



# 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 **second** 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 4<sup>th</sup> 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**  
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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/Surveilans/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 13<sup>th</sup> 2021

Dekan  
*Dean*

  
Prof. Dr. Lurah Wulan SRW, SKM, M. Kes  
NIP. 197204281997022001

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

  
dr. Agustyas Iptaningrum, Sp-PK  
NIP. 197208292002122001

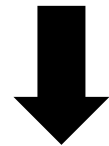
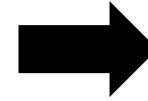
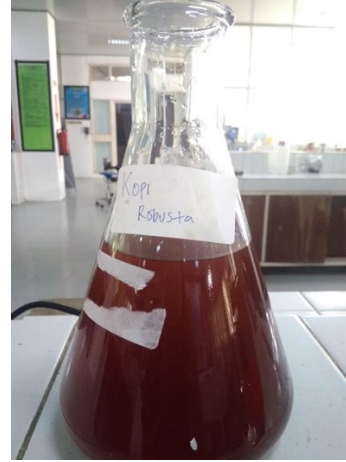
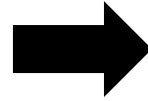
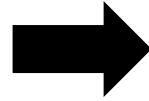
Keterangan/notes:

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

Diipinai dengan Cap/Bareng

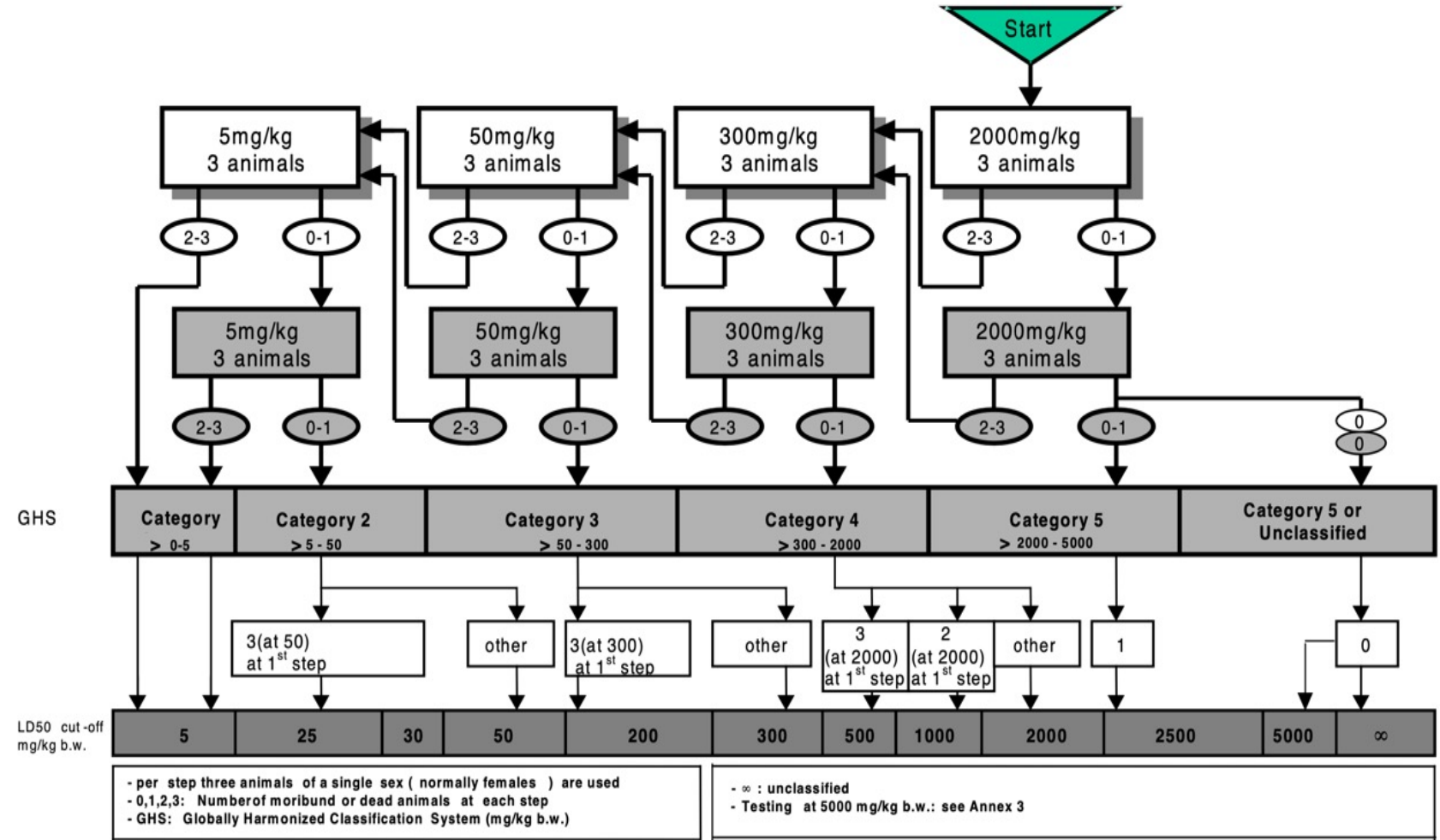
# METHOD

## Roasted Coffee Extraction



# 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 → observe 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



National Centre  
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Refinement & Reduction  
of Animals in Research

## The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring/brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<b>Orbital tightening</b> <ul style="list-style-type: none"><li>• Closing of the eyelid (narrowing of orbital area)</li><li>• A wrinkle may be visible around the eye</li></ul>			
<b>Nose/cheek flattening</b> <ul style="list-style-type: none"><li>• Flattening and elongation of the bridge of the nose</li><li>• Flattening of the cheeks (potentially sunken look)</li></ul>			
<b>Ear changes</b> <ul style="list-style-type: none"><li>• Ears curl inwards and are angled forward to form a 'pointed' shape</li><li>• Space between the ears increases</li></ul>			
<b>Whisker change</b> <ul style="list-style-type: none"><li>• Whiskers stiffen and angle along the face</li><li>• Whiskers may 'clump' together</li><li>• Whiskers lose their natural 'downward' curve</li></ul>			

Read the original paper:  
Sokolov IR, Sorge RE, Zelamus A, Tuttle AH, Mørth L-J, Wessely JB, Megretzke JCG, Weil P, Zhan S, Zhang S, McDougall JJ, King CD, Mogil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* 7:35. doi:10.1186/1744-5019-7-35

For guidance on using the Rat Grimace Scale, research papers that underpin this technique and for grimace scales in other species, visit [www.nc3rs.org.uk/grimacescales](http://www.nc3rs.org.uk/grimacescales). To request copies of this poster, please email [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk). The NC3Rs provides a range of 3Rs resources at [www.nc3rs.org.uk/resources](http://www.nc3rs.org.uk/resources).

Images kindly provided by Dr Jeffrey Mogil, McGill University

## 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	No death occurred in the first 24-72 hours, BUT rats no. 2 showed pain and toxicity sign after 72 hours induction												
Car													
Respiration Frequency	n	n	n	n	n	n	n	n	n	n	n	↑	n
Defecation	n	n	n	n	n	n	n	n	n	n	n	n	n
Excitability	-	-	-	-	-	-	-	-	-	-	-	-	-
Hair electricity	-	-	-	-	-	-	-	-	-	-	-	-	-

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

## RESULTS

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

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