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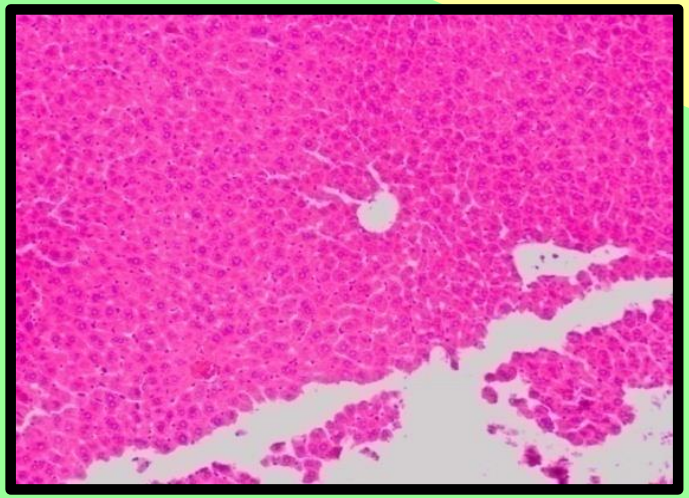
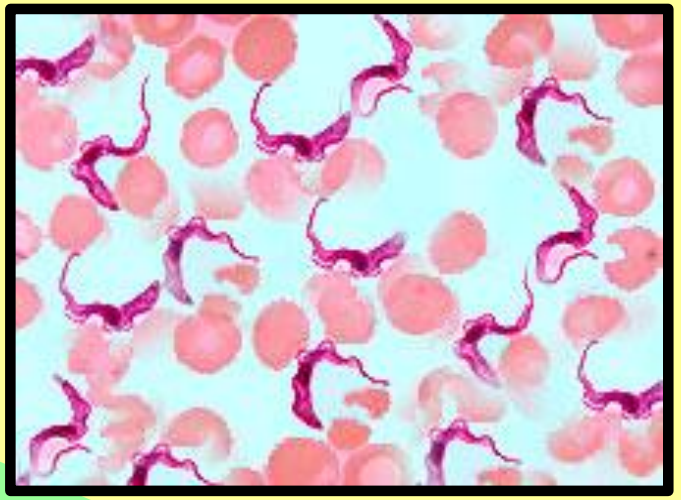
Garden of Knowledge and Virtue

Piper sarmentosum Leaf As a Promising Non-toxic Antiparasitic Agent Against *Trypanosoma evansi*-Induced Mice





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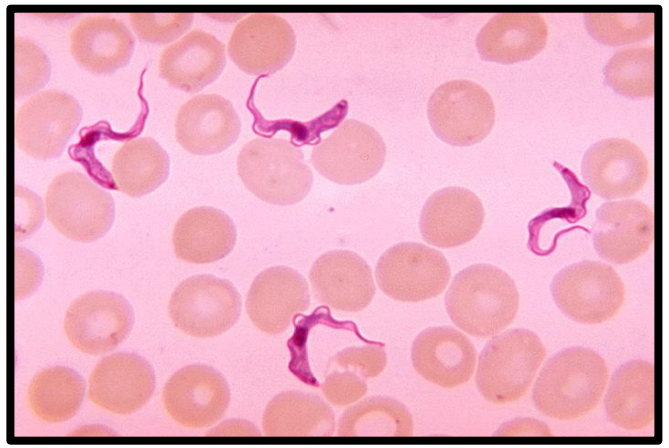
INTRODUCTION

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 mammals (mostly livestock)
- Wide variety of vectors → worldwide distributed
- Drug resistant in some regions → suramin, pentamidine, berenil



Vectors of *Trypanosoma evansi*

Hirudo medicinalis

Tabanus striatus

Desmondus rotundus

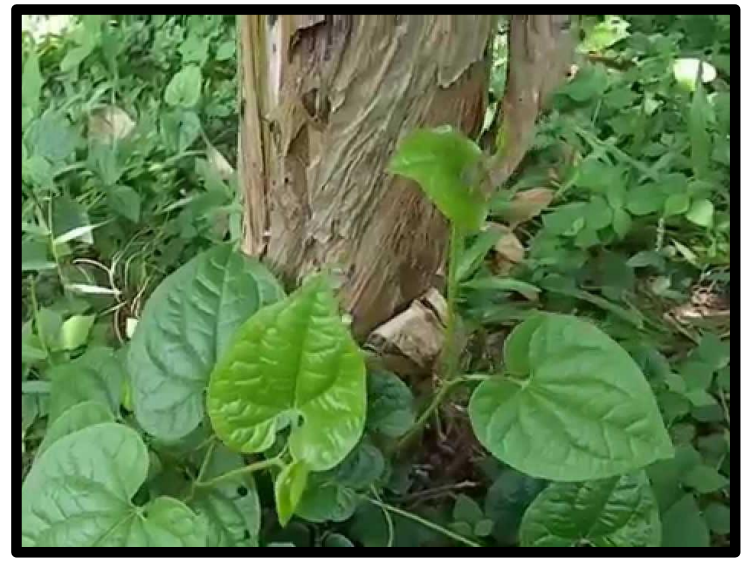
Triatoma infestants

Glossina morsitans

Anthomyia pluvialis

Piper sarmentosum

- A traditional herb & aromatic flowering plant locally known as “kaduk” in Malay



- Wildly & abundant in damp open areas, cleared riverbanks and under shady trees (Seyyedan *et al.*, 2013)

- Well growth on cultivated land in India, Sri Lanka & Southeast Asian region (Hussain *et al*., 2009).

- Variety of phytochemical constituents & groups identified from various parts of the plants → phenylpropanoids, α-asarone, asaricin, myricetin, sarmentamide A & B, piperitone, naringenin, spathulenol, farnesol, quercetin, etc…

Piper sarmentosum: The Testimonial

Significant antimicrobial activities (Chan & Wong, 2014):
- Antifungal
- Antiamoebic
- Antituberculosis
- Anti-dengue

Antiplasmodial & antileishmanial activities of Pellitorine compound (Souza Oliveira *et al*, 2018)

The flowers used to treat many chronic diseases (Shim & Gam, 2012):
- Hypertension
- Diabetes mellitus
- Asthma
- Atherosclerosis

Wide range of pharmacological properties (Syed Ab Rahman *et al*, 2014):
- Wound healing
- Antioxidant
- Anti-infammatory
- Anti-osteoperosis

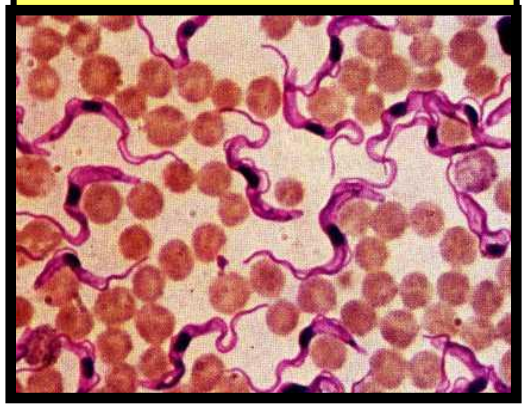
Antibacterial activities (Sanusi *et al*, 2017): *E. coli, MRSA, B. cereus, V. cholera & S. typhi*

Herbal remedies for many illnesses (Atiax *et al,* 2011):
- Feet dermatitis
- Toothache
- Headaches
- Coughs

MATERIALS & METHODS

Flow Chart

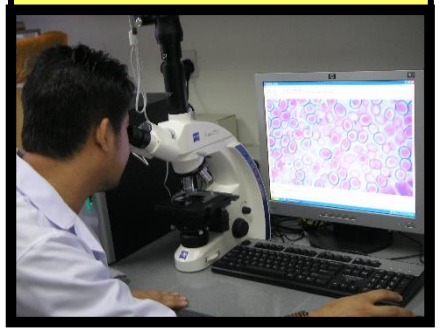
T. evansi stock



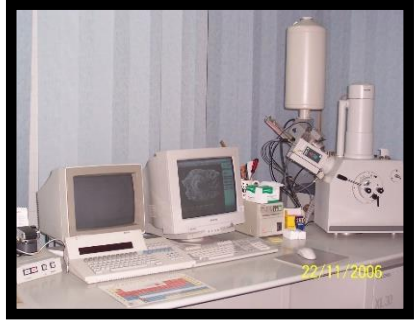
T. evansi administered *i.p.* (5 × 10^3 *T. evansi* / mice)

Orally administered of 0.1 mL 10 mg/kg bw of freeze-dried *P. sarmentosum* aqueous exract

Giemsa blood slide for inhibition rate evaluation



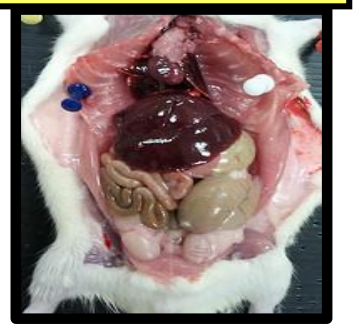
Blood slide for electron microscopic observation



