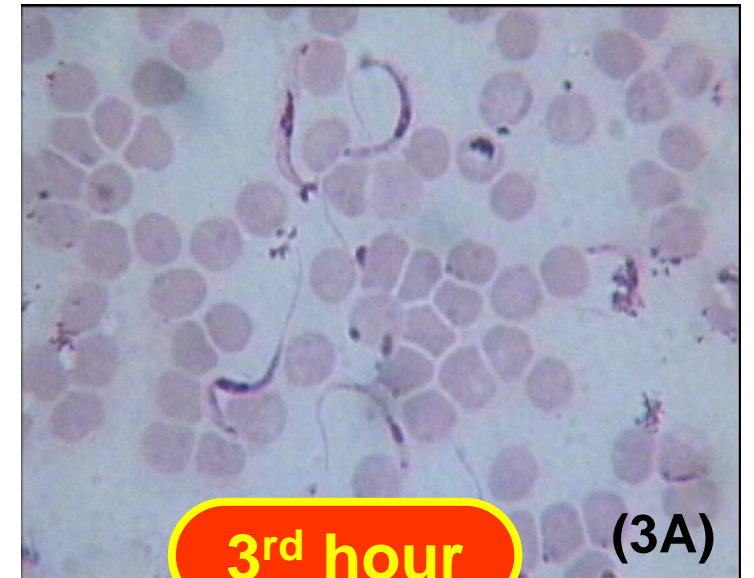


DAY 270

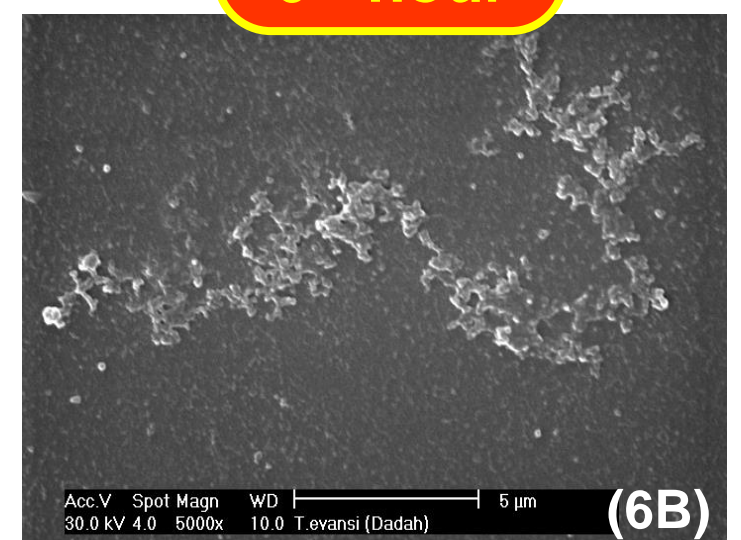
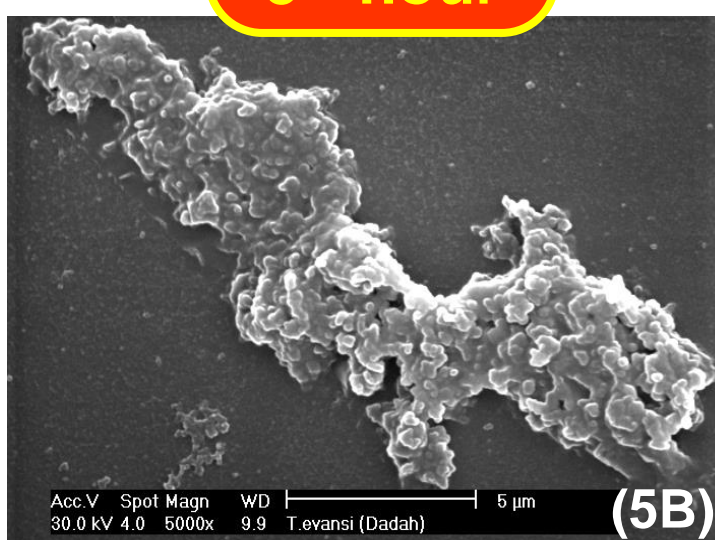
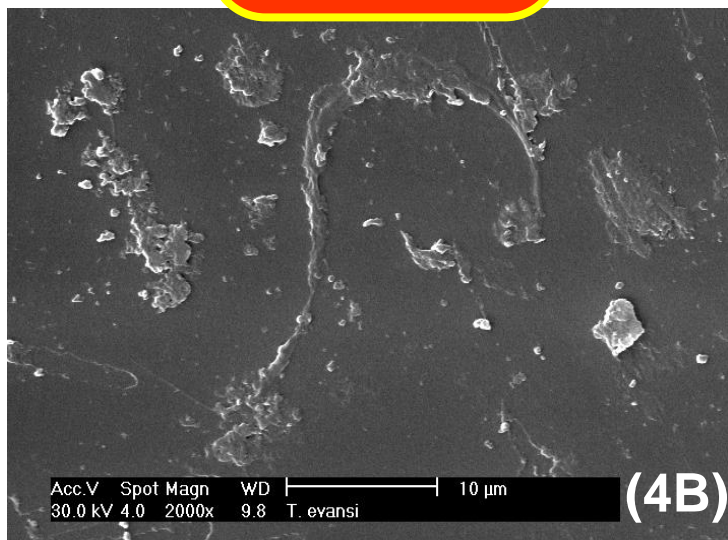


