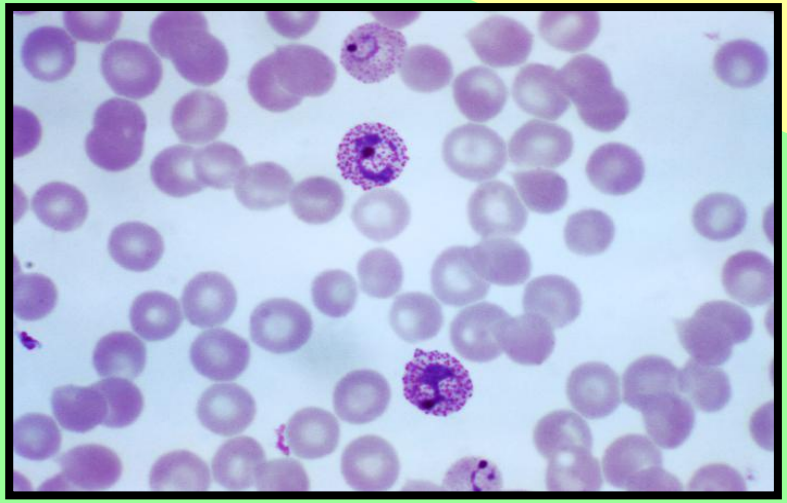
National University of Malaysia

University of Strathclyde Glasgow, UK

الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
Garden of Knowledge and Virtue

The assessment of 3-indole lactate as promising antimalarial agent isolated from endophytic *Streptomyces* SUK10 in *Shorea ovalis*



Mohd Shukri Baba, Noraziah Mohamad Zin and Jalifah Abdul Latip;

mohd_shukri@iium.edu.my

INTRODUCTION

MALARIA

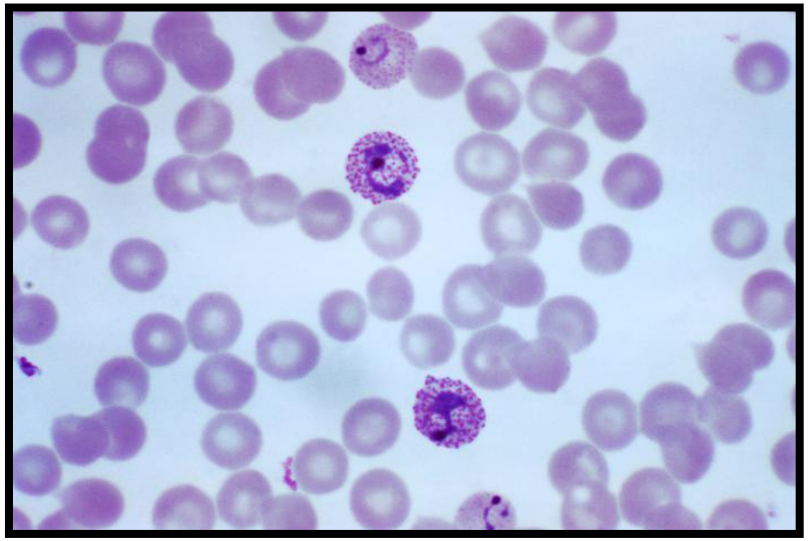
- Most threatening and devastating human parasitic vector-borne disease
- *Plasmodium falciparum* → resistance nearly to all current antimalarial drugs
- Side effects of anti-malarial drugs → sulfadoxin chloroquine, quinine, and pyremethamine
- Increasing in number of malaria cases due to unavoidable factors:

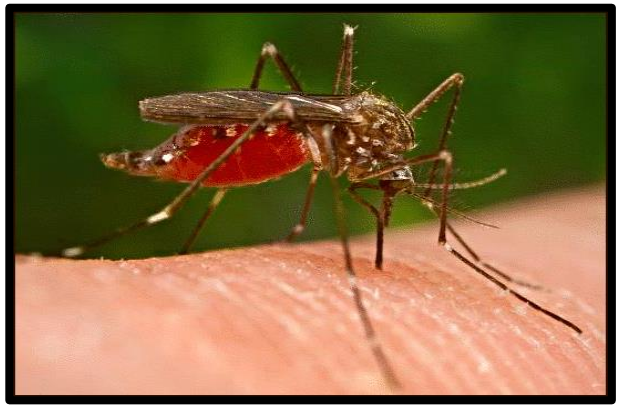
  (a) Wide spread of vector & parasite

  (b) *P. knowlesi* → new emerging spp.

  (c) Uncontrolled immigrant activity.

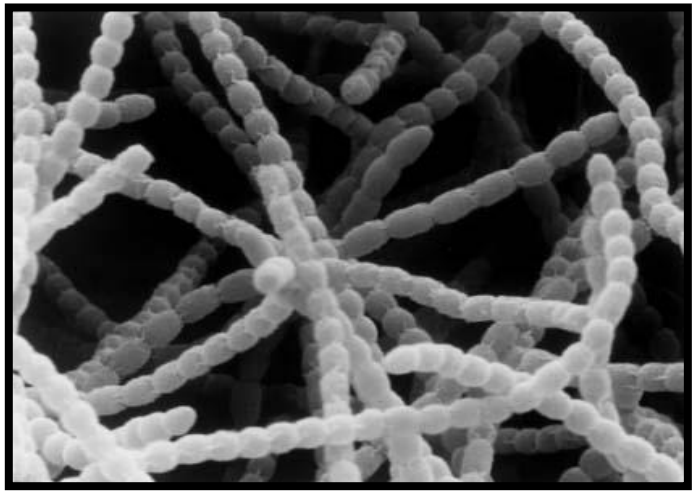
  (d) Antimalarial drug resistance strain





STREPTOMYCES

- Filamentous Gram (+ve) bacteria, high genomic G-C content and spores producer
- 85 % out of 500 species produced 2/3 of today's well known clinical antibiotics such as streptomycin, tetracycline, erythromycin, amphotericin B, ivermectin, chloramphenicol, vancomycin, hygromycin → profits to pharmaceutical company
- Some species living endophytically in the plants or other organisms
- Present study: *Streptomyces* SUK10 living endophytically in internal bark of *Shorea ovalis* tree.



SHOREA OVALIS

Family : Dipterocarpaceae

Also known as:
- Meranti kepong (Malay)
- Meranti merah (Sumatra)
- Damar lampong (Borneo)

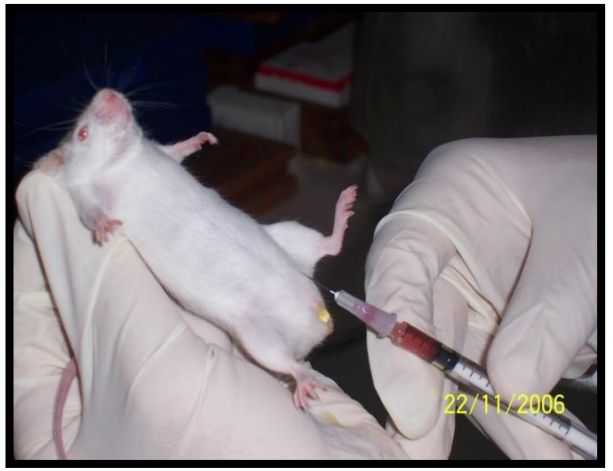
Present study → focused on the bark of *S. ovalis* tree