Physical observation of symptoms and mice survival

Blood biochemistry and renal function tests

Vital organ histology for toxicity assessment

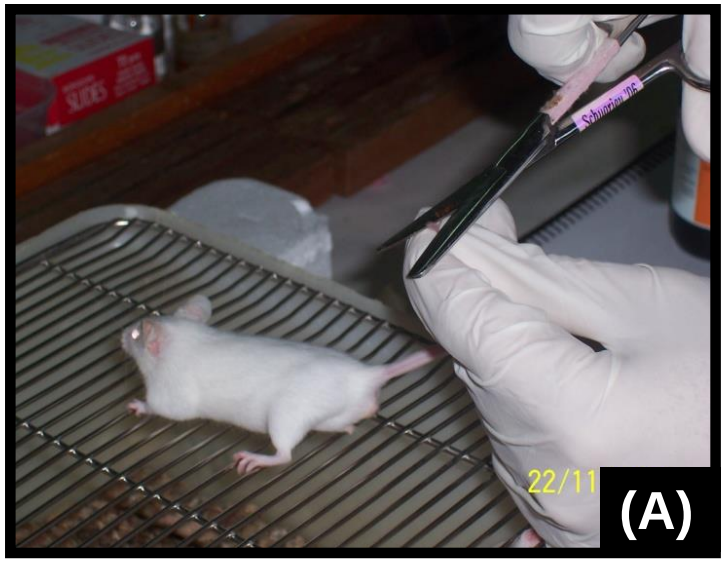


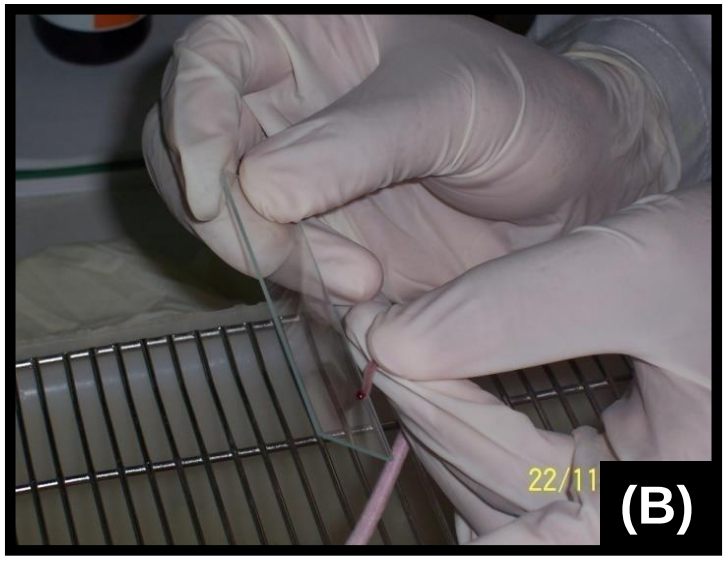
Experimental Design

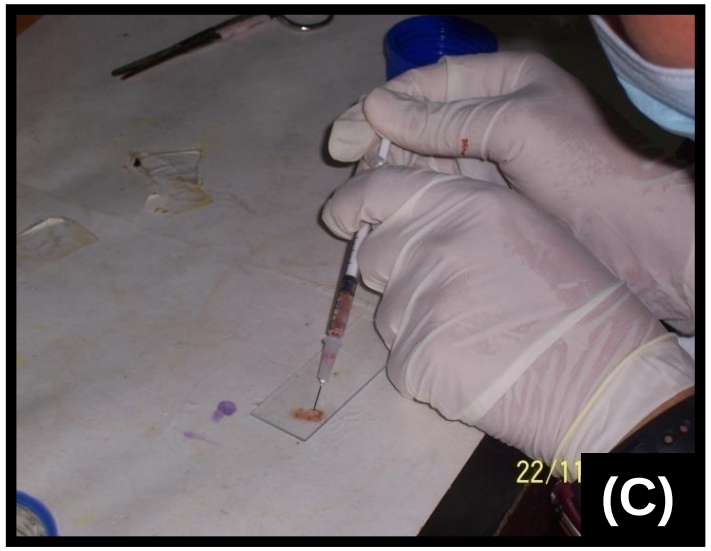
GROUP	REGIMENS	CODE	DESCRIPTION	*P. sarmentosum* DOSAGE
TREATMENT	PREVENTIVE	PRE14	14 days pre-infection	0.1 mL 10 mg/kg bw sdH_2O-extract
		PRE7	7 days pre-infection	0.1 mL 10 mg/kg bw sdH_2O-extract
		PRE3	3 days pre-infection	0.1 mL 10 mg/kg bw sdH_2O-extract
	CURATIVE	CUR3	3 days post-infection	0.1 mL 10 mg/kg bw sdH_2O-extract
		CUR5	5 days post-infection	0.1 mL 10 mg/kg bw sdH_2O-extract
		CUR7	7 days post-infection	0.1 mL 10 mg/kg bw sdH_2O-extract

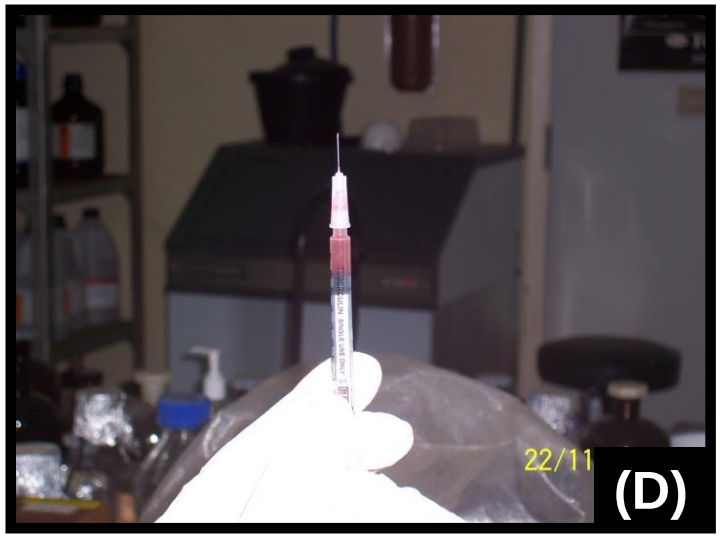
GROUP	REGIMENS	CODE	DESCRIPTION	CONTROL DOSAGE
CONTROL	POSITIVE	POS	Berenil (Sigma-Aldrich KL)	0.01 mL 3.5 mg/kg bw Berenil
	NEGATIVE	NEG	0.9 % Normal Saline	0.1 mL 0.9 normal saline (NS)
	LETHAL	LTN	Infection without treatment	5×10^3 *T. evansi* / mice (i.p.)

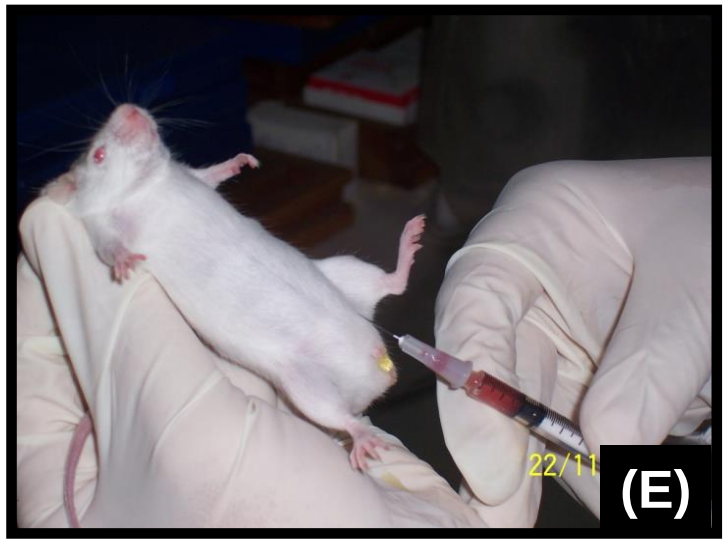
Parasite Administration And Animal Tagging

