

Potassium channel subunit *Kcnab1* identifies unilateral cortico-cortical projection neurons in the mouse cerebral cortex

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Cortical subplate (SP) neurons are heterogeneous and include projection neurons that form local and subcortical connections. We set out to characterize axon projection pattern of mouse SP neurons and to correlate it to specific gene expression. Fluoro-Gold (FG) was injected into the primary motor cortex (M1) or primary somatosensory cortex (S1) to retrogradely label neurons that project from S1 to M1, or to contralateral S1, respectively. Images of the whole FG-injected brains were taken with a fluorescence stereomicroscope (MZ 10F, Leica) paired with a ET UV LP filter set (Leica), and the brain sections were imaged with BZ-X700 (Keyence) using a FG ET DAPI/FluoroGold filter cube. Subsequently, *in situ* hybridisation (ISH) and immunocytochemistry (IHC) were performed to identify molecular properties of the tracer-labelled neurons. Fluorescence signals were imaged with a laser scanning confocal microscope (LSM 880 with Airyscan, Zeiss). We found retrogradely-labelled cells in SP of S1 only when tracer was injected into M1, but S1 SP neurons did not project to the other hemisphere. *Kcnab1* expression was limited to the SP during embryonic and neonatal stage, but scattered in other cortical layers of mature brains. ISH and IHC revealed that majority of the FG-labelled ipsilateral projection neurons in S1 SP was *Kcnab1*-positive. Our results suggest that a molecularly-discrete projection neuron subclass in SP predominantly project unilaterally from one cortical area to another and specific role of these neurons in brain network establishment is to be elucidated.

Systematic Significance of Leaf Epidermal Characteristics in *Shorea* Roxb. (Dipterocarpaceae) in Malaysia

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Keywords: Dipterocarpaceae, Shoreae, *Shorea*, leaf epidermal characteristics

The taxonomic problems in *Shorea* mostly involve identification and classification. The investigation presented in this study aims to prove that leaf epidermal characteristics have taxonomic value in *Shorea*. Epidermal peels were prepared using Jeffrey's solution. The underside of the leaf surface was scraped with a razor blade to remove loose cells and soaked for several days in Jeffrey's solution or bleaching agent Vortex. The cleared leaf was washed, stained with Safranin, dehydrated, mounted, photographed and observed as described. For observation under scanning electron microscope, 3 to 5 mm² leaf sections were washed in water, dehydrated through an alcohol series, were then placed between two glass slides, undergone critical point drying process, coated with gold palladium and were examined in a 2.7Å field emission of Cambridge Instruments Stereoscan 360 scanning electron microscope. Results have shown that the epidermis of the leaf surface under LM is useful for distinguishing *S. isoptera*, and *S. maxwelliana*, from the others and also between these two species themselves, so that it could be a diagnostic character. The study of the epidermal surfaces revealed a number of important micromorphological characters, and these characters exhibit interesting interspecific variations that are of significance for species identification and authentication. The presence of crustose wax with an abundance of flakes on the leaf surface may be characteristic of *S. parviflora*. The epidermal sculpturing has considerable diagnostic value, and may characterize some species and could serve as a criterion of distinguishing *S. bracteolata*. Results have shown that leaf epidermal features could be useful for authentication and identification purposes especially at species level, thus this study have shown taxonomic value of leaf epidermal characteristics in *Shorea*.

Occurrence of Gastrointestinal Parasites from Friesian Cattle from a Commercial Farm in Pekan

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Keywords: Friesian cattle, Goat, Gastrointestinal parasites

Gastrointestinal parasitic infections in cattle causes a reduction in production and affect the cattle's health. A study was carried out to investigate the parasite infection in cattle from commercial farm in Pekan, Pahang. A total of 152 Friesian cattle and calf samples were collected and examined by direct smear technique, simple flotation, and sedimentation techniques to determine parasite loads in different age groups. Parasitic eggs seen in cattle feces with the highest prevalence was *Eimeria* spp. (56.58%), followed by *Strongyle* spp. (9.87%), *Strongyloides* spp. (1.32%) and *Ascaris* spp. (0.66%). Calves below 6 months (85%) were more infected with parasites than older ones. This study shows that the most common parasite among Friesian cattle was *Eimeria* spp., so an effective farm management system and antiprotozoal treatment should be conducted to control the parasitic infections in cattle farms.