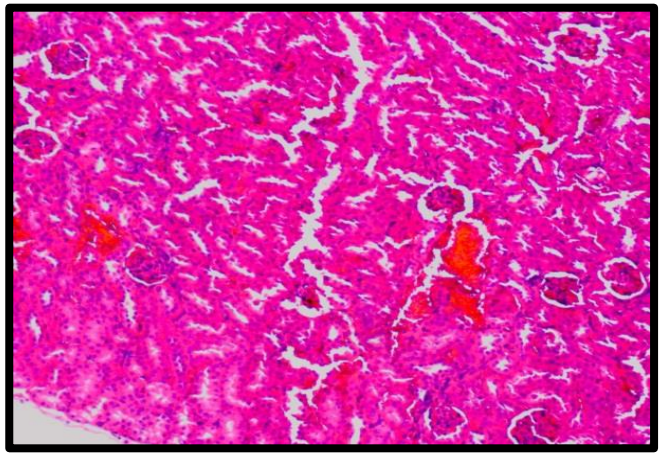
Well-distributed in:
- Malaysia
- Kalimantan
- Sumatra
- Thailand
- Indo-China

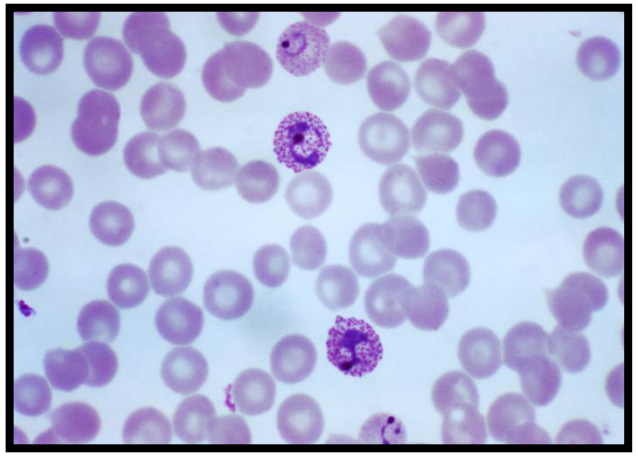
Rich with many compounds with promising bioactivities :
- Triterpenoid → good antifungal (Saraswathy *et al.* 1992)
- Hemsleyanols → agent for chemopreventive (Tanaka *et al.* 2001)
- Hopeaphenol → antidysentery (Subramaniam *et al,* 2015)
- α-Viniferin → cytotoxicity agent (Rohaiza *et al.* 2011)
- Acuminatol → antioxidant (Muhammad *et al.* 2012)
- Vaticanol B → antibacterial activity (Basri *et al.* 2012)

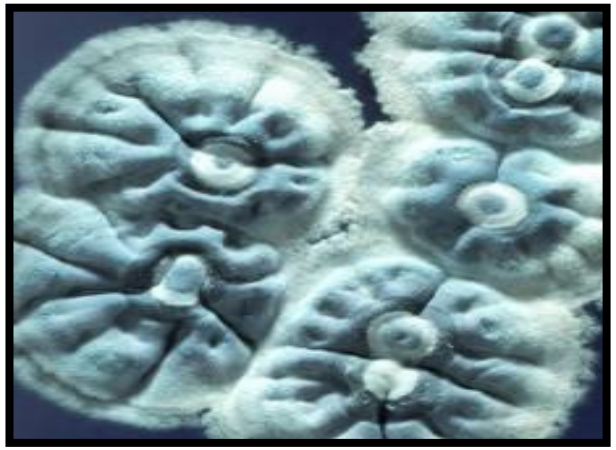
OBJECTIVES

1. To analyze the chemical structure of bioactive compound isolated from endophytic *Streptomyces* SUK10 strain lived in the bark of *Shorea ovalis* tree.
2. To assess the in-vivo antimalarial activity of 3-indole lactate isolated from *Shorea ovalis*
3. To determine the in-vivo toxicity test of 3-indole lactate in mice.





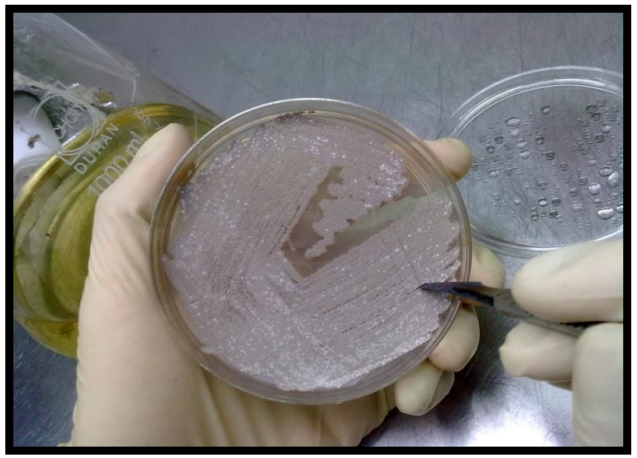




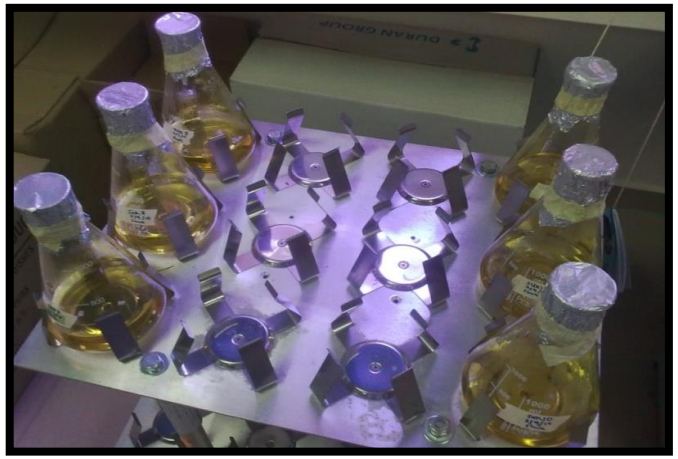
MATERIALS & METHODS

SUK 10 CRUDE EXTRACT PRODUCTION

To produce ethyl acetate-crude extract of *Streptomyces* SUK10



3-5 block of (5x5)mm ISP2 agar enriched with isolates



400 mL NB → on orbital shaker (200 rpm, RT)

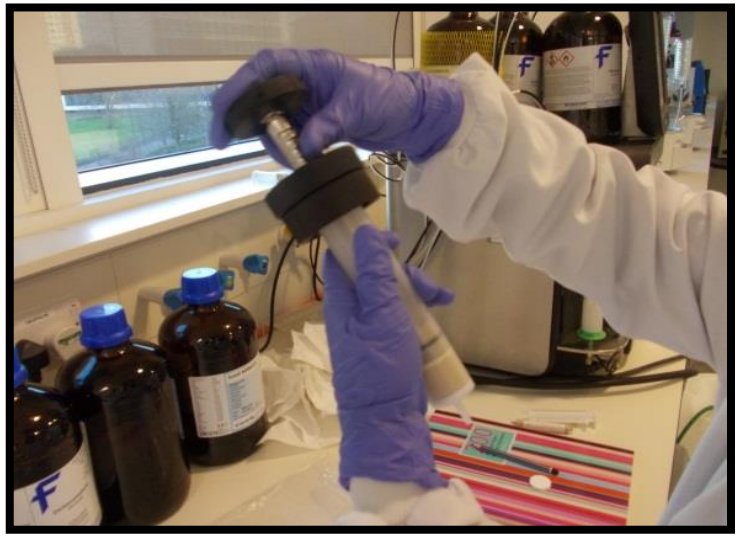
After 7 -14 days

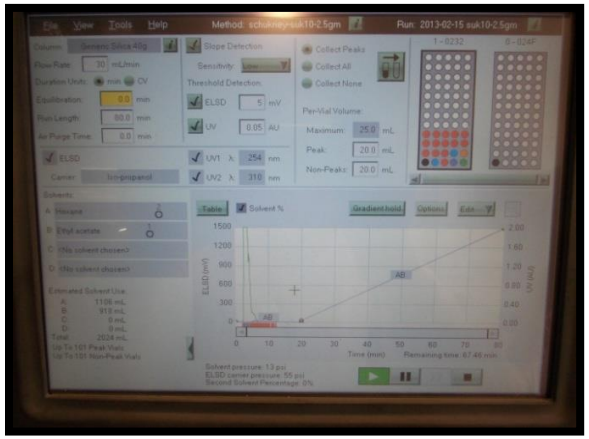
Filtration → extract the supernatant (sp) for 3x with ethyl acetate (EA) (sp : EA = 2:1)