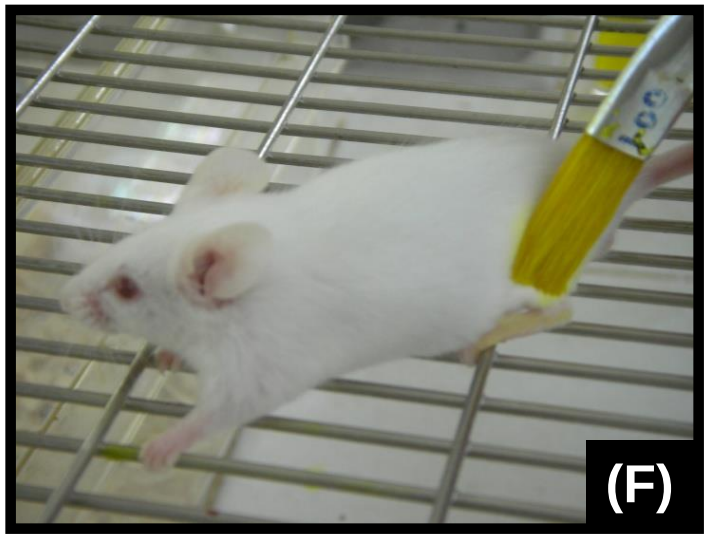




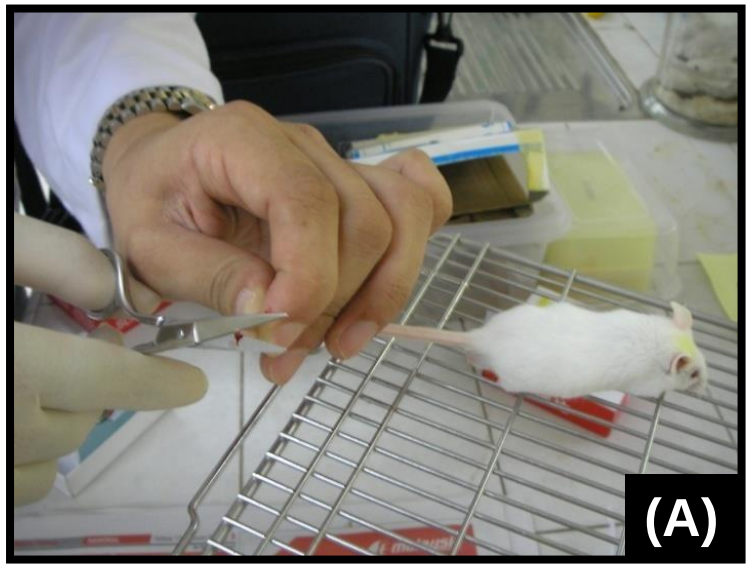


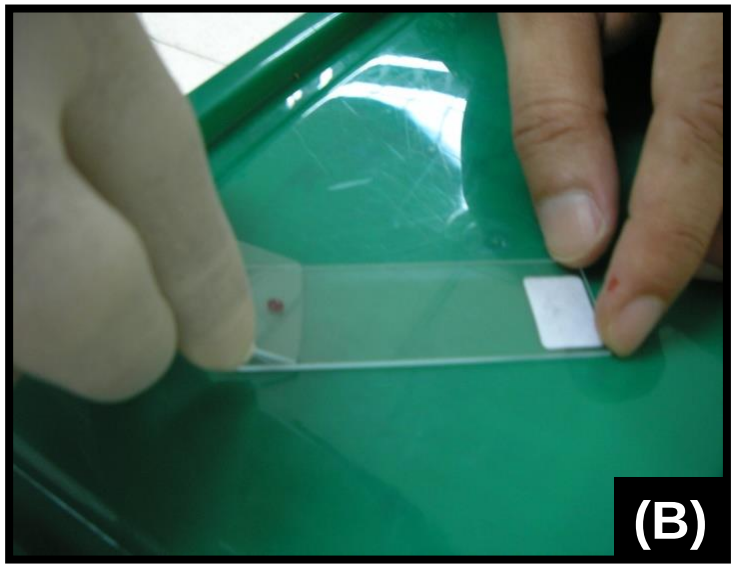


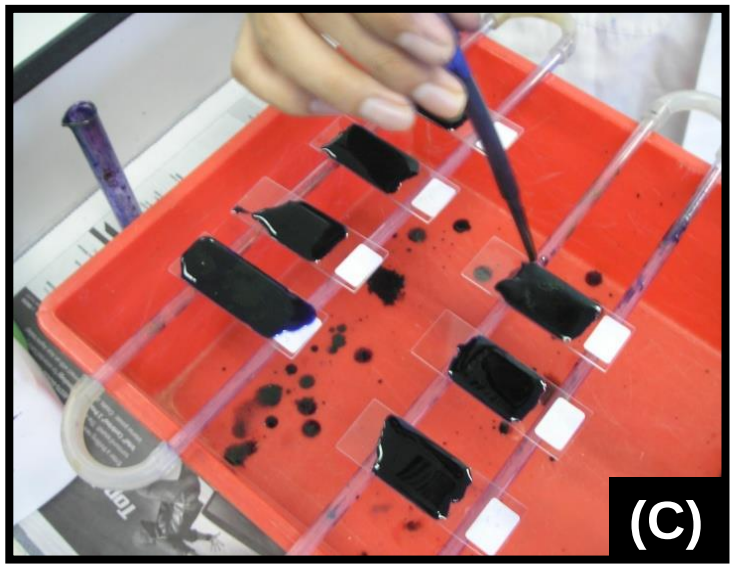


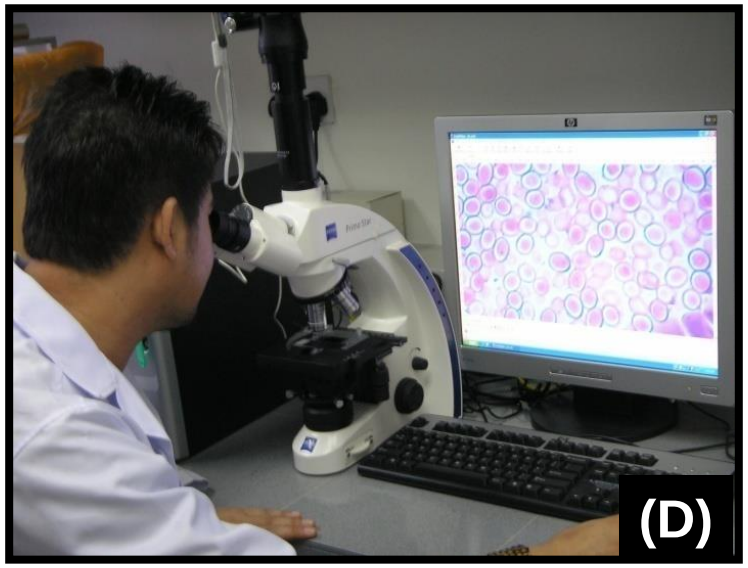


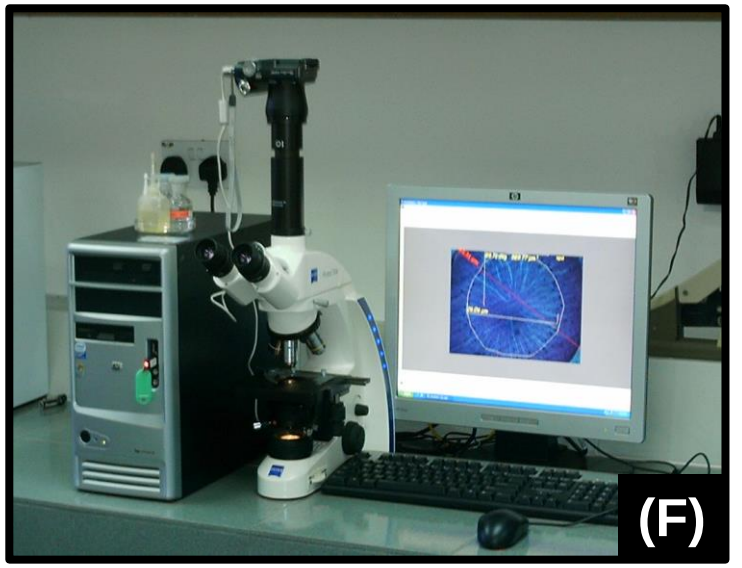
Giemsa Staining And Microscopic Observation











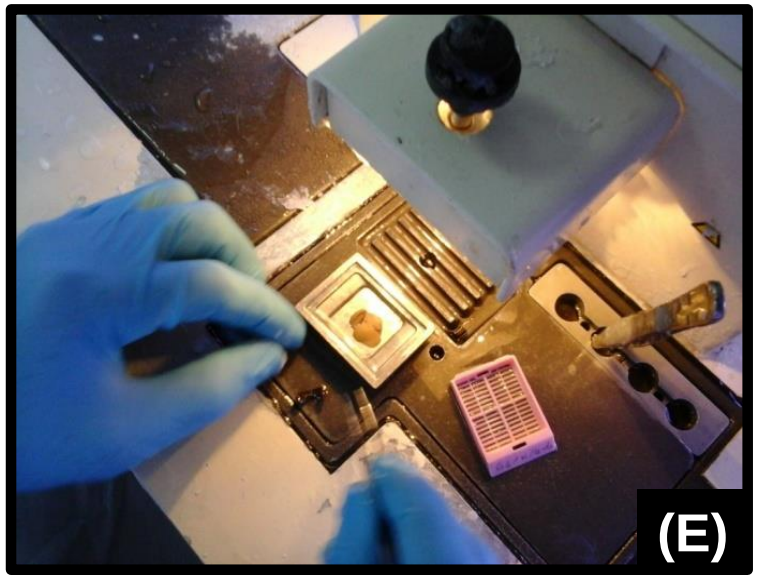
Biochemical Test And Histology Of Liver & Kidney


(A)


(B)


(C)


(D)

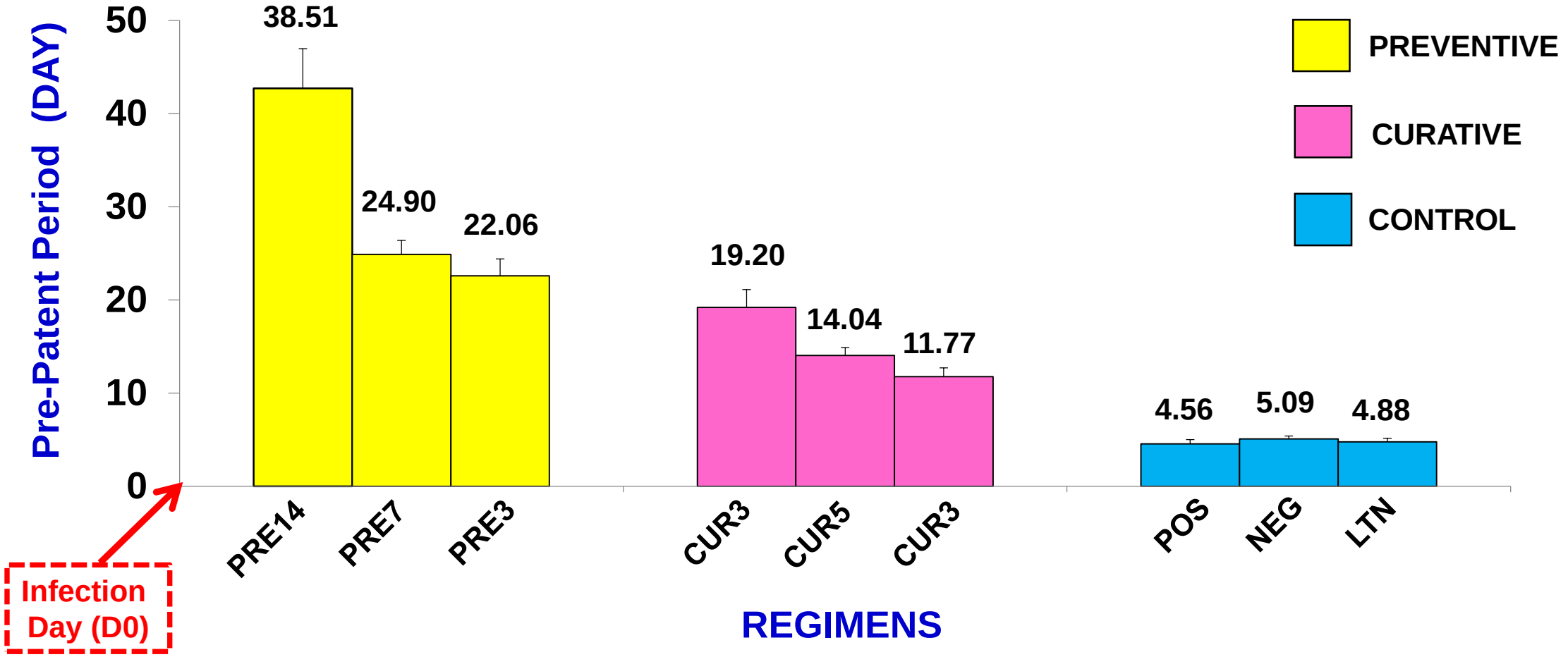

(E)

(F)

RESULTS & DISCUSSIONS

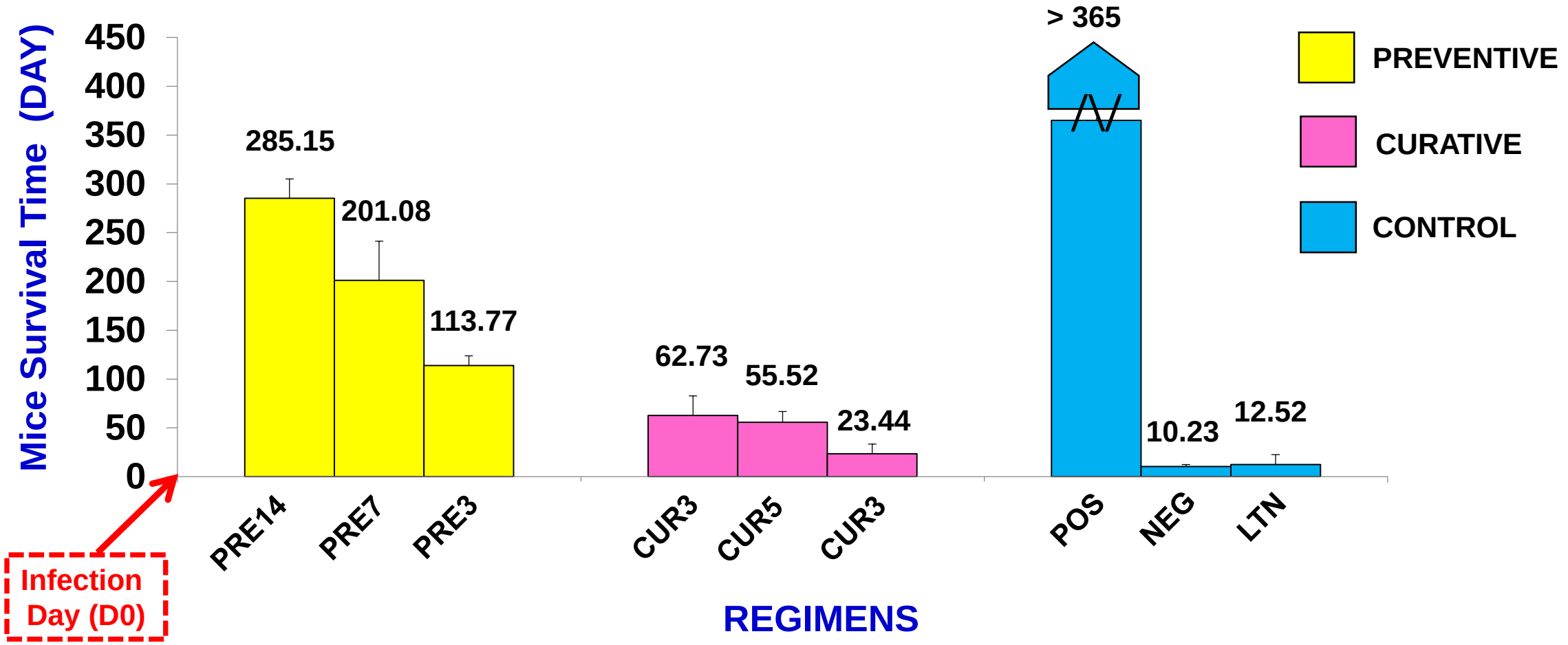
Parasite Pre-Patent Period (Day)

Pre-Patent Period (day) of the mice treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract on D3 post-infection at 5 × 10^3 *T. evansi* / mice (i.p.) as compared with 3 regimes of control