Giemsa thin blood smear of the mice from PRE14 mice group taken on day 270 post-infection as observed under x100 magnification of light microscope (A) and x4600 magnification of SEM (Leo 1450VP, Japan) (B)

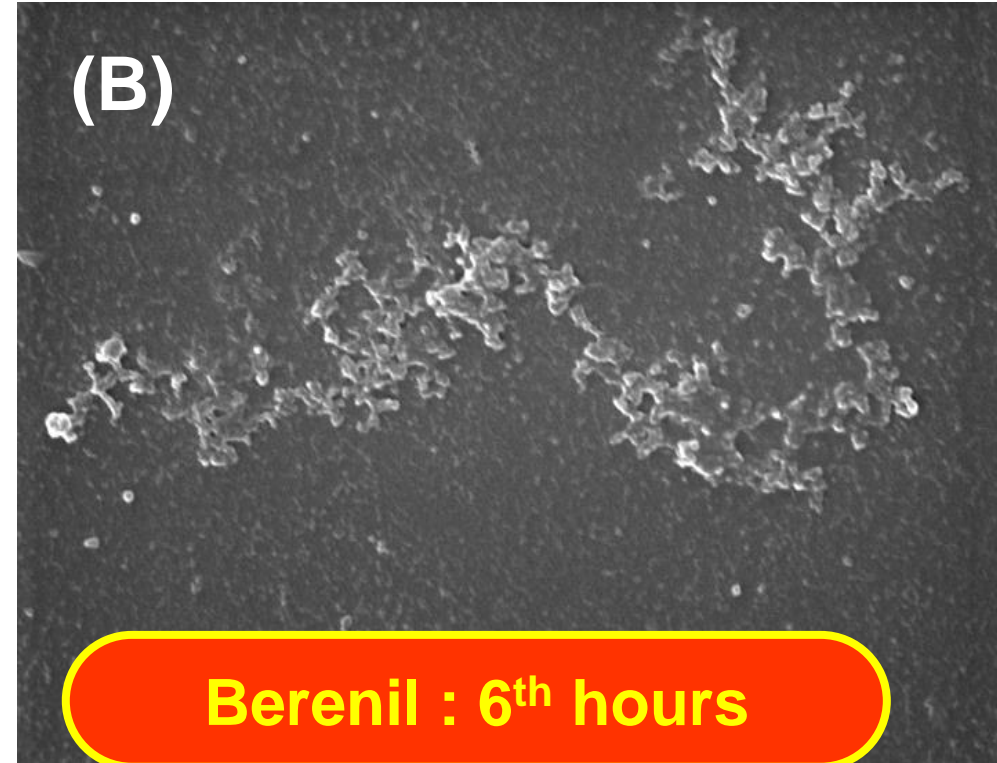
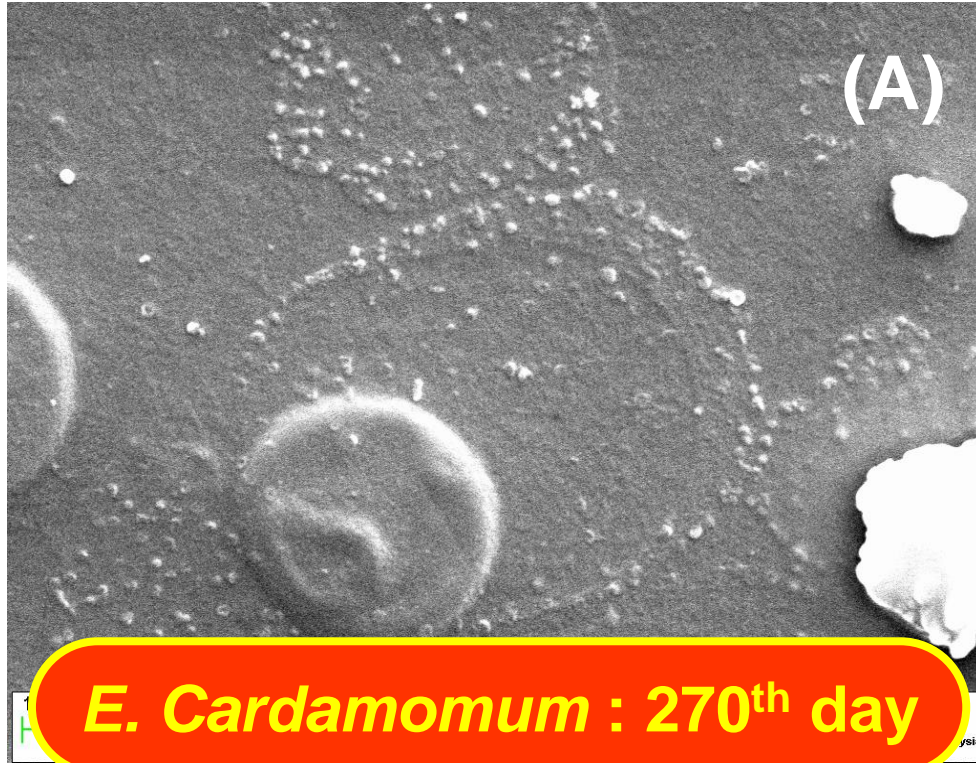
Parasite Growth in Berenil-Treated Group (POS)



