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Lipid profile of regular kratom (*Mitragyna speciosa* Korth.) users in the community setting

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ABSTRACT

The protocol describe the steps involved in conducting fasting lipid profile and liver function test from the blood samples of regular kratom users and healthy non-kratom using subjects in this study. This tests were conducted in an ISO 15189-certified laboratory at Advanced Medical and Dental Institute, Universiti Sains Malaysia. The blood parameters which were determined quantitatively included serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), total protein (TP), albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB) and direct bilirubin (DB). While globulin was determined from simple subtraction of albumin from total protein value, indirect bilirubin was determined by subtraction of direct bilirubin from total bilirubin value, and low-density lipoprotein (LDL) was calculated via Friedwald equation.

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Fasting lipid profile and liver function test

- 1 All participants were told to begin fasting at 21:00 and then arrive at the Advanced Medical and Dental Institute (AMDI), USM at 09:00 the following day for blood collection (i.e., 12 hours of fasting).
- 2 A blood sample was collected from each participant for fasting lipid profile (FLP) and liver function test (LFT). The blood collection was performed by a phlebotomist. These tests were conducted in an ISO 15189-certified laboratory at AMDI, USM.
- 3 Blood samples were transferred to the laboratory within 1 hour, and the FLP and LFT analyses were carried out on the same day.
- 4 Quantitative determination of serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), total protein (TP), albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB) and direct bilirubin (DB) were performed on a fully automated biochemistry analyser Beckman Coulter AU680. Whereas, globulin was determined from simple subtraction of albumin from total protein value, and indirect bilirubin was determined by subtraction of direct bilirubin from total bilirubin value. Low-density

lipoprotein (LDL) was calculated via Friedwald equation.

- 5 Total protein was quantified using biuret method which is traceable to National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 972c.
- 6 Albumin was quantified using bromocresol green method traceable to International Federation of Clinical Chemistry (IFCC) standard certified reference material (CRM) 470.
- 7 For ALP, it was quantified using kinetic colour (pNP) method traceable to Beckman Coulter master calibration, whereas ALT and AST both were measured using kinetic UV method which are traceable to IFCC.
- 8 Total bilirubin and direct bilirubin were quantified using photometric colour test (diazonium salt) which are traceable to Beckman Coulter master calibrator and NIST SRM 916a respectively.
- 9 The results of TP, albumin and globulin were expressed in g/L; ALP, ALT and AST in U/L, and bilirubin in $\mu\text{mol/L}$.
- 10 Quantitation of TG, TC and HDL level were determined using enzymatic color method.
- 11 The method for TG and TC is traceable to isotope dilution mass spectrometry and NIST SRM 909b Level 1 respectively.
- 12 For HDL, the method is traceable to the US Centre for Disease Control.

- 13** All the findings of FLP were expressed in mmol/L.
- 14** Calibration for all test methods were accomplished by the use of chemistry calibrator cat. 66300 except for HDL, a separate HDL-cholesterol calibrator ODC 0011 was used.
- 15** Internal quality control procedure was undertaken immediately following calibration in accordance to good laboratory practice.