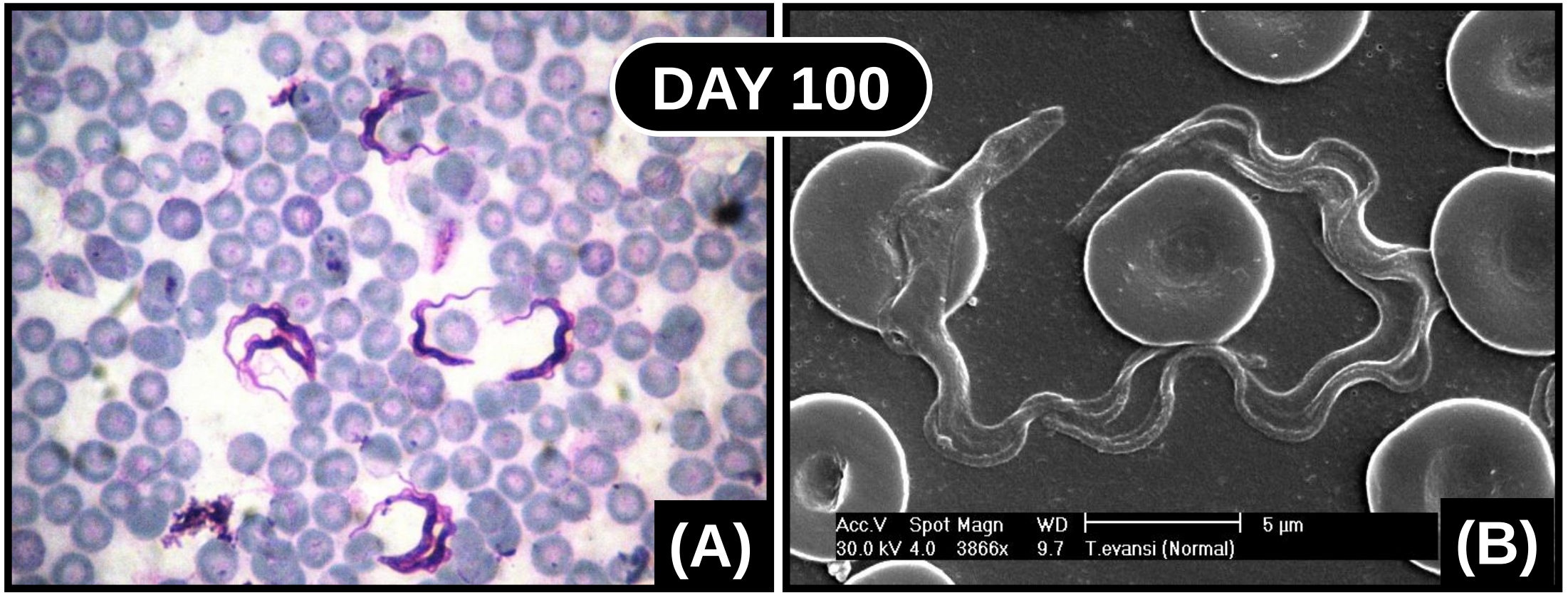


Mice Survival Time (Day)

Mice Survival Time(day) of the mice treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract after infection at 5 × 10^3 *T. evansi* / mice (i.p.) as compared with 3 regimes of control

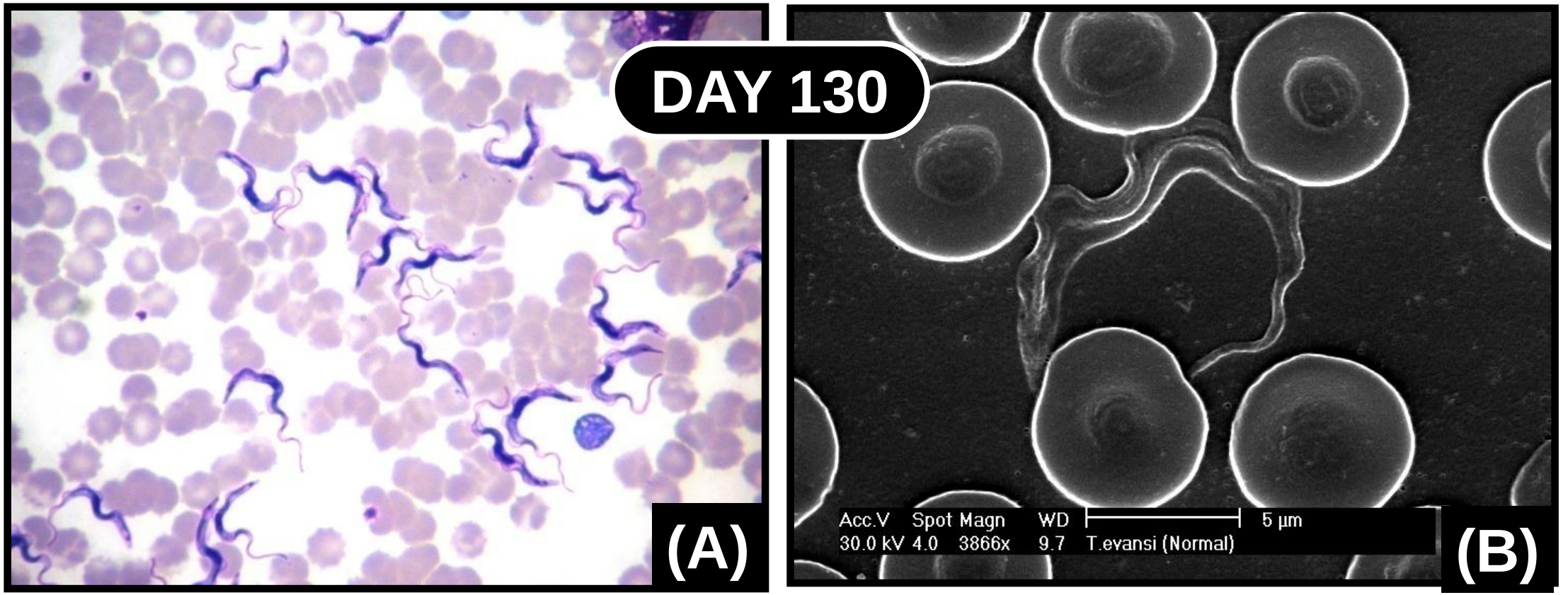


Parasite Survival In PRE14 Mice Group : 100th Day



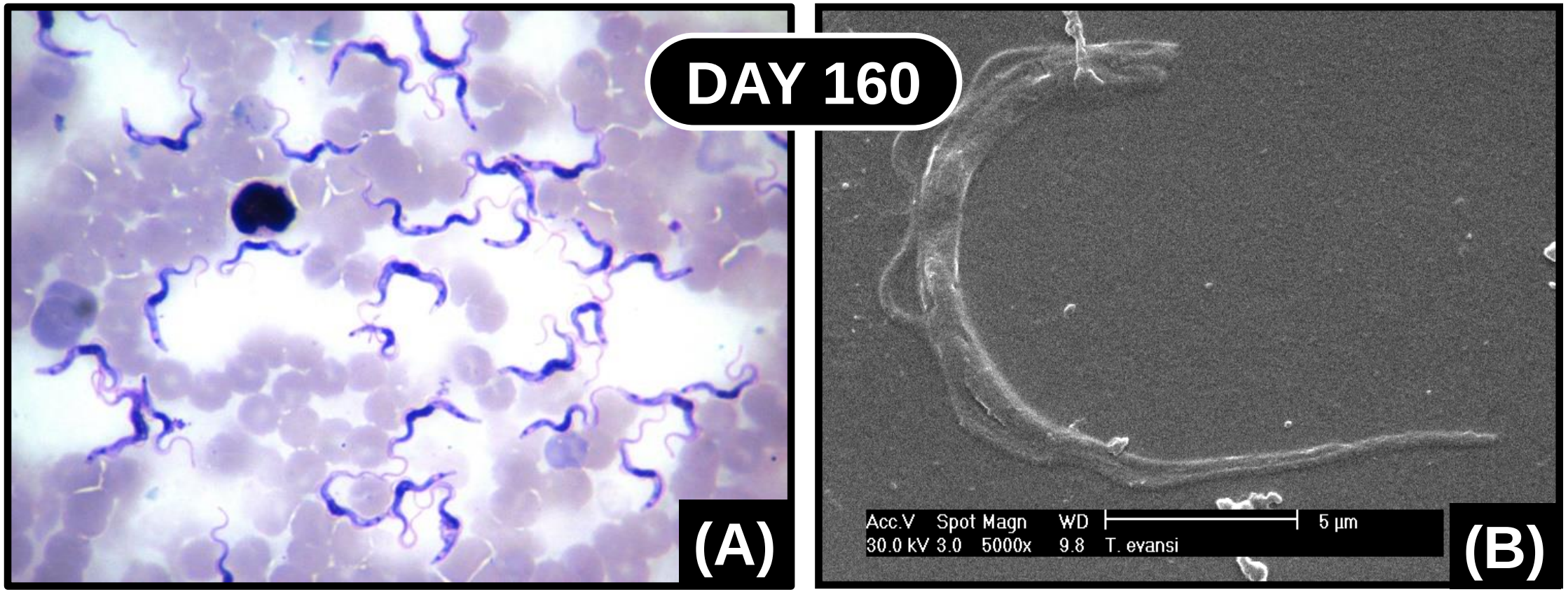
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 100 post-infection as observed under x100 magnification of light microscope (A) and x3866 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 130th Day



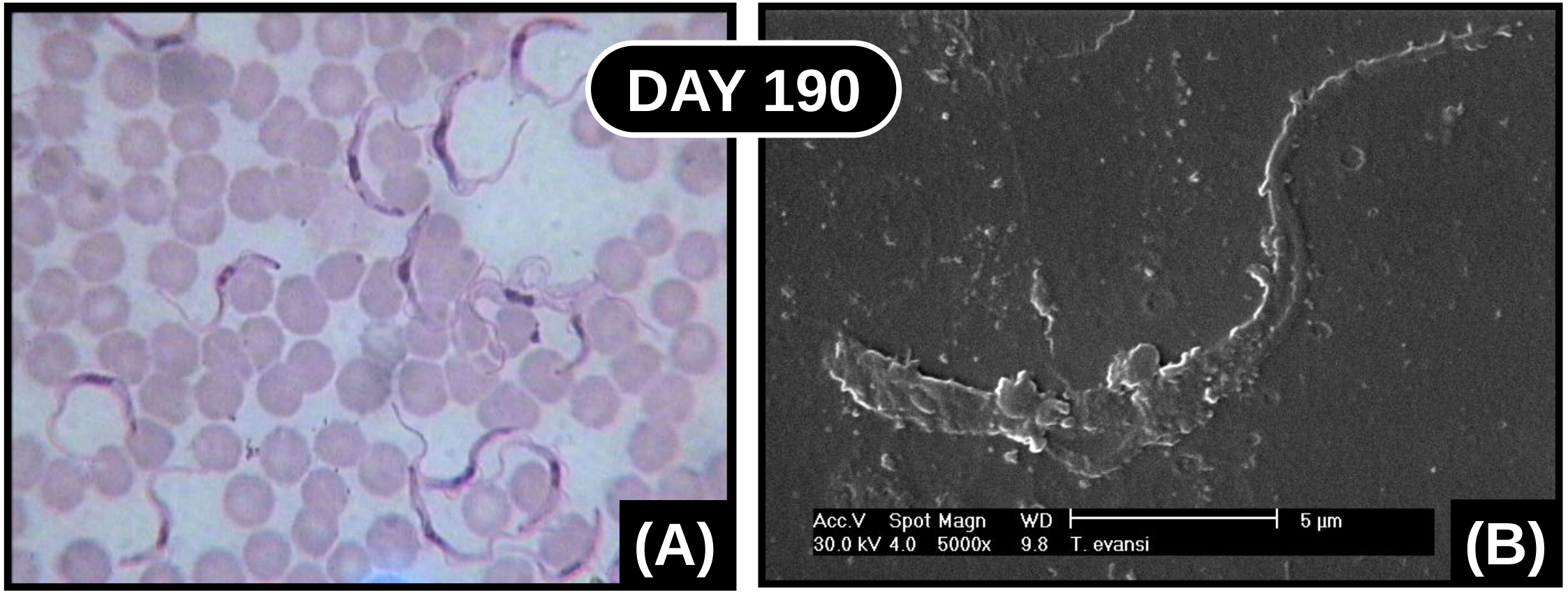
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 130 post-infection as observed under x100 magnification of light microscope (A) and x3866 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 160th Day