PCR-based detection and phylogenetic analysis of Torque Teno Virus (TTV) isolated in Malaysia

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Keywords: *Torque Teno Virus, polymerase chain reaction, phylogenetic analysis*

Torque teno virus (TTV) is a single-stranded DNA virus of ~3.8 kb in size with 95% of infection rate among healthy individuals and more than 70% in hepatitis patients. This small, non-enveloped and circular virus can be found in most organisms. Prevalence of TTV in Malaysia and its genetic information is currently unavailable, hence, this study was conducted. UTR PCR (5' UTR and 3' UTR) and N22 PCR were used for the detection of TTV in 137 plasma samples of Malaysian healthy individuals and hepatitis patients. TTV positive samples were then sequenced and phylogenetic analysis was performed. Results showed 70.80% of healthy individuals and 73.7% of hepatitis patients are positive for TTV, based on 5' UTR PCR. Phylogenetic analysis based on 3' UTR PCR from healthy individuals sequences clustering them into four genetic lineages. Similar analysis was also conducted on hepatitis patient's samples and revealed 5 cluster of isolated TTV on phylogenetic tree. TTV is common among Malaysian population (healthy and hepatitis patients) and TTV isolated in Malaysia is important for *Alphatorquevirus* databases record. The information obtained from this study may provide additional information of its genetic diversity, and it should be taken into consideration for pathogenicity study in the future.

Hepatoprotective Activity of Rebaudioside A from *Stevia Rebaudiana* Leaves against Acetaminophen-Induced Liver Toxicity

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Keywords: liver toxicity, *Stevia rebaudiana*, acetaminophen

Liver plays a paramount importance for the detoxification of foreign compounds and fulfils numerous vital functions associated with the endogenous metabolism of the organism. However, if prolonged exposure to high doses of acetaminophen (AAP) which is easily available as over-the-counter (OTC) drugs can lead to hepatotoxicity effect. Therefore, there is a need to find new hepatoprotective agents from natural sources to overcome the problems. *Stevia rebaudiana* (Bertoni) plant belonging to the family Asteraceae is an endemic herb from Paraguay and the neighboring Brazilian border. Due to the non-caloric nature, *Stevia* leaves and their compounds are used in many therapeutic applications such as diabetes, obesity, and plague retardant, indigestion, yeast infection, oral health, skin toning and healing burns and wounds. Rebaudioside A (Reb A) is one of several naturally-occurring steviol glycoside constituents of the *S. rebaudiana* plant. The proposed study aimed to investigate the protective effect of rebaudioside A against AAP-induced liver toxicity in experimental rats. Reb A was dissolved in distilled water (dH₂O) into the required doses (5, 25 and 50 mg/kg). Studies were conducted upon male Sprague Dawley (SD) rats with body weight between 180 to 200g. The rats were randomly divided into 6 groups with 6 rats per group (n=6) and received test solution; dH₂O, silymarin (200mg/kg) or Reb A (5, 25 and 50mg/kg) once daily for 7 days. AAP (3g/kg) was administered orally 3 hours after the administration of the test solutions on day 7. 48 hours after the inducer administration and the animal were sacrificed immediately to obtain liver for histopathological study. From the histological prospect, result indicated a significant hepatoprotective effects relative to 5 and 25 mg/kg of Reb A to the negative control (P<0.05). The result suggested that the Reb A exerts potential protective effect on hepatotoxicity induced by AAP. Further studies at the genetic and/or molecular levels are necessary to explain the protective effects of Reb A on AAP-induced hepatotoxicity.

Ultrastructural and Localization of Incidental Pollens on *Heterotrigona Itama* In Indo-Malayan Meliponine Repository Sekayu, Terengganu

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Keywords: *H.itama*, incidental pollen, selective pollen

Stingless bees play an important role as pollinators of plants. *Heterotrigona itama* is considered as important pollinator's bee and the most popular species in meliponiculture for high value honey. As a generalist stingless bee, they collected many types of pollen species. Thirteen (13) species of pollens which were collected by *H.itama* have been identified in Indo-Malayan Meliponine Repository Sekayu, Terengganu. They are *Antigonon leptopus* (Polygonaceae), *Cocos nucifera* (Arecaceae), *Capsicum annum* (Solanaceae), *Mimosa pudica* (Fabaceae), *Acacia auriculiformis* (Fabaceae), *Amaranthus spinosus* (Amaranthaceae), *Averrhoa carambola* (Oxalidaceae), *Cosmos sulphureus* (Asteraceae), *Hymenocallis littoralis* (Amaryllidaceae), *Sphagneticola tribolata* (Asteraceae); *Solanum melongena* (Solanaceae) and *Andrographis paniculata* (Acanthaceae). Those pollens were mainly load on corbiculae, and this is called as "selective pollen acquisition". Pollens were also adhered abundantly on other body regions such as antenna, compound eye, mandible, tongue, other legs, and abdomen; and also spread on the surface of both wings and on thorax. This is called as "incidental pollen acquisition". While the pollens on corbiculae or the selective pollens may not serve a reproductive role for the plant as they almost invariably moistened with nectar, the incidental pollen is believed play an effective role in plant pollination as the chances of losing individual grains to contact stigmas and perform pollination during floral visits.