Parasite Growth in Berenil-Treated Group (POS)



T. cucumerina VS Berenil



Scanning electron micrograph showed the morphological changes of *T. evansi* in PRE14 mice (0.2 mL 100 mg/kg bw *T. cucumerina* aqueous-extract) on 270th day post infection (A) and in POS mice at 6th hours post treatment (0.01mL 3.5 mg/kg bw Berenil) (B) as observed under x5000 magnification of SEM

Biochemical Test For Toxicity Assessment



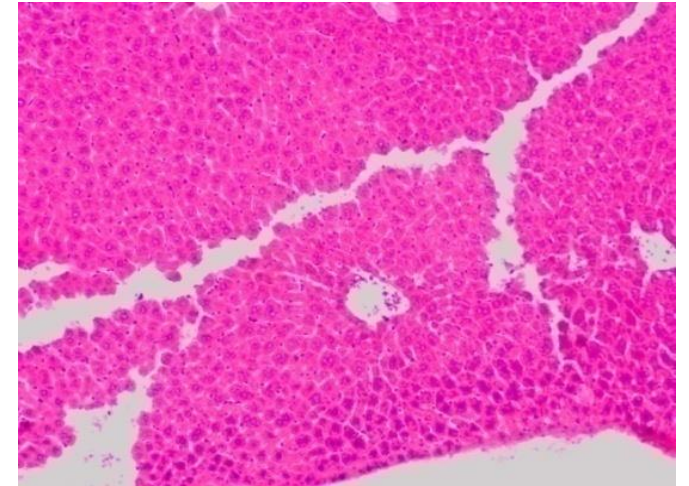
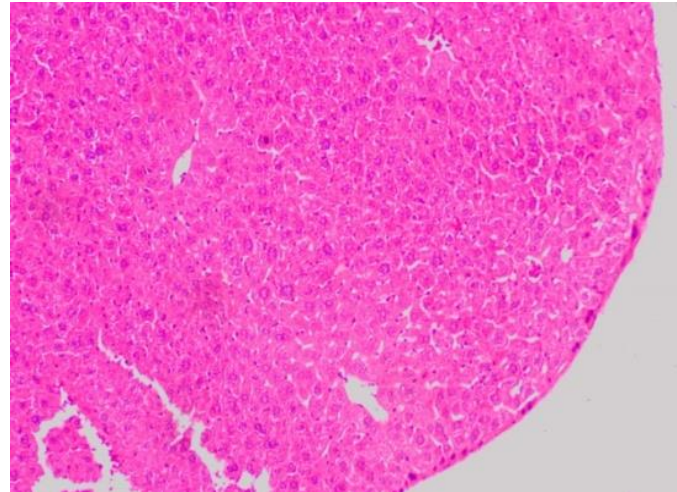
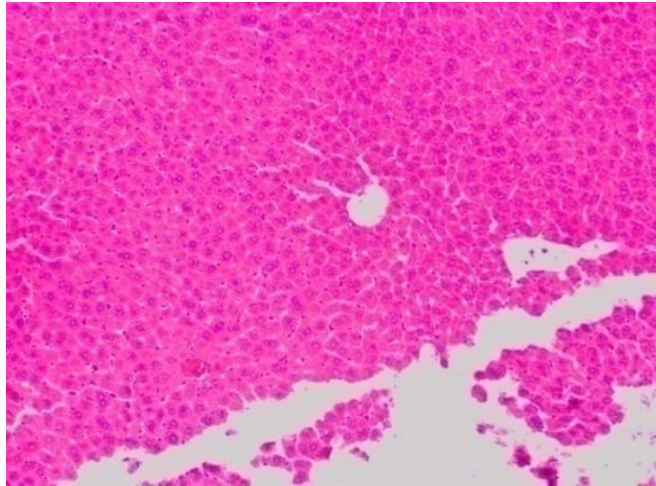
Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	40 – 93	IU/L
AST (*)	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	92 – 206	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	61.46 ± 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

