Abstracts

Abstracts of Theses Approved for the PhD/Msc at the School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

GENERATION OF RETICULOCYTES DERIVED FROM HUMAN PERIPHERAL BLOOD CD34+ HAEMATOPOIETIC STEM/PROGENITOR CELLS FOR *Plasmodium knowlesi* IN VITRO INVASION ASSAY

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Introduction: Reticulocytes are specialised host cells for *Plasmodium knowlesi (P. knowlesi)*, the fifth identified human malaria parasite. Yet, the availability of reticulocytes for *P. knowlesi* in vitro culture is restricted by the limited number of circulating reticulocytes in human peripheral blood (PB).

Objectives: Therefore, human PB-derived $CD34^+$ haematopoietic stem/progenitor cells (HSPCs) with high proliferative potential were utilised in the present study as a source to generate sufficient supply of reticulocytes for *P. knowlesi* in vitro invasion assay.

Materials and Methods: $CD34^+$ HSPCs were expanded for 5 days in serum-free medium supplemented with expansion cytokines and growth factors. Expanded $CD34^+$ HSPCs were then cultured with erythroid-supporting cytokines for 14 days for differentiation towards erythroid lineage to produce reticulocytes. The maturation of $CD34^+$ HSPCs into reticulocytes was characterised by the expression of cell surface markers as well as the morphology of cells undergoing differentiation. The susceptibility of generated reticulocytes to invasion by *P. knowlesi* and *P. falciparum* (as a control parasite) was determined.

Results: After five days of expansion, the total cell population increased approximately 2.10 ± 0.10-fold in a culture initiated with $CD34^+$ HSPCs. The commitment of $CD34^+$ HSPCs towards the erythroid lineage was identified through a high expression of CD36/CD71 on day 11 and a decrease in expression of CD34 and CD45. Down regulation of CD36/CD71 expression on day 14 indicated that the maturation of normoblasts into reticulocytes. The morphological analysis revealed the presence of proerythroblasts, a large nucleated cell on day 8. The progression of proerythroblasts into normoblast was observed on day 11 by a decrease in cell size. Enucleated cells with at least three dots of cresyl blue ribonucleic acid (RNA) were recognised as reticulocytes and reached its maximum at 30.00 ± 1.76% on day 14. The invasion assay showed that *P. knowlesi* invaded $CD34^+$ HSPC-derived reticulocytes, which was confirmed by Giemsa stained observations at 24-hour post-inoculation, however, with lower invasion index, 1.20 ± 0.33%. Meanwhile, *P. falciparum* efficiently invaded $CD34^+$ HSPC-derived reticulocytes which was observed at 41-hour post-inoculation with an invasion index of 2.60 ± 0.11%.

Conclusion: In conclusion, human PB-derived $CD34^+$ HSPCs could be considered as a potential source to generate reticulocytes required for *P. knowlesi* continuous in vitro culture.

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RESPIRATORY EFFECTS OF AFLATOXIN B1 AND RISK OF WORKPLACE TO HOME EXPOSURE AMONG SELECTED RICE MILLERS IN MALAYSIA

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Introduction: Rice milling process produces a huge amount of dust which may contain fungal toxins due to improper handling and storage at high humidity levels. Aflatoxin B1 (AFB1) is the most potent among the aflatoxins and carcinogenic to humans when ingested or inhaled that might cause hepatocellular cancer or lung cancer.

Objectives: This study aims to assess the exposure of AFB1 and its respiratory effects among selected rice millers in Malaysia.

Materials and Methods: Area and personal dust exposure were evaluated using a sampling train consist of a glass microfibre filter, IOM sampler and GilAir plus personal air sampling pump. The pump was worn for 8 working hours, attached to the workers' belt. Lung function test was performed pre- and post-shift. The palms of both hands of the workers were swabbed with sterile cotton pads wetted with phosphate buffered saline Tween-20 solution (PBST), pre- and post-shift. Home dust sampling was done by vacuuming the living room using a DUSTREAM collector containing mesh nylon filter attached to a vacuum cleaner. The results were then compared with the control group involving Universiti Sains Malaysia administration workers. Questionnaires were given to collect information

on sociodemographic data, occupational details, personal protection equipment (PPE) compliance, workplace practices, respiratory symptoms and factors that lead to take-home exposure to AFB1. Altogether, there were 115 subjects participated in the study, 77.5% of them are Malay.

Results: AFB1 was detected in 14.1% (n = 10) of personal airborne dust samples (median: 0.16, IQR: 0.14, 0.90). Post-shift hand swab was positive with AFB1 in two rice millers 13.0% (n = 2) (median: 0.24 ng/mL, IQR: 0.24, 0.27 ng/mL). However, AFB1 was non- detected on hand swabs among office workers (< LOQ: 0.24 ng/mL). There was no significant difference was found in post-shift lung function between rice millers and controls (FVC: P = 0.911, FEV1: P = 0.637, FEV1/FVC: P = 0.385, PEF: P = 0.160) after controlled for gender and smoking as confounders. The AFB1 home dust level among rice millers was 0.18 ng/kg (IQR: 0.08–8.32). There was no significant difference in AFB1 levels in home dust between both groups.

