SINGLE DOSE ACUTE TOXICITY OF ROASTED ROBUSTA COFFEE EXTRACT (*COFFEA CANEPHORA*) IN SPRAGUE-DAWLEY WHITE RATS (*RATTUS NORVEGICUS*) USING OECD NO. 423 GUIDELINE

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INTRODUCTION

- The coffee beans → represent the s**econd** most important commodity in the world after oil
- The drink resulting from these coffee beans (coffee) → is ranked in **4th position** after tea, milk and beer
- **Indonesia has been ranked in 4th position** as the highest exporter of coffee bean after Brazil, Vietnam, and Columbia
- Lampung → **center of coffee plantation** → highest in Robusta

INTRODUCTION

- Coffee contains more than dozen of bioactive compound, mostly formed during roasting process
- Three of the highest concentration → caffeine, diterpene alcohols and phenolic compound
- Caffeine → known principally for its **stimulant effects**
  - Increase alertness and concentration
  - Can disrupt the sleep
- European Food Safety Authority → amount of caffeine considered **no danger is 200 mg**
- Single dose of 400 to 800 mg caffeine → cause adverse biological effects → insomnia, headache, nervousness, anxiety, tachycardia, and tremor

INTRODUCTION

- Coffee → dose 333 mg reported → infertility, decrease mandibule density, affect cardiovascular
- In contrast, previous research → coffee known to be beneficial as **antioxidant, antibacterial, anti-inflammatory, anticancer and neuroprotective agents**
- Safe dose → **NO INFORMATION**

PURPOSE

To evaluate the single dose acute toxicity of roasted robusta coffee extract in rats model using OECD No. 423 Guideline

ETHIC PERMISSION

KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET, DAN TEKNOLOGI
UNIVERSITAS LAMPUNG
FAKULTAS KEDOKTERAN
Jalan Prof. Dr. Sumantri Brojonegoro No. 1 Telp/Fax (0721) 7691197 Bandar Lampung 35145
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PERSETUJUAN ETIK
ETHICAL APPROVAL

No: 1827 /UN26.18/PP.05.02.00/2021

Komite Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Lampung, dalam upaya melindungi hak asasi dan kesejahteraan subjek penelitian kesehatan dan menjamin bahwa penelitian yang menggunakan formulir Survei/Registrasi/Surveilens/Epidemiologi/Humaniora/Sosial Budaya/Bahan Biologi Tersimpan/Sel Punca dan non klinis lainnya berjalan dengan memperhatikan implikasi etik, hukum, sosial dan non klinis lainnya yang berlaku, telah mengkaji dengan teliti proposal penelitian berjudul:

The Health Research Ethics Committee, Faculty of Medicine, University Lampung, in order to protect the rights and welfare of the health research subject, and to guaranty that the research using survey/questionnaire/registry/surveillance/epidemiology/humaniora/social-cultural/archived biological materials/stem cell/other nonclinical materials, will carry out according to ethical, legal, social implications and other applicable regulations, have been thoroughly reviewed the proposal entitled:

"Uji Toksisitas Akut Dosis Tunggal Ekstrak Kopi Robusta (*Coffea canephora*) pada Tikus Putih (*Rattus norvegicus* Berkenhout, 1769) Galur Spraque-Dawley Menggunakan *Guideline* Uji OECD No. 423"

"Single Dose Acute Toxicity of Robusta Coffee Extract (Coffea canephora) in Spraque-Dawley Rats (Rattus norvegicus Berkenhout, 1769) using OECD No. 423 Guideline"

Nama Peneliti Utama : Selvi Rahmawati, S. Si., M. Sc
Principal researcher
Nama Institusi : Fakultas Kedokteran Universitas Lampung
Institution : *Faculty of Medicine University of Lampung*

Proposal tersebut dapat disetujui pelaksanaannya.
Hereby declare that the proposal is approved.