Drying up the organic solvent by rotary evaporator

SUK10 CRUDE EXTRACT FRACTIONATION

- Instrument: Biotage Flash Chromatography (GRACE REVELERIS)
  - Mobile phase : Hexane (A) & Ethyl acetate (B)
  - SUK10 crude extract net weight : 2.8 g
  - Column : Generic Silica 40 g
  - Elution flow rate : 35 mL/minute
  - Run length : 80 minutes
  - UV_1 wavelength absorption : 254 nm
  - UV_2 wavelength absorption : 310 nm

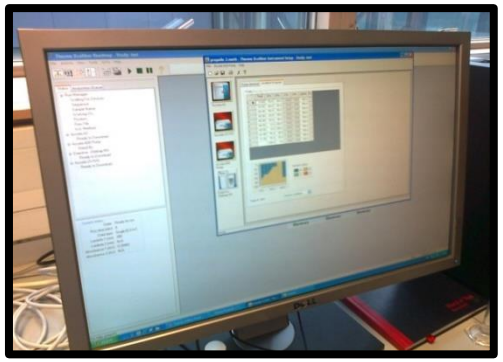


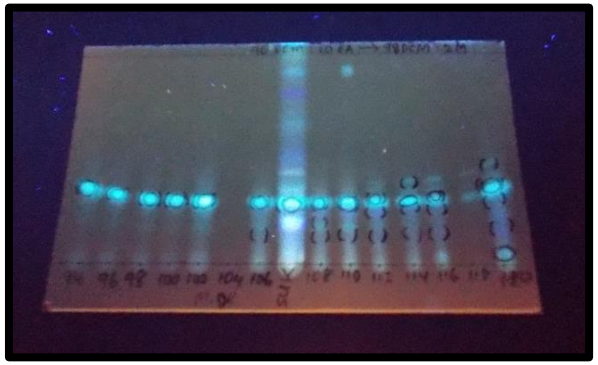


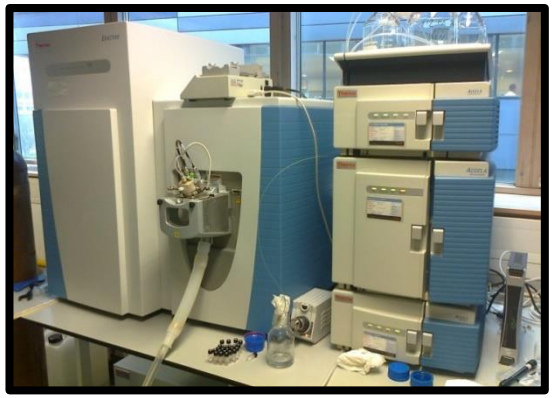
TLC, NMR & HR-LCMS

- NMR Solvent : Chloroform-D
- Test : ^{1}H, C^{13}, HMBC, HMQC, Depth, COSY (400Mz) & ROESY (600Mz)
- All samples were sent for HR-LCMS
- Info obtained : RT, molecular formula & weight and compound name & data
- Comparison : AntiBase Library (Version 2013)

- TLC solvent system :
  - 70-H : 30-EA (V1 – V3)
  - 80-H : 20-EA (V4 – V10)
  - 40-H : 60-EA (V11 – V18)
  - 98-DCM : 2-M (V19 – V22)

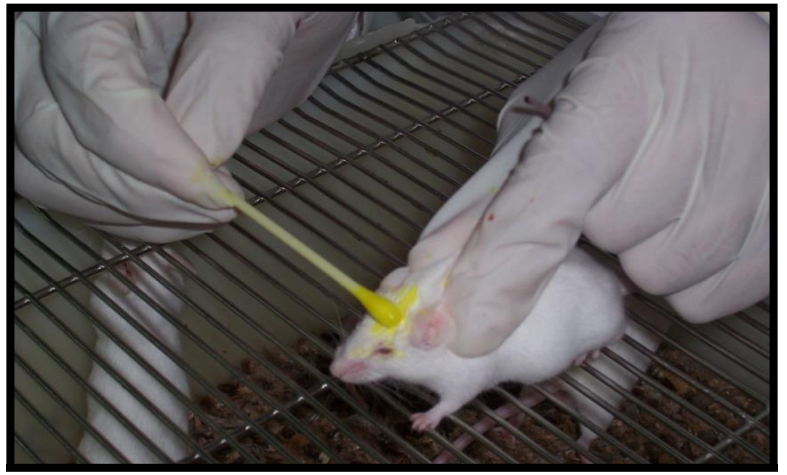


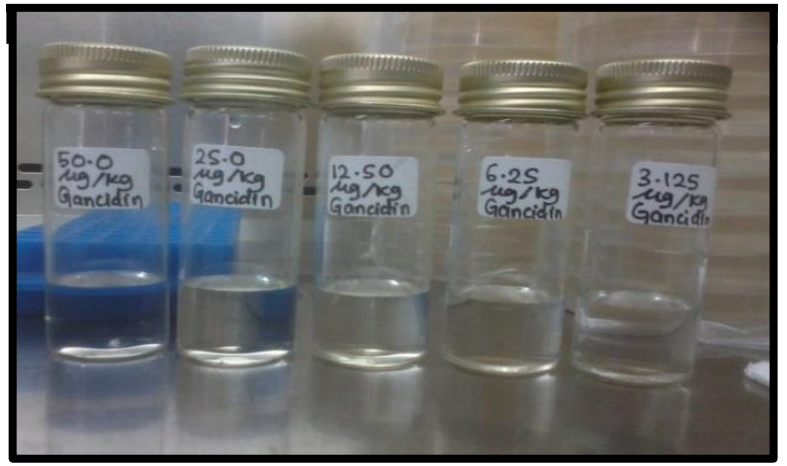




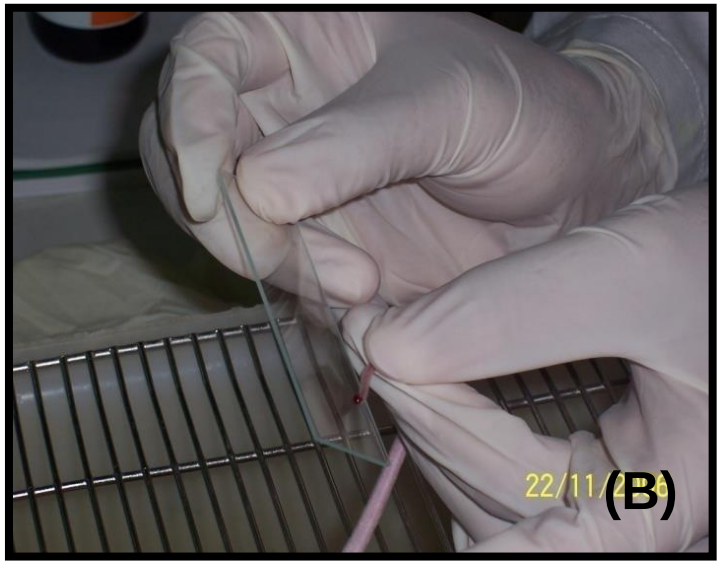
ANTIMALARIAL ACTIVITY OF 3-INDOLE LACTATE