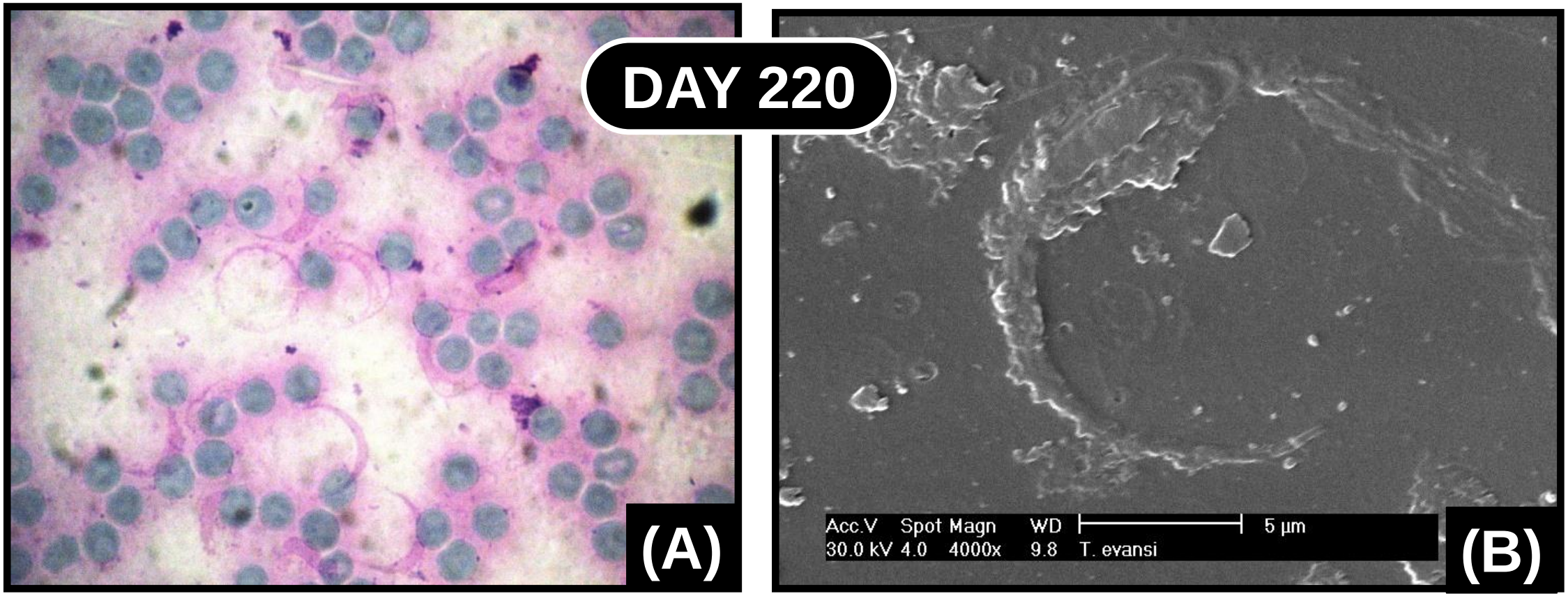
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 160 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 190th Day



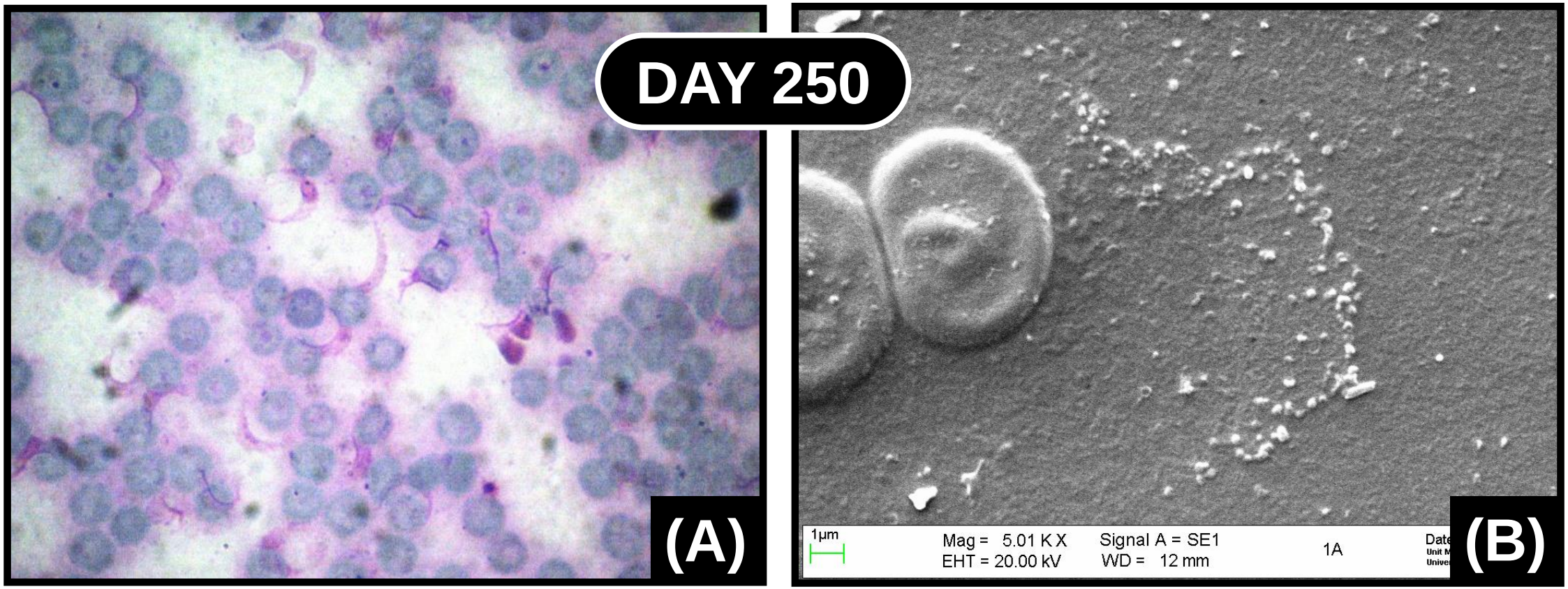
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 190 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 220th Day



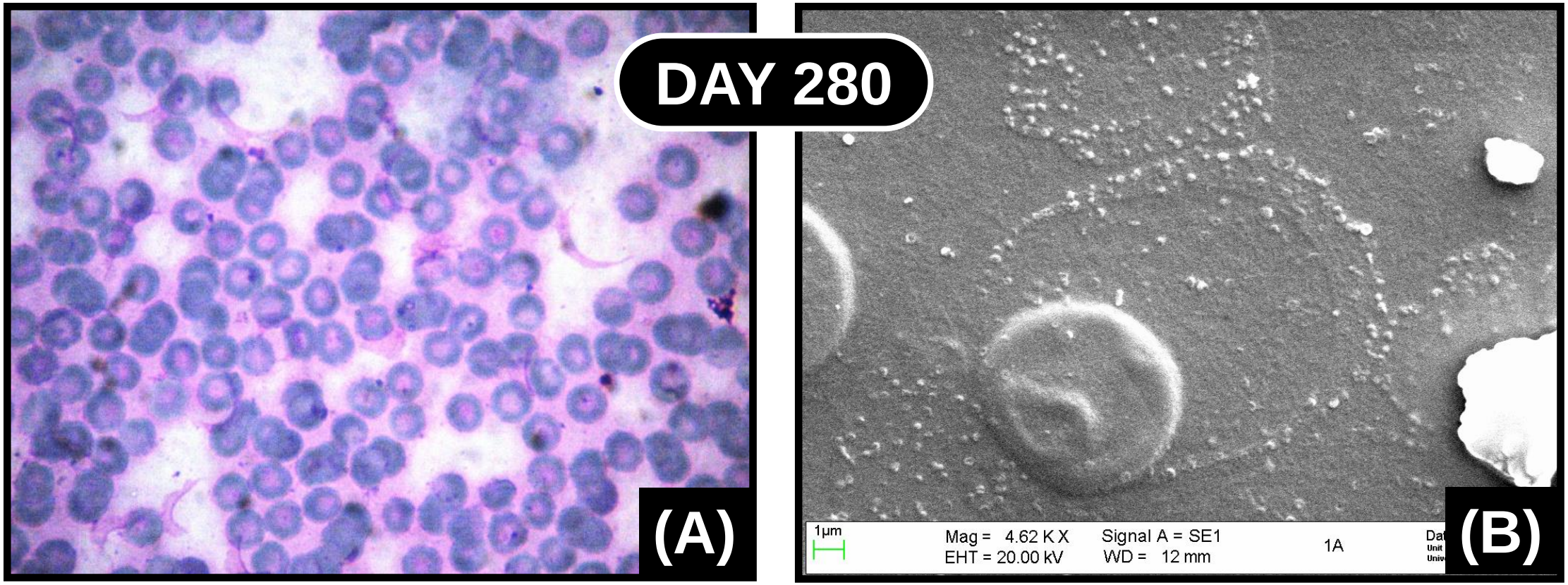
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 220 post-infection as observed under x100 magnification of light microscope (A) and x4000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 250th Day



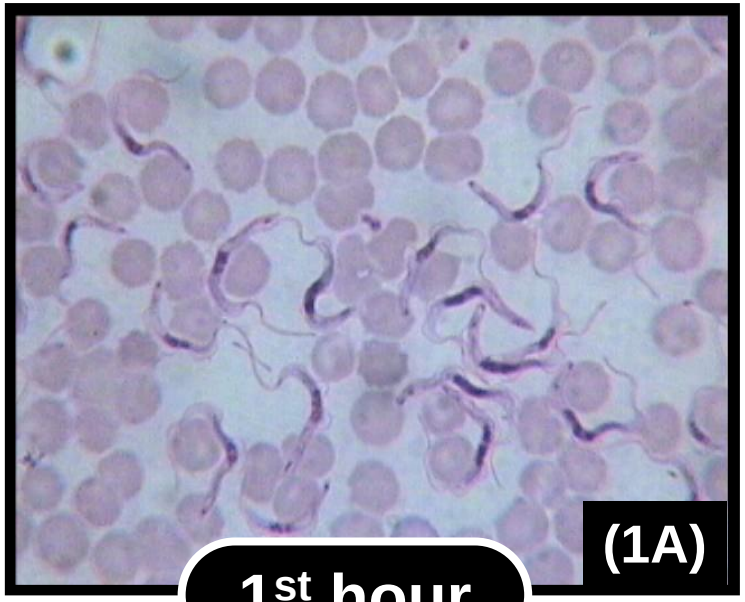
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 250 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Survival In PRE14 Mice Group : 280th Day