Bandar Lampung, 13 Juli 2021
Bandar Lampung, *July 13th 2021*

Dekan
Dean

Prof. Dr. Dyah Wulan SRW, SKM., M. Kes
NIP. 197206281997022001

Komisi Etik Penelitian Kesehatan
Fakultas Kedokteran Universitas Lampung
Health Research Ethical Commission
Faculty of Medicine University of Lampung

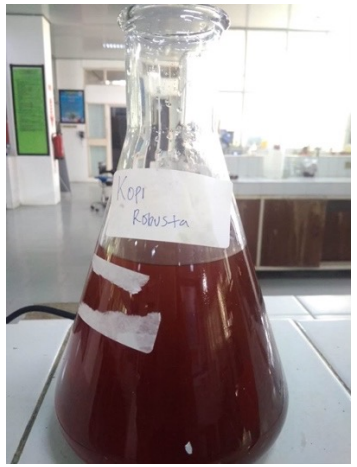
dr. Agustyas Tjiptaningrum, Sp.PK
NIP. 197208292002122001

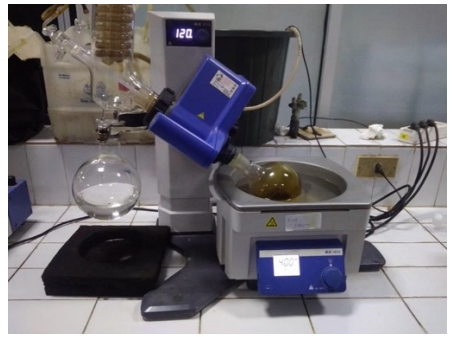
Keterangan/*notes*:

Persetujuan etik ini berlaku selama satu tahun sejak tanggal ditetapkan
This ethical clearance is effective for one year from the due date