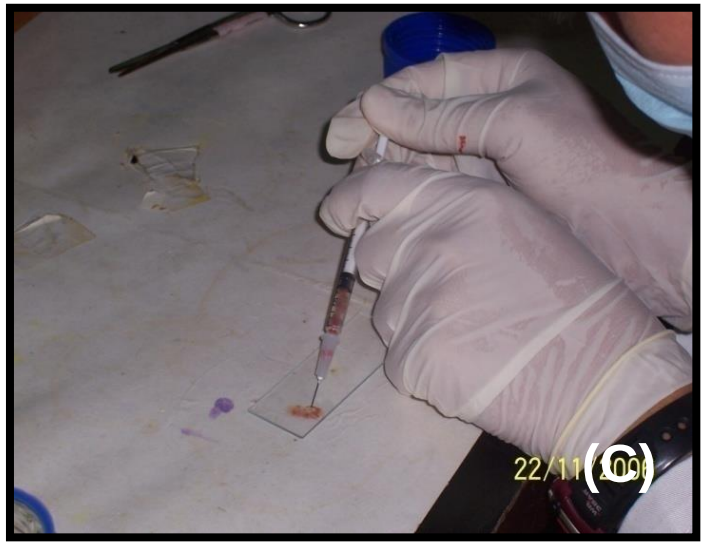
- Parasite : *Plasmodium berghei* NK65
- Mice : ICR / ♂ / 25-30 g / 6-8 weeks / *ad-libitium* feed
- 4D suppression test
  - ~ Parasitemia density (%)
  - ~ Parasitemia inhibition (%)
  - ~ Survival time (day)
- Concentration (doses of 0.1 ml)
  - ~ 50 µl/kg bw (1x)
  - ~ 25 µl/kg bw (1/2x)
  - ~ 12.5 µl/kg bw (1/4x)
  - ~ 6.25 µl/kg bw (1/8x)
  - ~ 3.125 µl/kg bw (1/16x)
- (+ve) control : 0.1 ml of 10 mg/kg dH_2O diluted CH
- (-ve) control : 0.1 ml of 0.9% normal saline

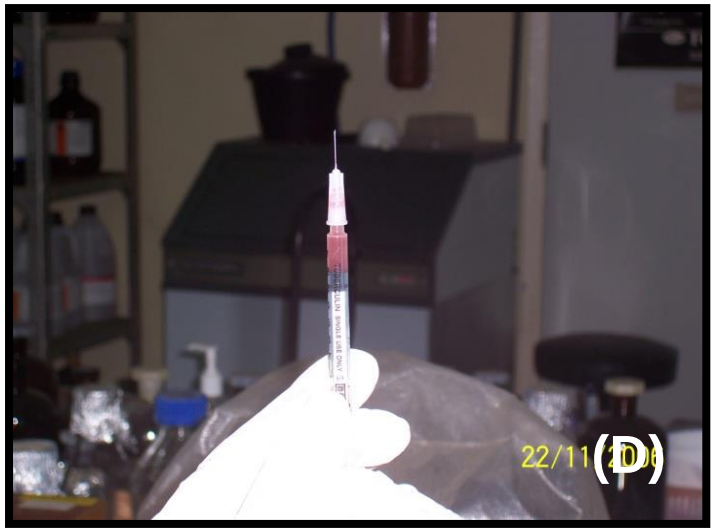


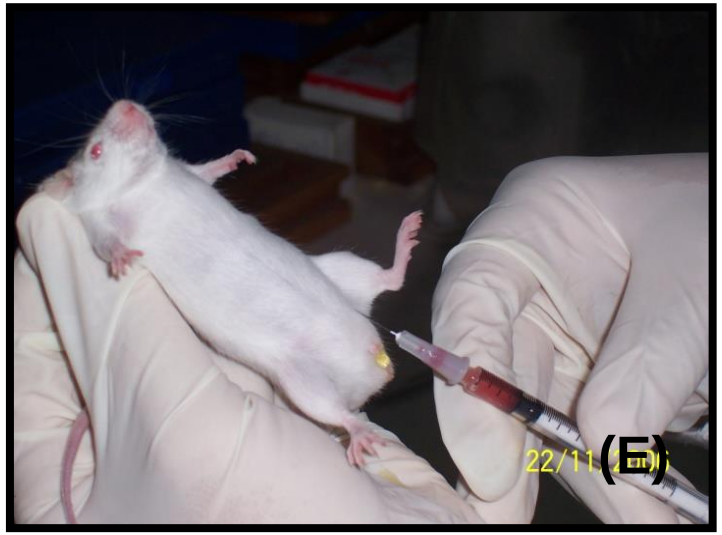


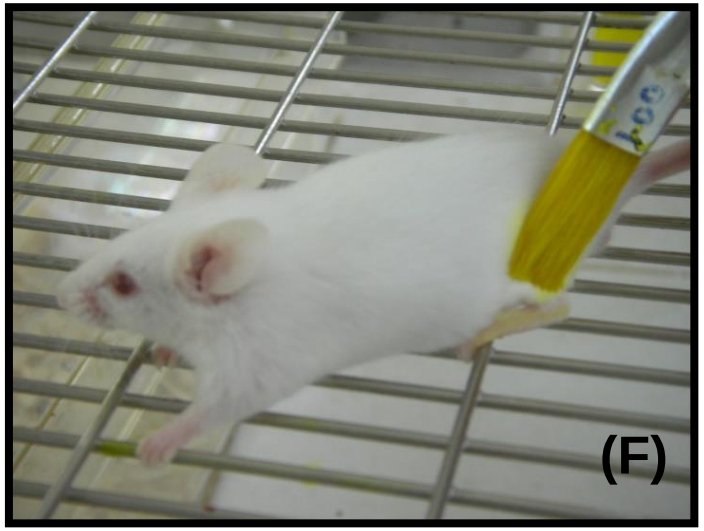
PARASITE ADMINISTRATION AND ANIMAL TAGGIING