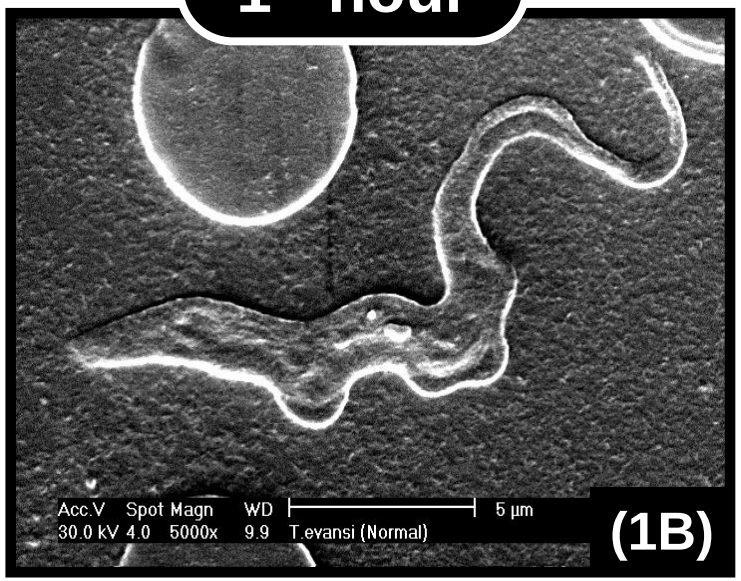


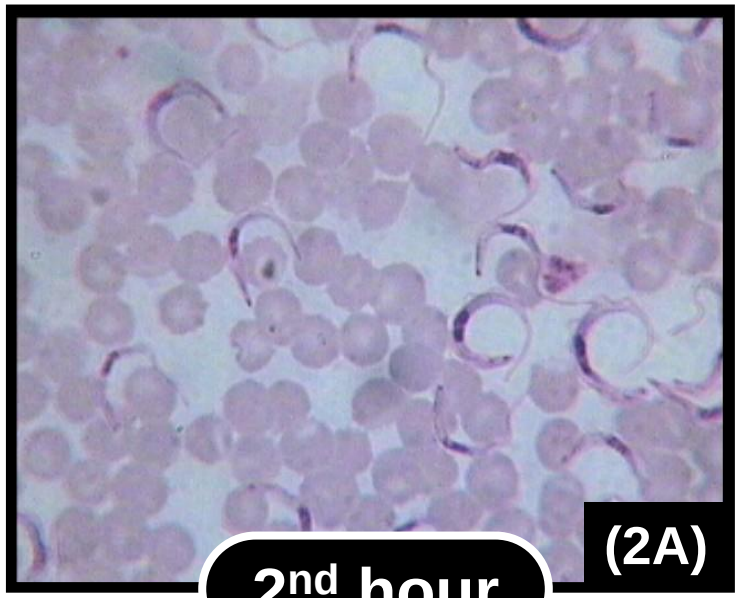
Giemsa thin blood smear of the mice from PRE14 mice group that treated with 0.1 mL 10 mg/kg bw sdH_2O–*P. sarmentosum* extract as observed on day 280 post-infection as observed under x100 magnification of light microscope (A) and x4600 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Growth in Berenil-Treated Group (POS)

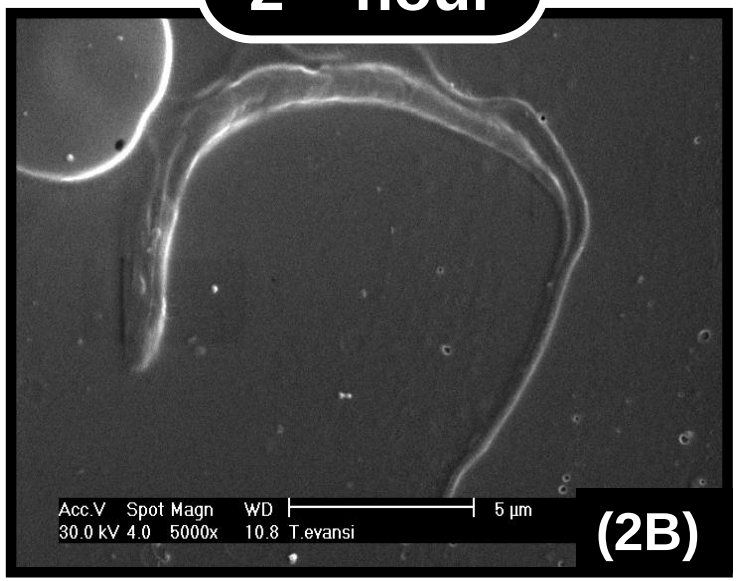


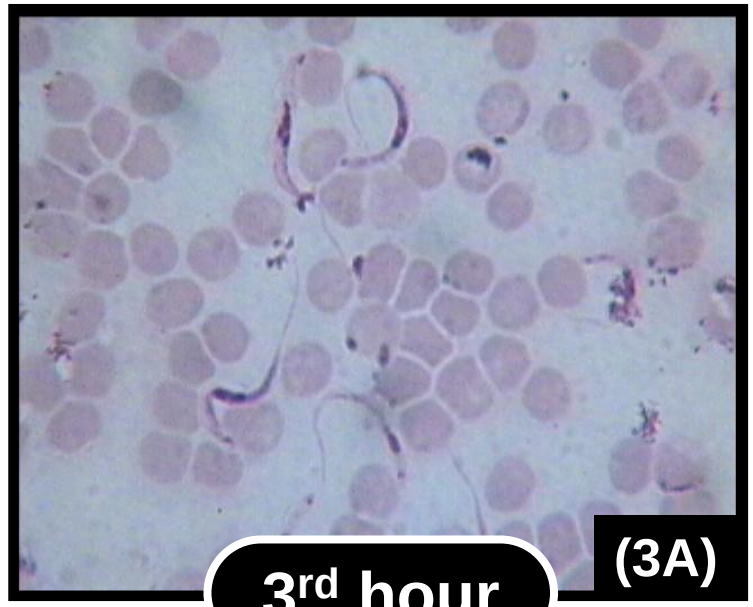
1st hour



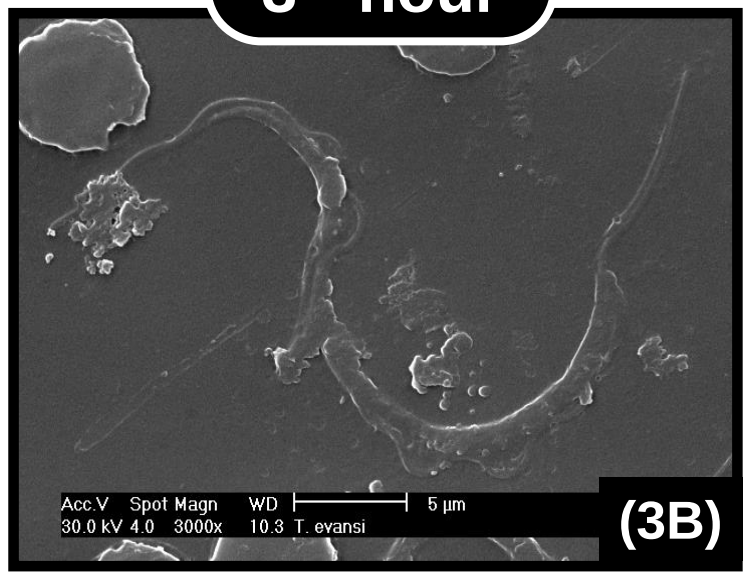


2nd hour

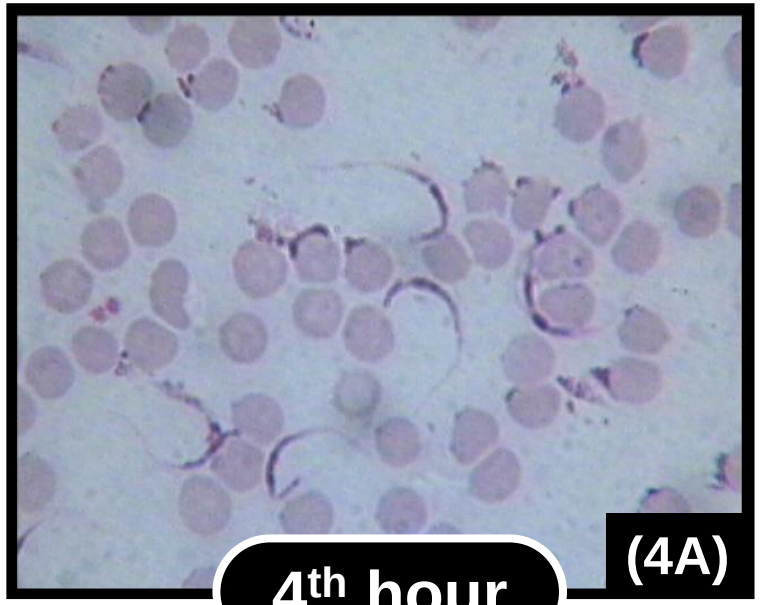




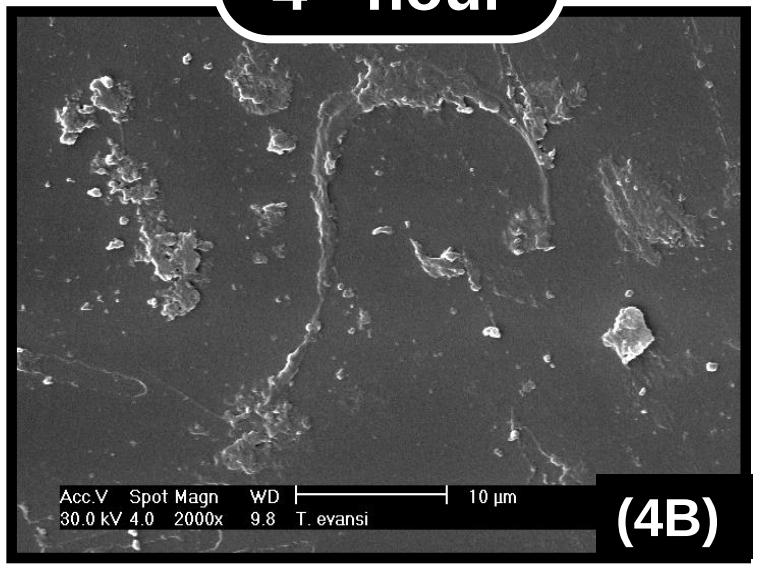
3rd hour

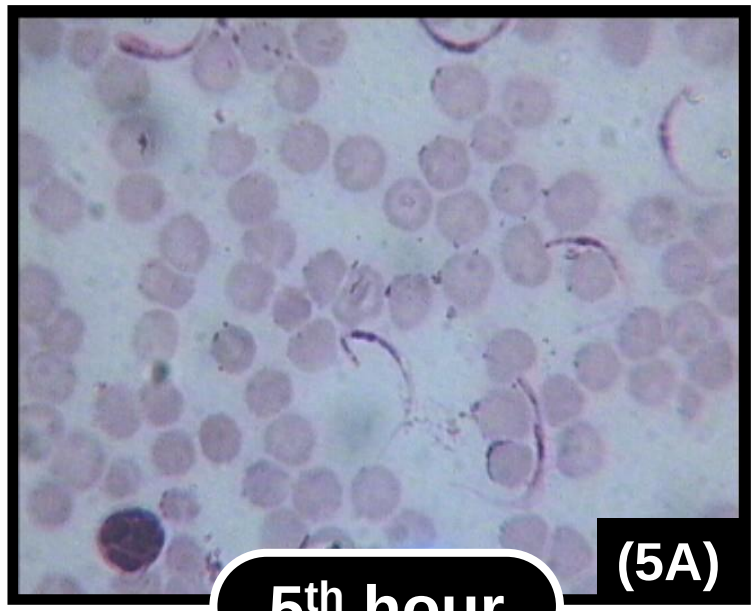


Parasite Growth in Berenil-Treated Group (POS)