METHOD

Roasted Coffee Extraction

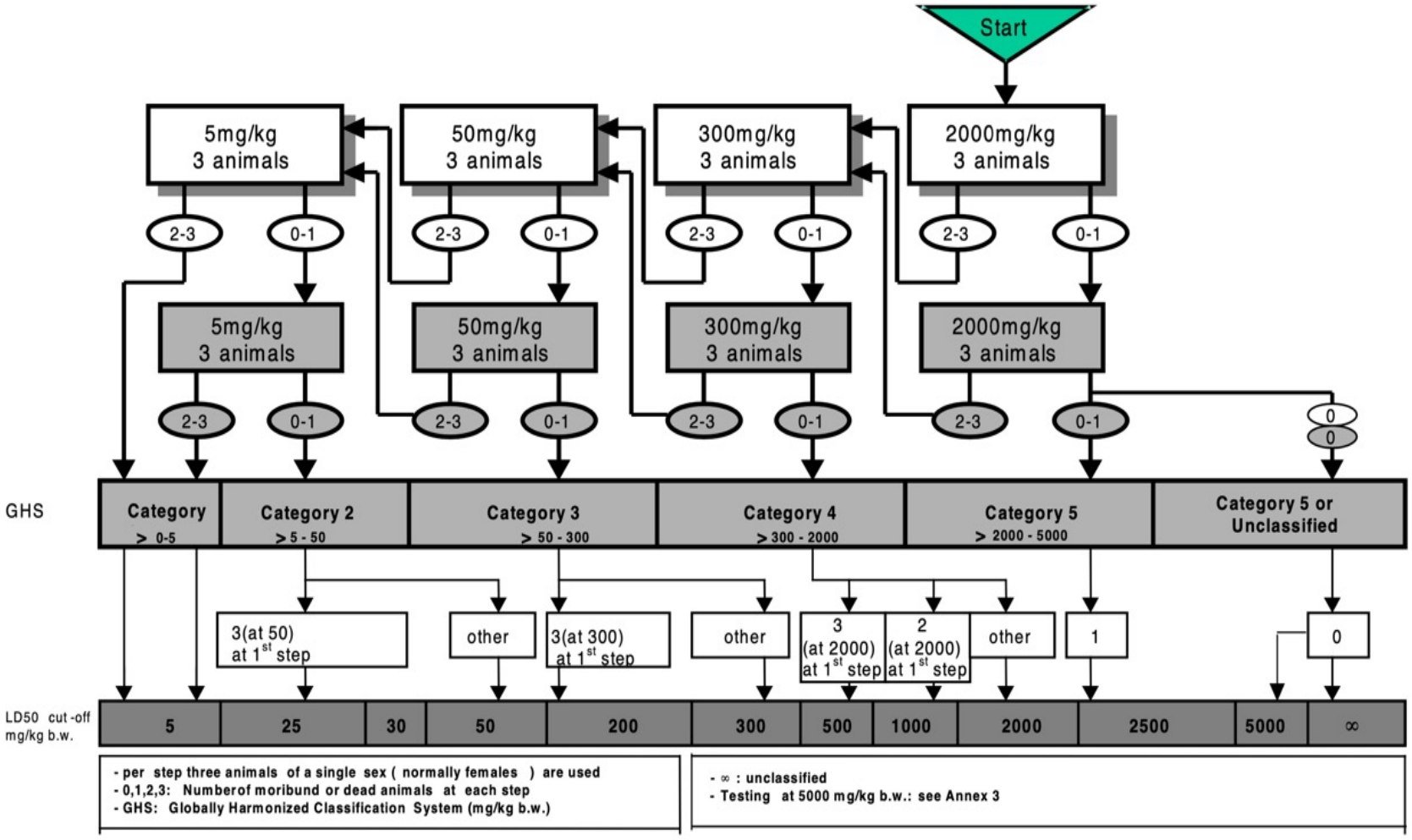




METHOD

Toxicity test

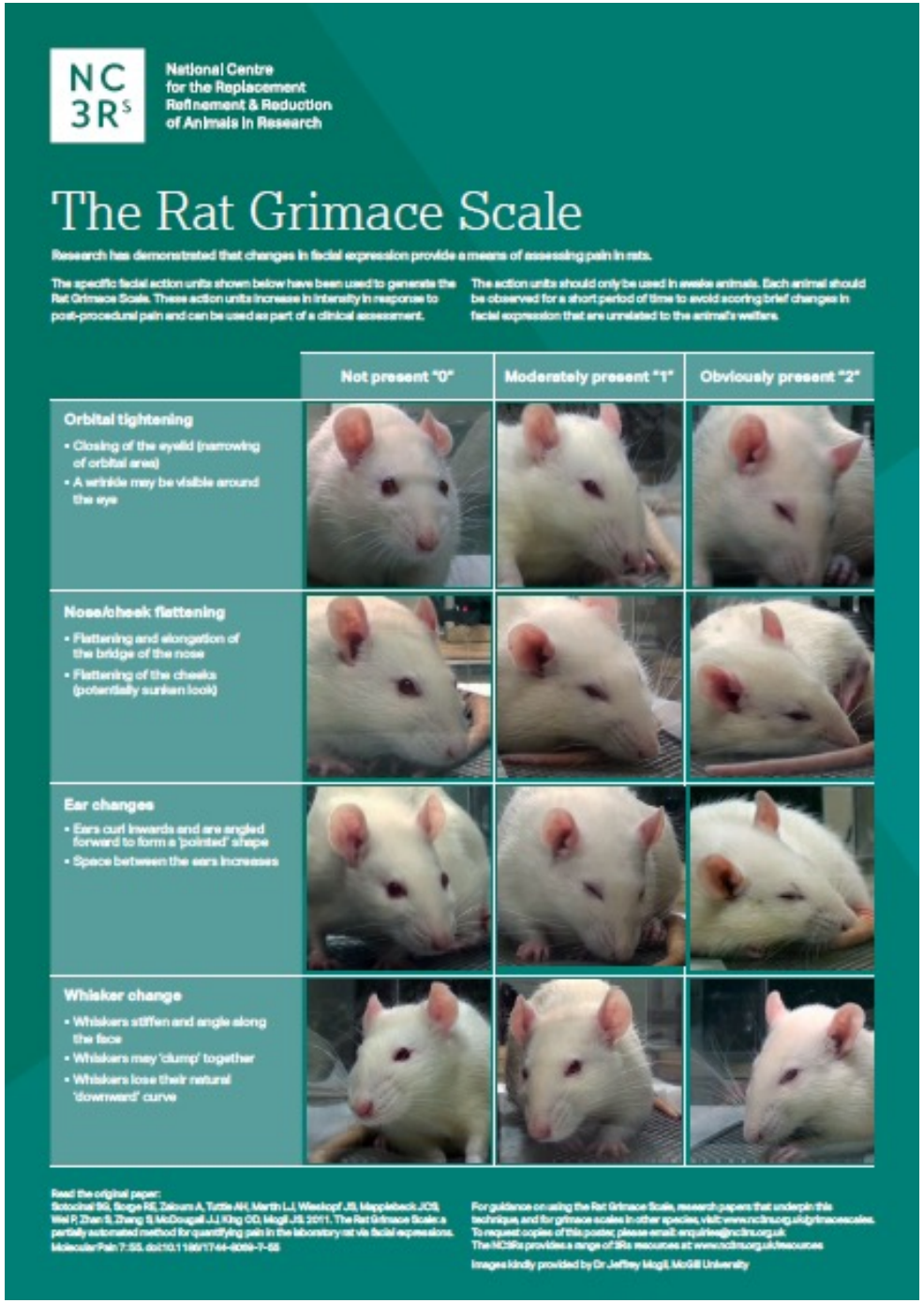
ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