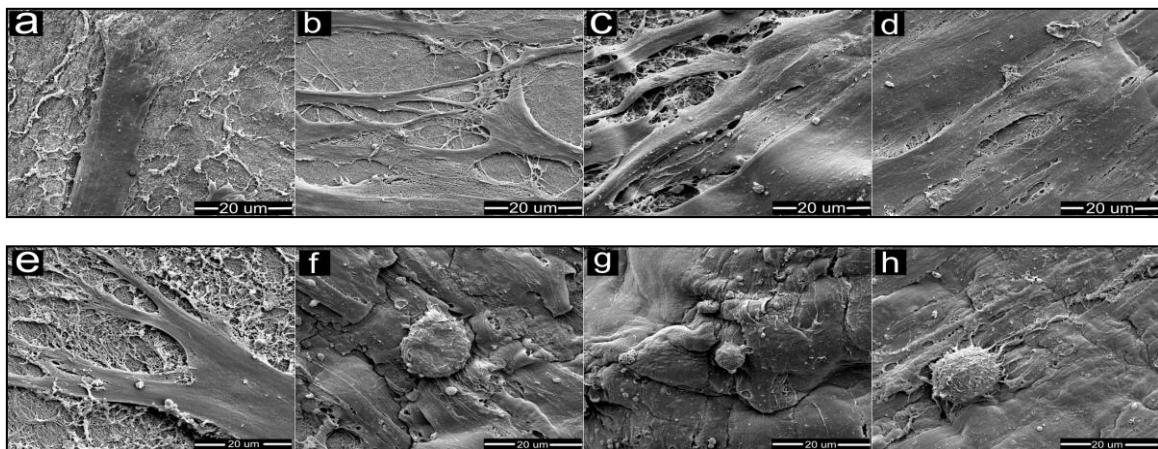
The Ultrastructure of Odontoblast-like Cells Differentiated from Stem Cells from Human Exfoliated Deciduous Teeth (SHED) Cultured on Human Amniotic Membrane

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This study aims to visualise the ultrastructure of odontoblast-like cells differentiated from SHED using scanning electron microscope (SEM). SHED was cultured on AM containing BMP-2 for 1, 7, 10 and 14 days, respectively. SHED cultured on AM without BMP-2 served as control. Samples for SEM were gold-coated with sputter coating machine SCD0005 and viewed by SEM FEG450 at 5000x magnification. SHED displayed cell extensions with small finger-like or web-like projections at the very end of the cell after a day of BMP-2 treatment. On the following days, SHED lost the fibroblast-like morphology, became smaller and round-shaped. By day 14, strong mineralisation on the cell body was observed, suggesting the morphology of a matured odontoblast. In the control group, SHED appearance as fibroblast-like cell morphology remained up until day 14 of culture. In conclusion, morphological evaluation via SEM confirms complete differentiation of SHED into odontoblast-like cells by day 14 of culture.



Chronic Low Dose Organic Arsenic Induced Liver Structural Damage

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Keywords: *organic, arsenic, liver.*

Over decades, organic arsenic has been thought to be less toxic than inorganic arsenic. Monosodium methylarsonate (MSMA) is a potent organoarsenical herbicide that is still being used in most Asian countries, even though in some countries the use has been restricted. Other organic studies reported the effects mainly on gastrointestinal system. However, the evidence on the severity of it to the liver is still insufficient. The study objective was to investigate the effect of organic arsenic (MSMA) exposure on hepatocytes and liver sinusoidal endothelial cells (LSEC). Rats were exposed to MSMA at 63.20 mg/kg daily for 6-months duration through oral gavage daily. Control rats received distilled water *ad libitum*. At the end of the duration, they were sacrificed and underwent liver perfusion for tissue preservation. Liver tissue was prepared for light microscopy, scanning and transmission electron microscopy. Histopathological and ultrastructural comparison between control and treated rats were qualitatively described. Histopathological and ultrastructurally, MSMA has caused necrotic and apoptotic changes of the liver with reduction of organelles in hepatocytes and capillarization of LSEC. Chronic low dose organic arsenic exposure showed evidence of toxicity to hepatocytes. Interestingly, LSEC demonstrated survival accommodation.

Effect of *Electeriospermum tapos* (*E.tapos*) extract as coadjuvant in ameliorating maternal obesity on female offspring at postnatal day 21 on histopathological changes.