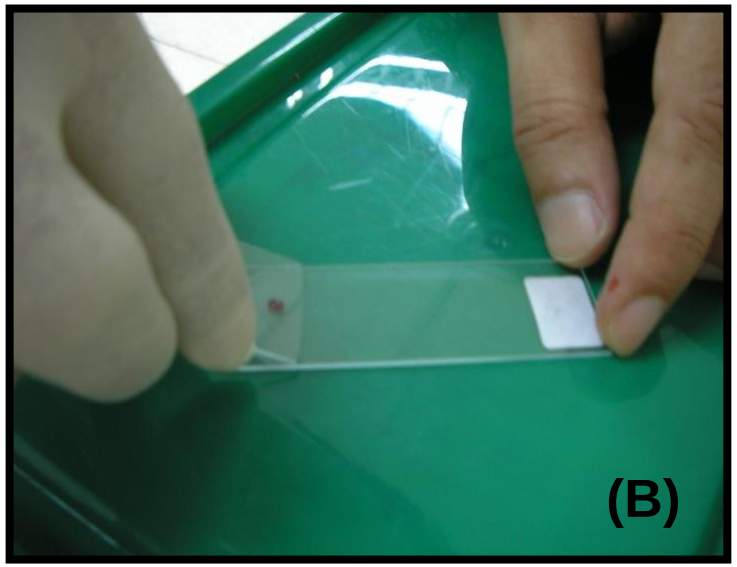






GIEMSA STAINING AND MICROSCOPIC OBSERVATION


(A)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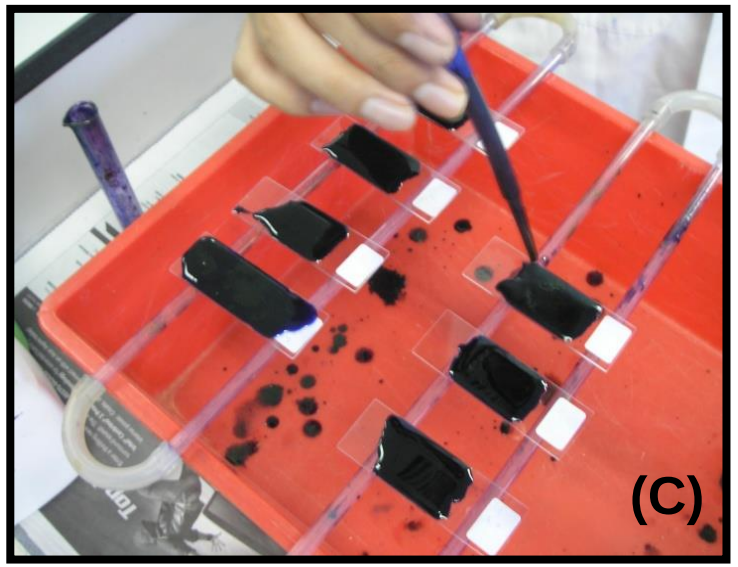
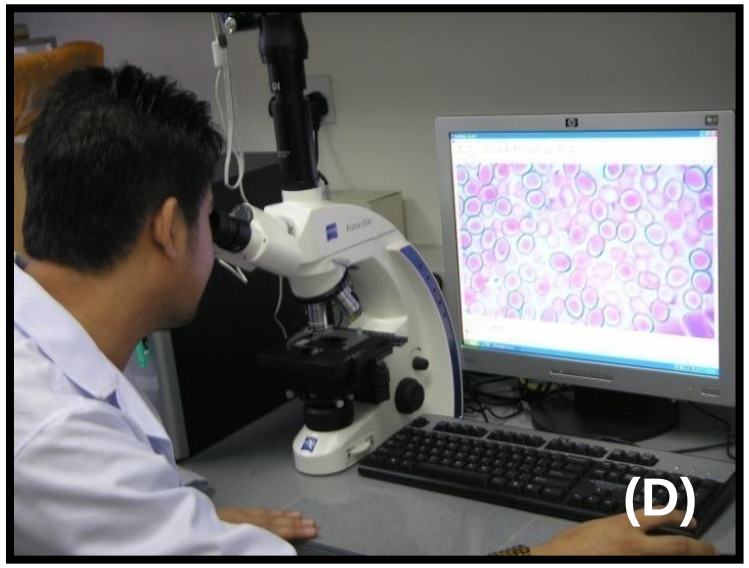
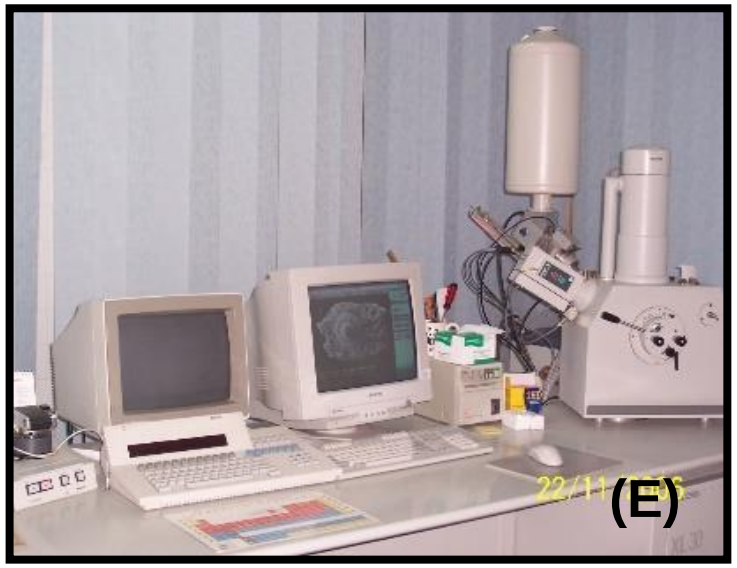
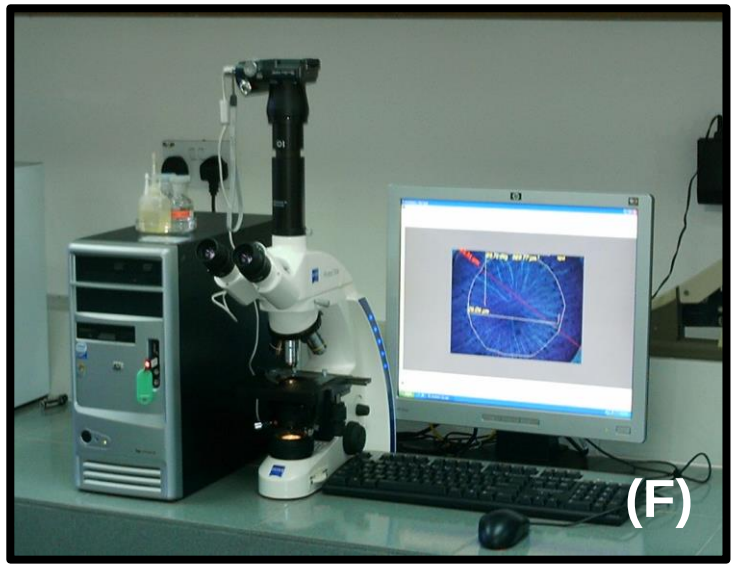
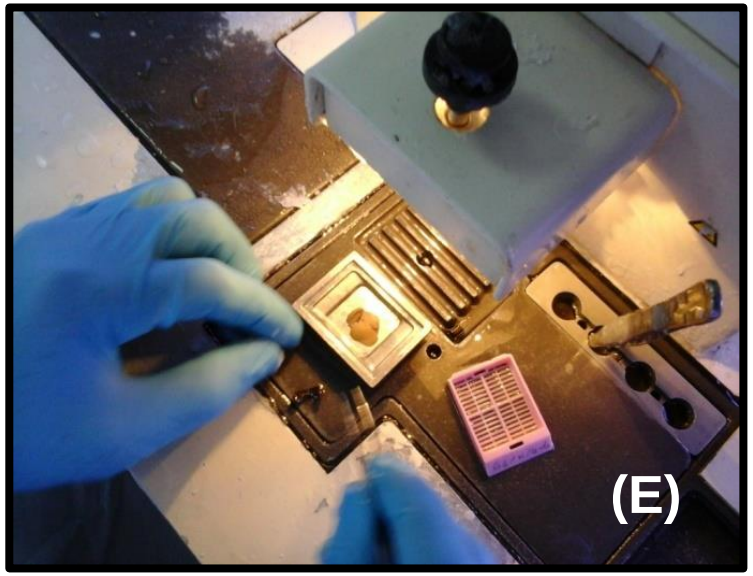

(C)


(D)


(E)


(F)