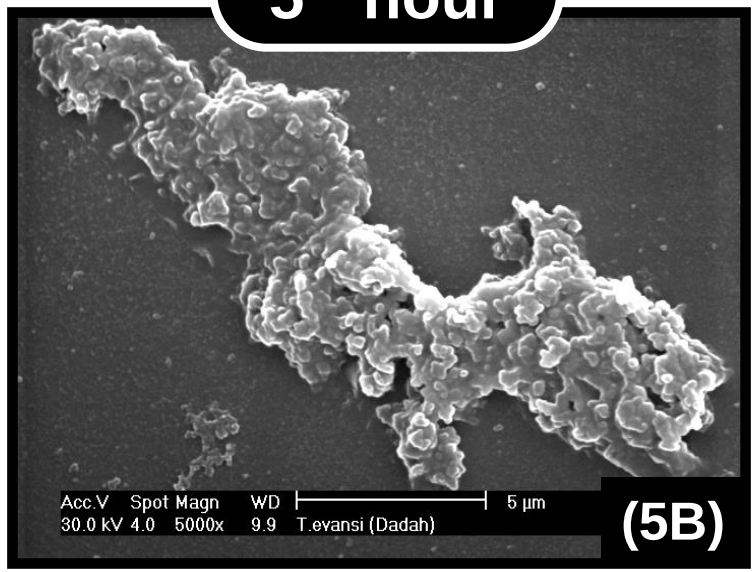


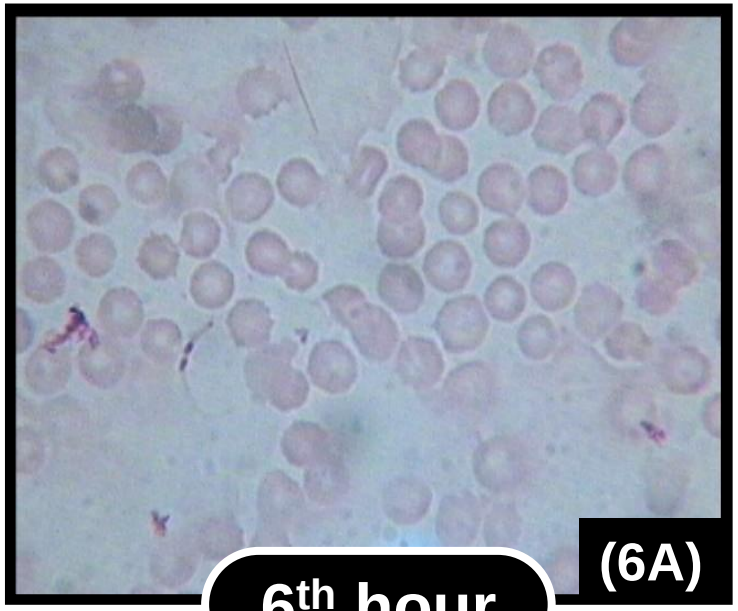
4th hour



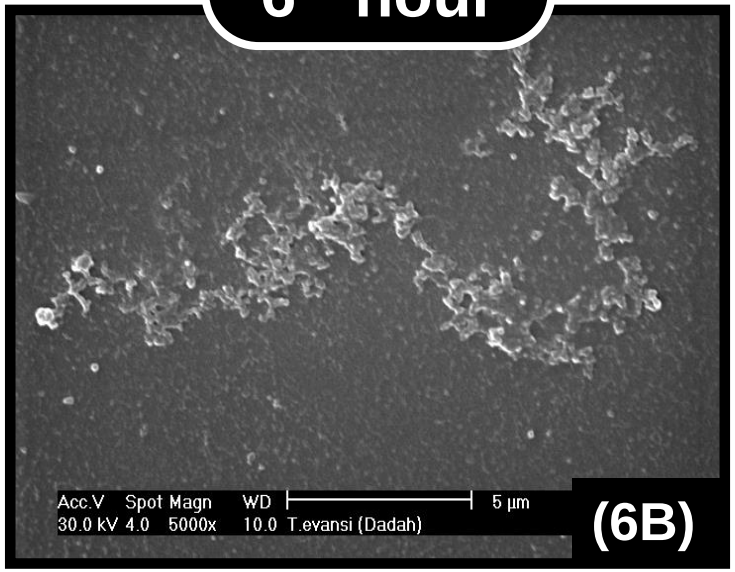


5th hour

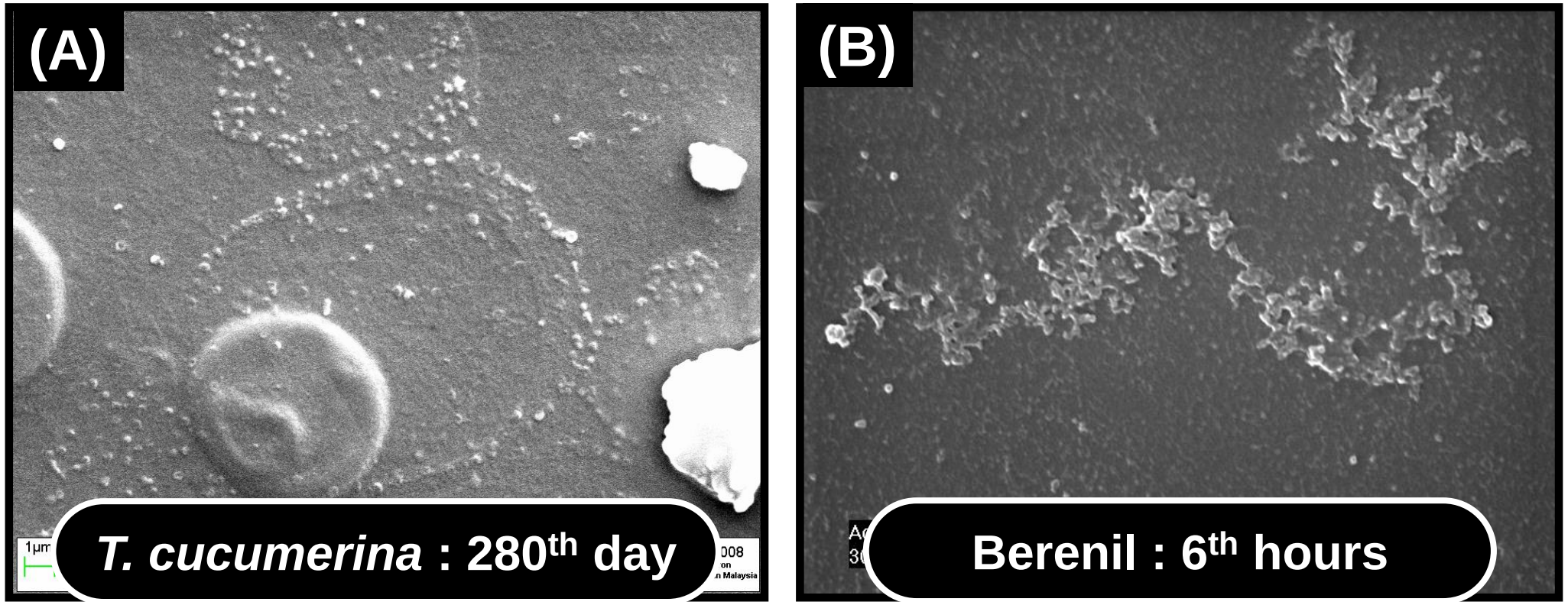




6th hour

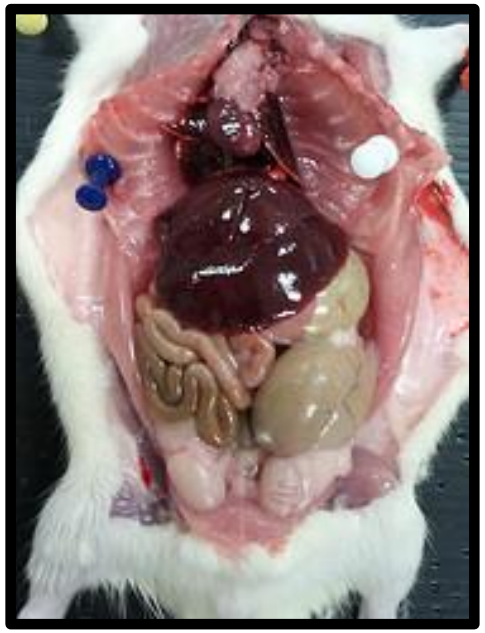


P. Sarmentosum vs Berenil



Scanning electron micrograph showed the morphological changes of *T. evansi* in PRE14 mice (0.1 mL 10 mg/kg bw of sdH_2O-*P. sarmentosum* extract) on 280th 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