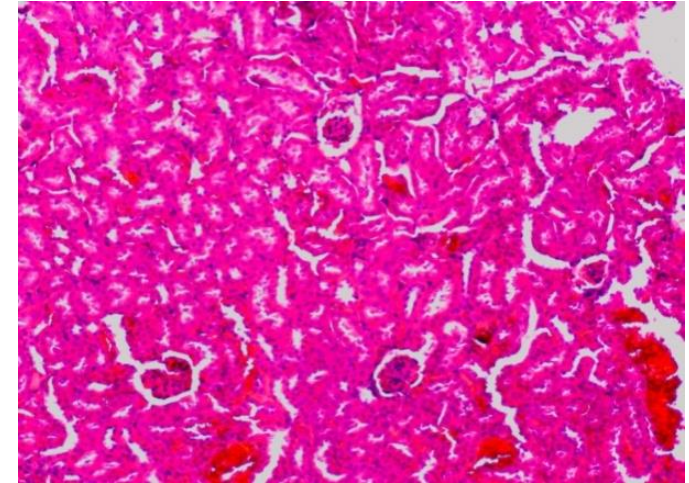
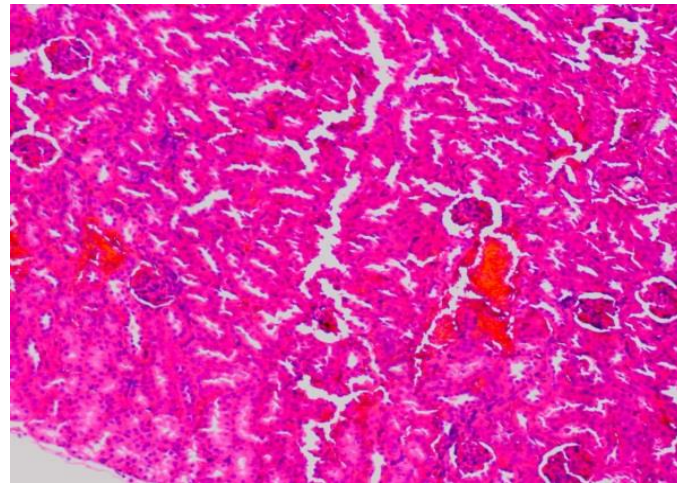
(*) All values were expressed as mean ± standard deviation (sd)

Organ Histology For Toxicity Assessment

Liver



Kidney



Treatment (Acute)

Treatment (Sub-acute)

Control

CONCLUSIONS



Hypothesis

- New wave of infection → mice is susceptible to infection (Kurup and Rajamohan 2011)
- Curative regime are less effective than other treatment regimens.
- Longer prophylactic duration → longer survival period of the host.
- A bioactive compound, nerolidol in *E. cardamomum* able to prolong the survival rate of the *Leishmania*-infected mice although the parasitemia density is high (Romão et al. 2006)
- OH approaches within multidisciplinary collaboration is required to combat AHT & Surra disease.

Suggestions

Various solvents
of *T. cucumerina*
extract

Mechanism
of action

In-vitro
anti-trypanosomal
screening

Concentration- &
time-dependant
alteration



Clinical &
molecular
approaches

Screening
against *T. cruzi*
and *T. brucei*

Absolute Hypothesis

CONSUME CARDAMOM..!



Absolute Hypothesis



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Acknowledgement





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يُونِيْڤَرْسِيْتِيْ اِسْلَامِيَّةٌ اِنْتَارْ اَبَحْسِيَا مَلِيْسِيَا

Thank You