BIOCHEMICAL TOXICITY TEST AND ORGAN HISTOLOGY



RESULTS & DISCUSSIONS

TLC

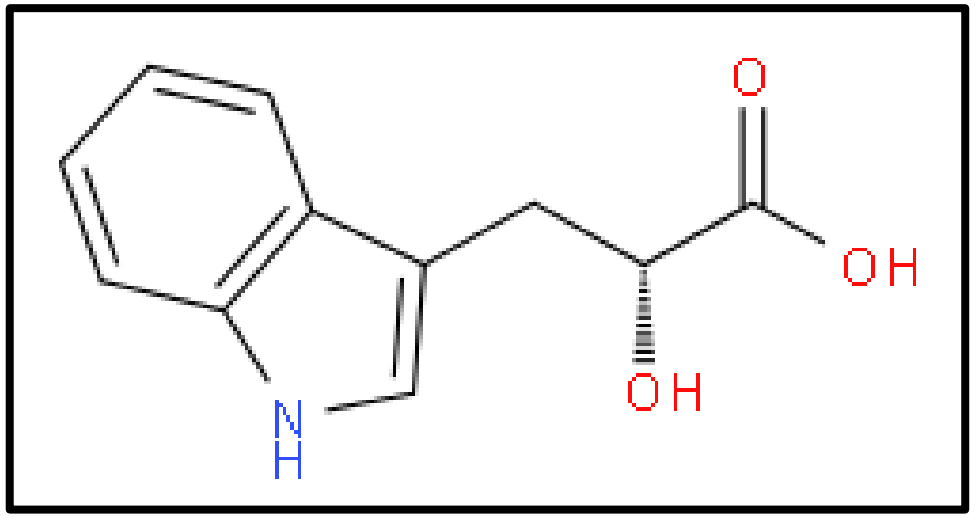
22 combine fractions out of 123 fractions number were obtained and labeled as V1 – V22

Solvent system	Vial label	Fraction number	Nett weight (g)
(70-H : 30-EA)	V1	2 – 10	0.1106
	V2	11 – 15	0.0271
	V3	16 – 25	0.8132
	V4	26 – 30	0.1255
	V5	31 – 36	0.0890
(80-H : 20-EA)	V6	37 – 42	0.0393
	V7	43 – 48	0.0162
	V8	49 – 55	0.0185
	V9	56 – 63	0.0125
	V10	64 – 71	0.0301
(40-H : 60-EA)	V11	72 – 75	0.0249
	V12	76 – 81	0.0287
	V13	82 – 86	0.0283
	V14	87 - 92	0.0191
(90-DCM : 10-M)	V15	93 – 98	0.0162
	V16	99 – 100	0.0113
	V17	101 – 103	0.0276
	V18	104	0.0082
	V19	105 – 110	0.0300
	V20	111 – 116	0.0498
	V21	117 – 119	0.0142
	V22	120 – 123	0.0184

NMR & HR-LCMS

- NMR spectrum analysis :
  - V3 = V4
  - V5 = V6
  - V7 = V8
  - V12 = V13
  - V15 = V22

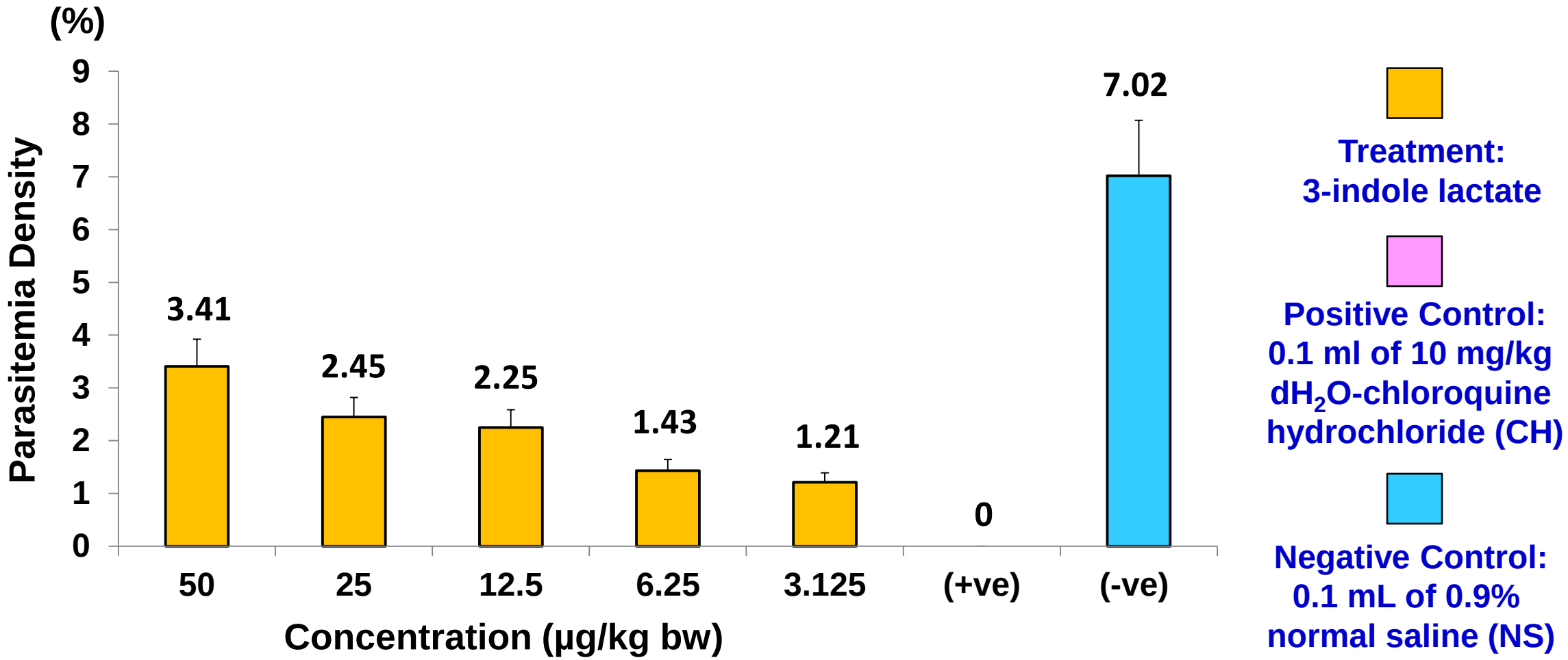
3-indole lactate
(indole-3-lactic acid)
Fraction V11
($C_{11}H_{11}NO_3$)



- Structure analysis :
  - V2 to V8 = long carbon chain (triglyceride fatty acids)
  - V11 = promising pure compound based on:
    - ^{1}H NMR analyses
    - promising net weight (g) in TLC
    - interesting signals/peaks (corresponding to major constituent)
  - V11 structure = 3-indole lactate or indole-3-lactic acid ($C_{11}H_{11}NO_3$)