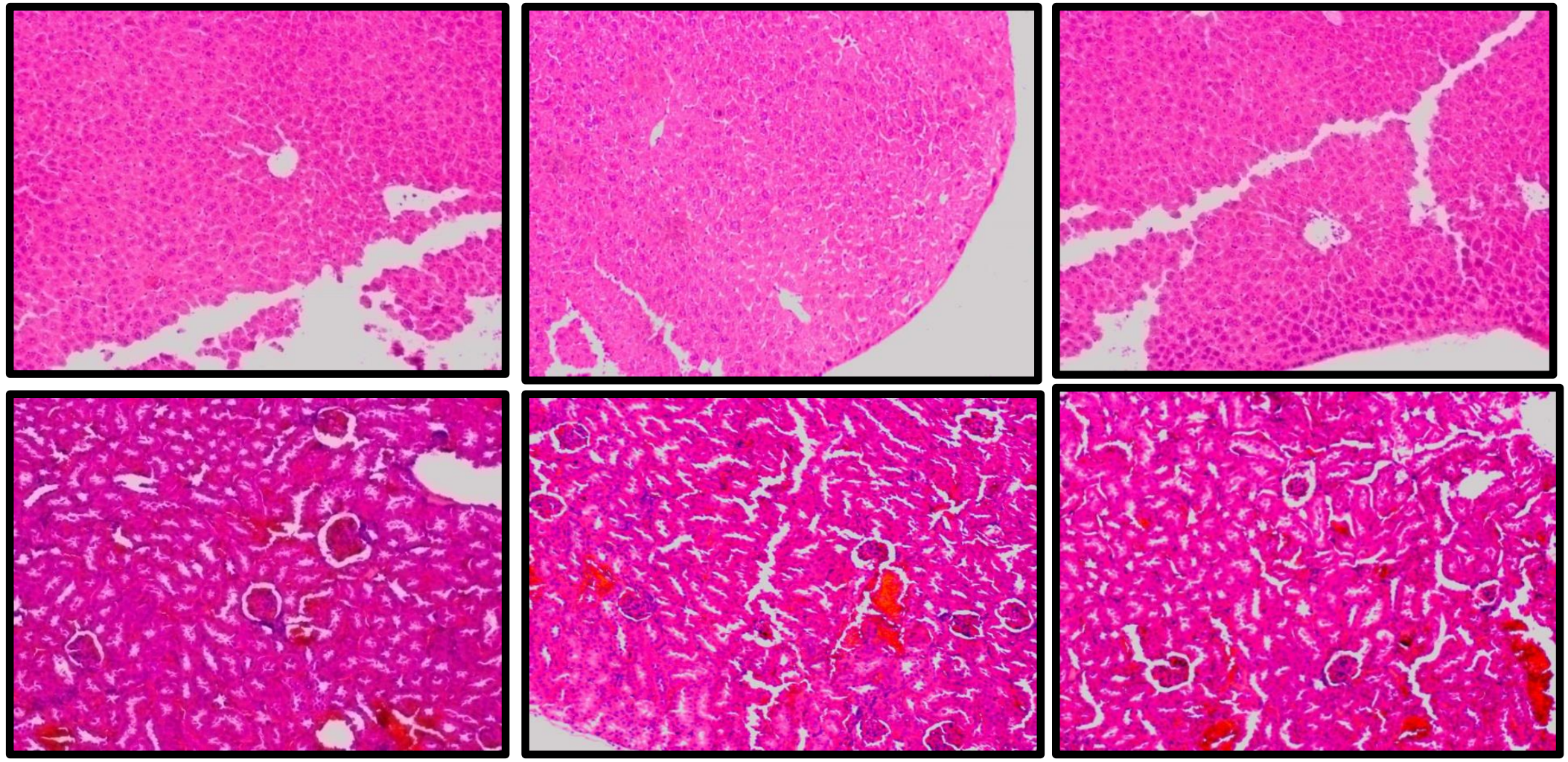


Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	**40 – 93**	IU/L
AST (*)	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	**92 – 206**	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	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 errors (se)*
(*) *All NR values were referred from Research Animal Resources, University of Minnesota, USA*

Organ Histology For Toxicity Assessment



CONCLUSIONS

Hypothesis

- Tryptophol toxin and stochastic genetic modification of VSA is still the best ‘weapon’ for *T. evansi* survival (Otto *et al.* 2010).

- New wave of infection & periodic changes of antigenic variation → changes in parasitemia peaks → longer survival time of the parasite & chronic infection on host (Salleh *et al*. 2009)

- The action of pellitorine ($C_{13}H_{25}ON$) molecule in *P. sarmentosum a*gainst –thiol group of parasite enzymes in which crucial for parasite proliferation (Souza Oliveira *et al*, 2018).

- Bioactive compound of Sarmentamide A in *P. sarmentosum* inhibited the important enzymes (alcohol dehydrogenase, cysteine proteinase and thioredoxin reductase) for the stability of the redox reaction in fungal cells such as *A. fumigatus* & *C. albians* (Tuntiwachwuttikul *et al*., 2006)

Future Plans

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

EAT KADUK..!

NO HARM TO EAT AS MUCH AS YOU CAN

Absolute Hypothesis



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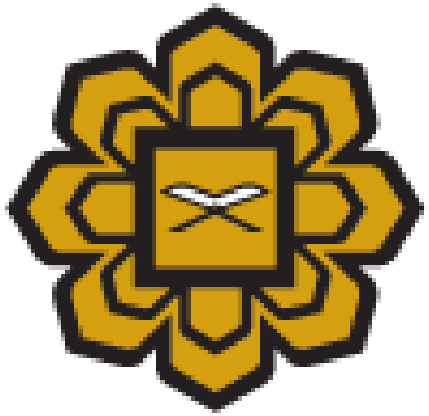

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KEMENTERIAN PENDIDIKAN MALAYSIA

Thank You

Rationale Of The Study

Reliability of Anti-Trypanosomial Drugs

- Resistant issues in India, Thailand & Indonesia
- Unaffordable → expensive in certain regions
- Wrong dosage & concentration → side effects

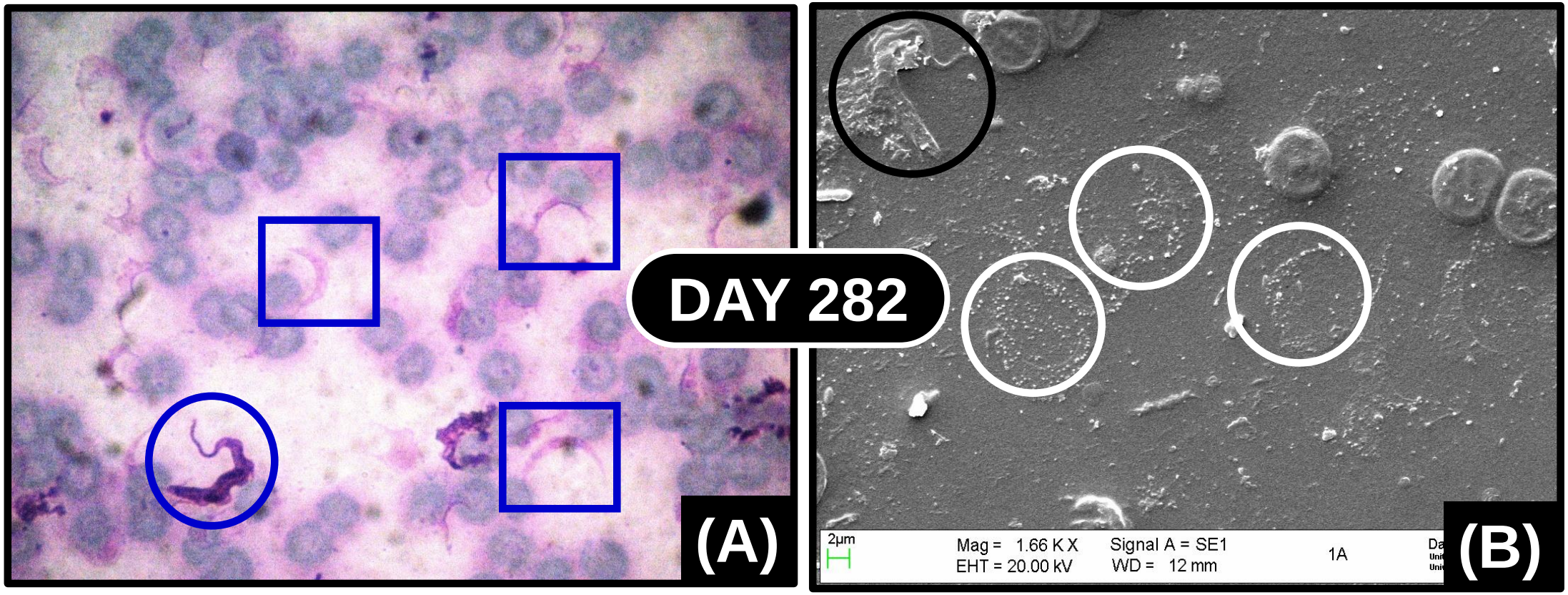
Economic Growth & Biotechnology Sector

- Biotechnology → main focus in the next decade
- Snake gourd → consumable & easily manipulated
- AHT & Surra → influenced productivity of human & livestock

Current Issues of *T. evansi*

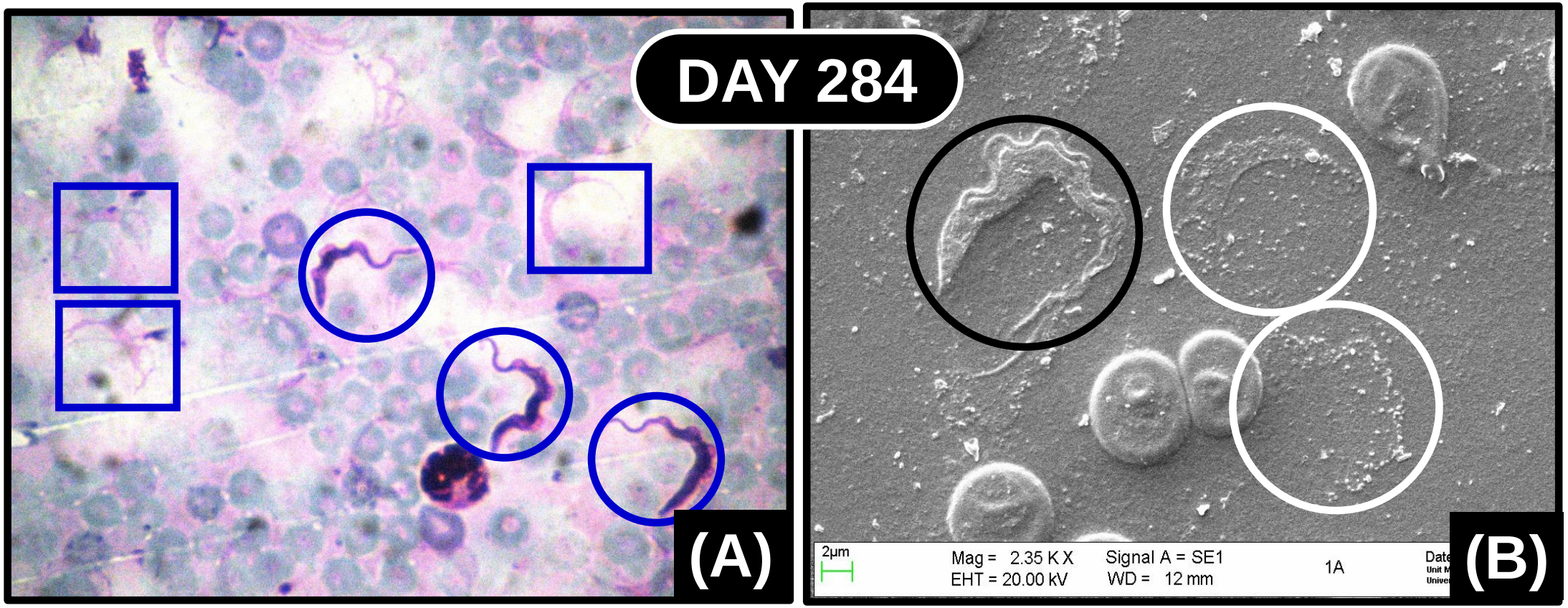
- Trans-host boundary : animal → human (Assam India 2008)

Parasite Survival In PRE14 Mice Group : 282nd Day



Reemerged of *T. evansi* which survived in PRE14 group mice on day 282 due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as observed under x100 magnification of light microscope (A) and x1600 magnification of SEM (Leo 1450VP, Japan) (B).

Parasite Survival In PRE14 Mice Group : 284th Day



Reemerged of *T. evansi* which survived in PRE14 group mice on day 284 due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as observed under x100 magnification of light microscope (A) and x2300 magnification of SEM (Leo 1450VP, Japan) (B). Later the mice died on day 286

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 Glycoprotein (VSG)

- 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