METHOD

Observation

- For the first 30 minutes, 4 hour and 24 hours → observate for **mortality or symptom of toxicity**
- NO DIE → evaluate for 14 days in max
- Evaluate for organ functions → ureum, creatinine, AST, ALT
- Evaluate for histological structure



RESULTS

Different clinical signs observed after a single dose induction of roasted coffee extract at the doses of 2000 mg/kg BW

PARAMETER	30 min			4 hour			24 hour			72 hour		
Mo												
Car												
Respiration Frequency	n	n	n	n	n	n	n	n	n	n	↑	n
Defecation	n	n	n	n	n	n	n	n	n	n	n	n
Excitability	–	–	–	–	–	–	–	–	–	–	–	–
Hair electricity	–	–	–	–	–	–	–	–	–	–	–	–

No death occurred in the first 24-72 hours, BUT rats no. 2 showed pain and toxicity sign after 72 hours induction

*n = normal; X = death, (–) = none, (+) = present

RESULTS

Different clinical signs observed after a single dose induction of roasted coffe extract at the doses of 5000mg/kg BW

PARAMETER	30 min			4 hour			24 hour		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Motoric activity	↓	↓	↓	X	↓	X	X	X	X
Two deaths occurred in the first 4 hour, last rat showed toxicity sign									
Urination	n	n	n	X	n	X	X	X	X
Excitability	+	-	+	X	-	X	X	X	X
Hair electricity	+	-	+	X	-	X	X	X	X

*n = normal; X = death, (−) = none, (+) = present

RESULTS

- This excitation is due to caffeine → increases **neuromuscular transmission** → increases the **neural excitability**
- Gradually, as time elapses, the rats were less agitated.
- After four hours, a general state of drowsiness sets in. This drowsiness resulting in **difficulty moving** → relate with decrease of motoric activity
- It is assumed that coffee could be attributed to **the sedative and anesthetic properties**

CONCLUSION

- Toxicity test of a single dose of robusta coffee show that the LD50 value (lethal dose 50) is **between 2000 and 5000 mg/kg BW** and is in the **5th category of toxicity based on the Global Harmonized System.**

NEXT PROJECT

- Further analysis of **organ function** and **histological structure evaluation**
- Study to evaluate the **toxicity effect on histological evaluation**
- Evaluate the safe amount of **repeated dose** of coffee consumption
- Evaluate in the level of **subchronic and chronic toxicity**
- Study to evaluate the **concentration of caffeine and other compounds** from the extract

THANK YOU