ANTIMALARIAL ACTIVITY OF 3-INDOLE LACTATE

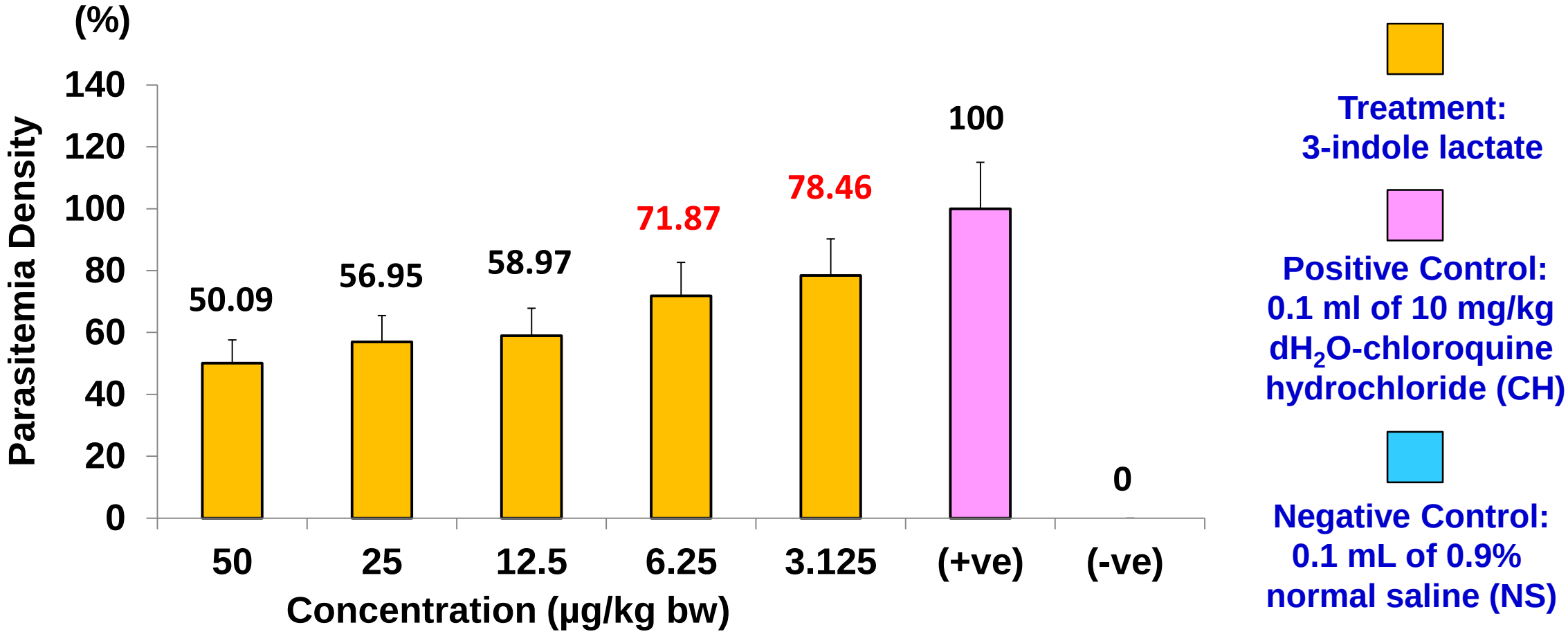
Parasitemia density (%) of the mice treated with 3-indole lactate on D4 post-infection at five different concentration (µg/kg bw)



ANTIMALARIAL ACTIVITY OF 3-INDOLE LACTATE

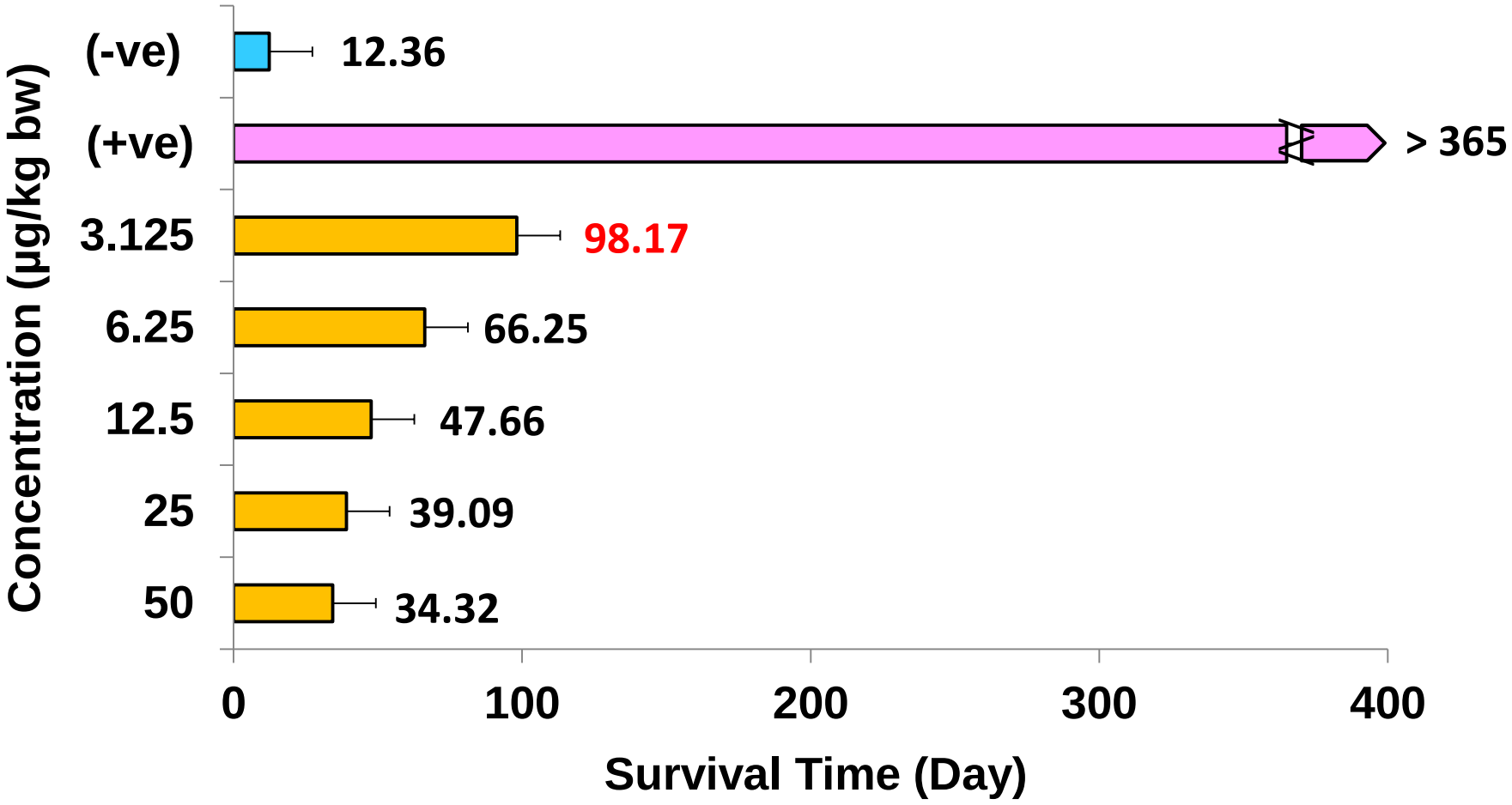
Inhibition Rate (%) of the mice treated with 3-indole lactate on D4 post-infection at five different concentration (µg/kg bw)

(*) *Inhibition rate at >70 % is considered as having antimalarial activity*



ANTIMALARIAL ACTIVITY OF 3-INDOLE LACTATE

Survival Time (days) of the mice treated with 3-indole lactate on D4 post-infection at five different concentration (µg/kg bw)