Conclusion: To conclude, detected AFB1 at rice mills (airborne filter and hand swabs) were lower than the permissible limit (30 ng/m3). Consequently, there is no significant correlation between workplace and home AFB1 levels. Thus, the presence of AFB1 at home could be influenced by environmental factors such as human activities at home. Cumulative effects may pose risks over the years so proper control measures such as hygiene practices at work and preventing take-home exposure are highly recommended to reduce the levels of AFB1 among the workers and their family members.

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EVALUATION OF AQUEOUS EXTRACT OF *Syzygium polyanthum* LEAVES AS ANTI-HYPERTENSIVE AGENT IN SPONTANEOUS HYPERTENSIVE RAT

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Introduction: Hypertension is associated with significant morbidity and mortality. The use of medicinal herbs as alternative medicines to manage hypertension is increasing exponentially. *Syzygium polyanthum* (*S. polyanthum*), has been claimed traditionally as an antihypertensive agent.

Objectives: This study aimed to determine the antihypertensive effects of the aqueous extract of *S. polyanthum* (AESP) leaves and its mechanisms using spontaneously hypertensive rats (SHR).

Materials and Methods: The phytochemical profiling, antioxidant properties and antihypertensive activity were evaluated. Fifty male SHR were divided equally into five groups; untreated-SHR, Losartan-treated, three groups of AESP-treated with different dosages (1500 mg/kg, 1750 mg/kg and 2250 mg/kg) and 10 male WKY rats as control. All treatments were given orally for 12th weeks. Systolic blood pressure (SBP) was measured biweekly. Whereas, the biochemical analysis, oxidative stress markers and angiotensin-converting enzyme (ACE) level were evaluated at the end of the study. The histology of thoracic aorta and kidney were assessed using haematoxylin and eosin (H&E) staining, and scanning electron microscope (SEM).

Results: AESP contains flavonoids and phenols with gallic acid detected. AESP showed high in vitro antioxidant and ACE-inhibitory activities. In AESP-treated SHR; SBP reduced significantly, improved renal function and oxidative stress markers. However, only AESP (2250 mg/kg) significantly reduced ACE concentration. There was also histology improvement of the thoracic aorta and renal in AESP-treated SHR.

Conclusion: The study suggests that antihypertensive properties of AESP are due to its antioxidant (mainly gallic acid) and ACE inhibitory activity. Thus, this study reveals the antihypertensive mechanism of AESP exerted inhibitory activity through suppression of the ACE action which might involve RAAS pathway.

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ANTIMALARIAL ACTIVITY, TOXICITY AND PHYTOCHEMICAL SCREENING OF *Quercus infectoria* GALL CRUDE EXTRACTS

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Introduction: The reduced efficacy of the mainstay antimalarial drugs due to widespread of drug-resistant *Plasmodium falciparum* has necessitated efforts to discover new antimalarial drugs with new targets. *Quercus infectoria* galls have been used traditionally as a herbal remedy for post-partum medication and treatment of parasitic diseases. However, the antimalarial activity of the galls has not been reported.

Objectives: Thus, this study was aimed at evaluating the *in vitro* antimalarial activity of *Q. infectoria* gall crude extracts. This study was also designed to evaluate the toxicity profiles and screen the phytochemical constituents.

Materials and Methods: The antimalarial potential of acetone, methanol, ethanol and aqueous extracts against the chloroquine-sensitive strain (3D7) of *P. falciparum* was assessed via malarial SYBR green-I fluorescence-based

(MSF) assay. The cytotoxicity of the extracts was evaluated against mouse fibroblast cell (NIH/3T3), monkey kidney epithelial cell (Vero) and primary human umbilical vein endothelial cell (HUVEC) via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition to the haemolytic assay, a 2,2-diphenyl-1-picrylhydrazyl (DPPH)-based antioxidant assay of the extracts was performed to observe its connection with haemolysis of human erythrocytes (A^+, B^+, AB^+ and O^+ blood groups). The effect of acetone extract which previously exhibited the most promising antimalarial activity and have satisfactory selectivity index (SI) values on the pH of the parasite's digestive vacuole was examined using a ratiometric fluorescent probe, fluorescein isothiocyanate (FITC)-dextran incorporated into mid trophozoite stage-infected erythrocytes and analysed by flow cytometry.

Results: Only acetone and methanol extracts showed a promising antimalarial activity with 50% inhibitory concentration (IC_{50}) of 5.86 (1.64) and 10.31 (1.90) µg/mL, respectively. The acetone and methanol extracts showed 50% cytotoxicity concentration (CC_{50}) ranged from moderate toxic to non-toxic against all tested normal cells. The cytotoxicity evaluation using a brine shrimp lethality test (BSLT) showed that all extracts were non-toxic according to Meyer's toxicity index. No haemolytic effect was observed on the erythrocytes treated with all extracts. All extracts exhibited excellent DPPH radical scavenging activities. The concentration of heavy metals (lead, zinc, chromium, copper and cadmium) analysed with atomic absorption spectroscopy (AAS) in all extracts was below the permissible level according to WHO guidelines. The phytochemical screening revealed the presence of tannins and flavonoids, and high amount of total phenolic content (TPC) and total flavonoid content (TFC) in all extracts. The pH of the digestive vacuole of acetone extract-treated parasites was significantly altered in a concentration-dependent manner compared to the untreated parasites ($P < 0.001$).

Conclusion: Overall, this study provides valuable insights of *Q. infectoria* gall capability as a safer and promising antimalarial candidate.

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