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Keywords: *Elateriospermum tapos*, maternal obesity, obesity

Maternal obesity refers to the condition in which pregnant women are excessively overweight and this will lead to childhood obesity. This study is aimed to investigate the effect of *Elateriospermum tapos* supplementation prior to pregnancy and its association with their offspring. Thirty female Sprague Dawley rats were assigned to 2 groups, 6 rats were assigned to normal control group (DCG) fed with standard chow and the remaining rats were assigned to high-fat diet group (HFD) to generate obesity. Next, obese rats were further divided into 4 groups after confirmed as obese. Negative control (DNG) rats with normal saline, positive control (DPG) with 200mg/kg of Orlistat, treatment 1 (DTX1) with 200 mg/kg of *E.tapos* seed and treatment 2 (DTX2) with 200 mg/kg *E.tapos* shell daily. After 6 weeks of treatment, rat were mated. The dams and weaners were culled at postnatal day21 (PN21). Tissue were collected during weaning period. Histopathological grading of the liver section of DNG, offspring from negative and positive group displayed changes with score 1 with the presence of ballooning hepatocyte. Histology of retroperitoneal white adipose tissue (RpWAT) of DTX1, DTX2, offspring from treatment 1 and treatment 2 group showed normal adipocytes with similar pattern of DCG and offspring control group. Atherosclerotic lesion were not present in group DTX1, DTX2, OTX1 and OTX2 compared to DNG and ONG group. In conclusion, *E.tapos* shell has a greater effect on ameliorating maternal obesity on female offspring at PND21 especially in histology changes of liver and RpWAT due to obesity.

Bone-graft interface in New Zealand White rabbit's model: A novel Malaysian-made biomaterial, "osteopaste"

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Keywords: *Bone-graft interface, calcium phosphate, critical size defect*

Injectable self-hardened synthetic bone cement (Osteopaste) was developed from limestone for the purpose to promote healing of bone fracture or in reconstructed bone defects. Excellent biocompatibility of biomaterial results in close cellular contact with biomaterial surface. The aim of this study was to evaluate the bone cell response produced at the bone-biomaterial interface using light microscopy (LM) and scanning electron microscopy (SEM). Osteopaste was implanted in a critical size defect model in rabbits' tibiae and then, compared with commercialized bone graft; JectOS (calcium phosphate) and MIIG-X3 (calcium sulphate). The animals were sacrificed at 6, 12 and 24 weeks post-surgery and bone tissues containing biomaterials were harvested. The specimens were then processed for viewing under LM and SEM to access the histological analysis, surface of biomaterials and biomaterial-bone interface. This visualization showed that no intervening soft tissue seen between new bone and Osteopaste which enable osteoblast cells adhered and spread on the surface of Osteopaste compared with JectOS and MIIG-X3. In conclusion, Osteopaste is biocompatible, osteoconductive and showed osteointegration with surrounding host bone to support ingrowth of new bone.

Anti-Obesity Effect of *Elateriospermum Tapos* in Female Adult Offspring of High-Fat Diet-Induced Obese Dams

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Keywords: Maternal obesity, *E.tapos*, offspring

Maternal obesity during pregnancy associated with adverse maternal and fetal pregnancy outcomes, as well affect health outcomes in offspring. High flavonoid and α -linolenic acid in *E. tapos* required to boost weight loss. This research was aim to explore the impact of *E. tapos* supplementation in obese dams prior pregnancy on adults female offspring's body weight and histopathological changes of organs. Thirty female Sprague Dawley rats were used. Six rats were assigned to normal diet group (DND). The remaining rats were fed with high-fat and cafeteria diet (HFCD) to generate obesity for 5 weeks. Next, obese rats were further divided into 4 groups: Negative Control (DNC; HFCD), Positive Control (DPC; Orlistat 200mg/kg BW), treatment 1 (DTX1; *E. tapos* seed 200 mg/kg BW) and treatment 2 (DTX2, *E. tapos* shell 200mg/kg BW). Treatments were given daily for 6 weeks prior mating. At weaning, female offspring were then designated into 6 groups according to their dam's group (n=6/group): OND from DND, OCD from DND, ONC from DNC, OPC from DPC, OTX1 from DTX1 and OTX2 from DTX2. All groups were fed with standard chow and cafeteria diet except for OND (standard chow only). The offspring were cull at 12 weeks of age. We observed that HFCD feeding significantly increased the offspring's body weight while histopathological study shows ballooning degeneration of liver cell and increases size of adipocytes. However, supplementation of *E.tapos* seed and shell in dams protected the liver and adipocytes of their offspring (OTX1 and OTX2) from the damage caused by cafeteria diet. This highlights *E. tapos* as a supplement that could provide a long term protective effect against pathological changes in obesity among the offspring generation.