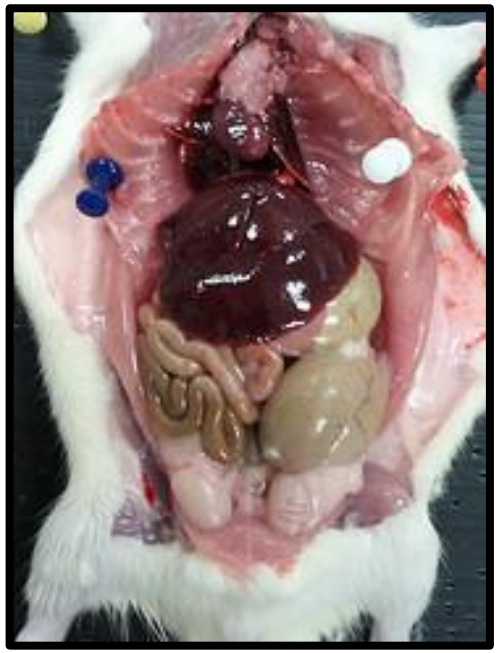


Treatment: 3-indole lactate

Positive Control: 0.1 ml of 10 mg/kg dH_2O-chloroquine hydrochloride (CH)

Negative Control: 0.1 mL of 0.9% normal saline (NS)

BIOCHEMICAL TEST FOR TOXICITY ASSESSMENT

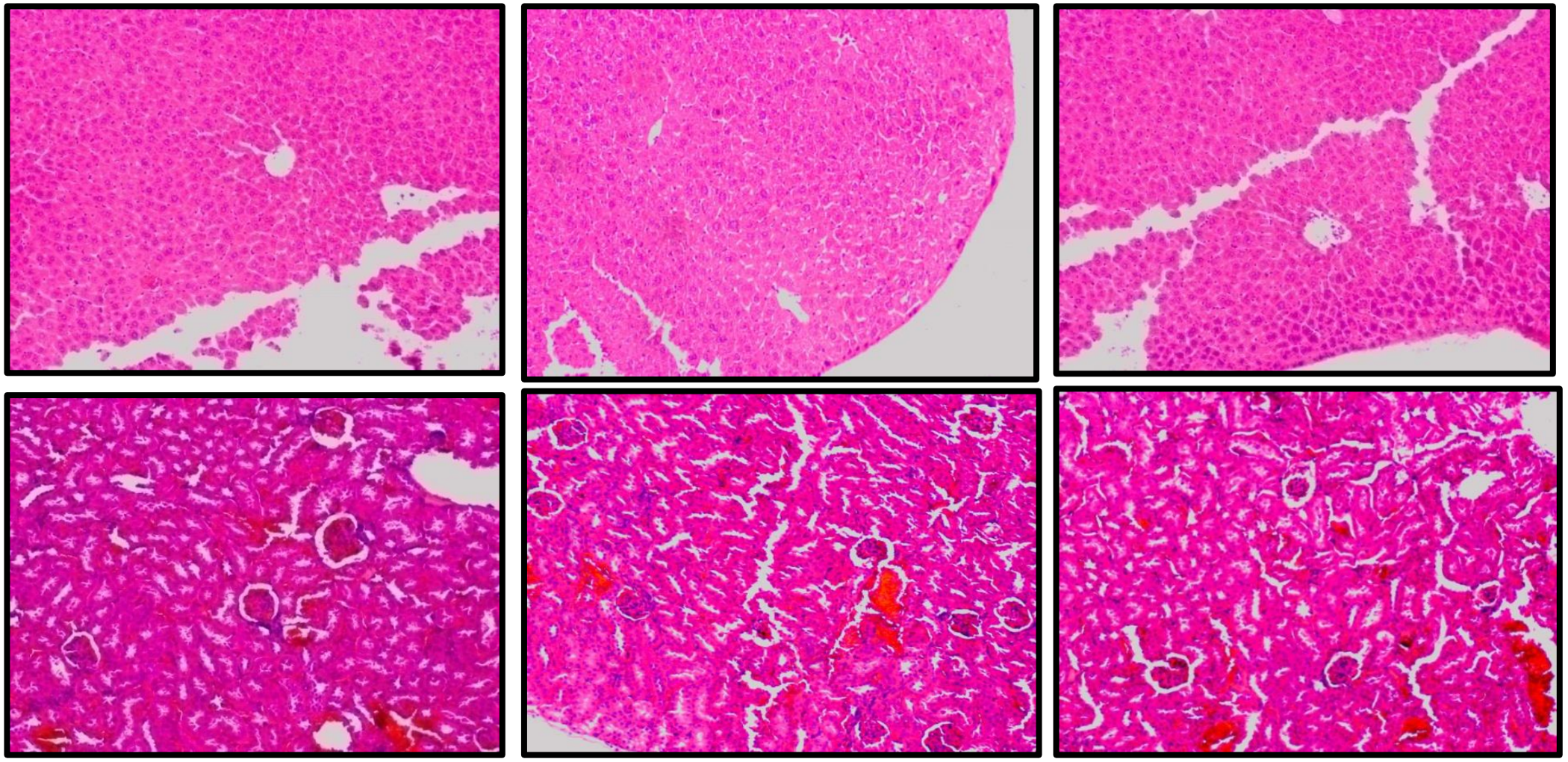


Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	**40 – 93**	IU/L
AST (*)	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	**92 – 206**	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10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

CONCLUSION

- 50% of the mice treated at 3.125 µg kg^{-1} bw 3-indole lactate were survive up to ≥100 days post-infection → one quarter of normal mice life span (Sage et al. 2010; Szenczi et al. 2012).
- No toxicity effects were discovered on the hosts treated with 3-indole lactate at all concentrations
- Naturally, indole lactate produced by many endophyte microorganisms and displayed a wide diversity of structures and biological functions (Samaoui et al. 2012) → making 3-indole lactate as useful chemical entity for the discovery and development of new drugs.
- 3-indole lactate derivatives were already demonstrated to have a good biological activities → antibacterial, antiviral, antitumor, fungicidal, herbicidal , etc.. (Magyar et al. 1999).
- Although 3-indole lactate was previously reported from other *Streptomyces* sp. (Rhee 2002, Ben Ameur Mehdi et al. 2004, Bin et al. 2009), its antimalarial properties has not been revealed.

RECOMMENDATION

FUTURE WORKS

1. Identifying biosynthesis pathways of 3-indole lactate in *Streptomyces* gene cluster
2. Determine the mechanism of action of 3-indole lactate
3. Play around with different concentrations of 3-indole lactate to determine therapeutic dosage
4. Consider curative and preventive regimes of the treatment
5. In-vitro antimalarial activities of the compound
6. Screening towards any other MDR agents (*M. tuberculosis*, MRSA, VRSA, etc..)

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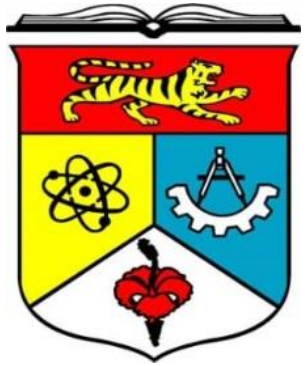
ACKNOWLEDGEMENT



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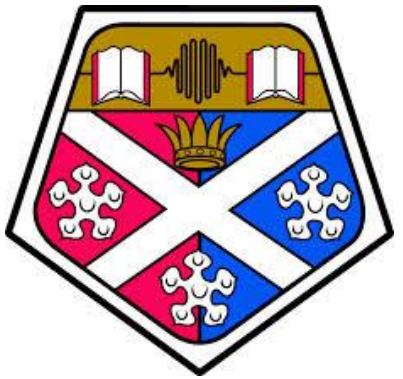

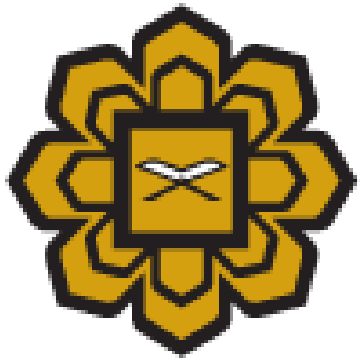

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