Pharmacogn J. 2021; 13(6): 1384-1388
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Subchronic Toxicity of Lunasin Targeted Extract (ET-Lun) from Soybean Seed (*Glycine max* (L.) Merr.): Perspective from Liver Histopathology, SGOT, and SGPT Levels in Sprague Dawley Rats

Numlil Khaira Rusdi[1,2], Weri Lia Yuliana[2], Erni Hernawati Purwaningsih[3,4], Andon Hestiantoro[5], Kusmardi Kusmardi[1,4,6,7,*]

Numlil Khaira Rusdi[1,2], Weri Lia Yuliana[2], Erni Hernawati Purwaningsih[3,4], Andon Hestiantoro[5], Kusmardi Kusmardi[1,4,6,7,*]

[1]Doctoral Program for Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, INDONESIA.
[2]Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. Hamka, Jakarta, INDONESIA.
[3]Department of Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta, INDONESIA.
[4]Drug Development Research Cluster, Indonesian Medical Education and Reseach Institute, Universitas INDONESIA.
[5]Department Obstetrics and Gynaecology, School of Medicine, Universitas Indonesia, Dr Cipto Mangunkusumo Hospital, Jakarta, INDONESIA.
[6]Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, INDONESIA.
[7]Human Cancer Research Cluster, Indonesian Medical Education and Reseach Institute, Universitas INDONESIA.

Correspondence

Kusmardi Kusmardi

Drug Development Research Cluster, Indonesian Medical Education and Reseach Institute; Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia; Human Cancer Research Cluster, Indonesian Medical Education and Reseach Institute, Universitas INDONESIA.

E-mail: kusmardi.ms@ui.ac.id

History

- Submission Date: 01-09-2021;
- Review completed: 08-09-2021;
- Accepted Date: 14-09-2021.

DOI : 10.5530/pj.2021.13.175

Article Available online

http://www.phcogj.com/v13/i6

ABSTRACT

Background: Lunasin Targeted Extract (ET-Lun) has a pharmacology effect in inhibiting inflammation by decreasing COX-2 and iNOS expression. ET-Lun could increase apoptosis and decrease dysplasia (p > 0,05). In addition, ET-Lun could decrease EGFR expression in breast cancer rats. The acute toxicity showed ET-Lun has LD50 more than 5000 mg/kg BW and was practically non-toxic. **Objective**: this study aimed to determine the subchronic toxicity of ET-Lun. **Methods**: Male and female Sprague Dawley rats (n=40) were divided into 4 groups, the control group and treatment group ET-Lun dose of 250 mg/Kg BW, 500 mg/kg BW, and 750 mg/kg BW. The ET-Lun was administered for 90 days. On the 91st day, the animals were dissected and examined for SGOT-SGPT levels, liver histopathology, and diameter of the central vein. **Results**: The SGOT-SGPT levels showed no significant difference between the treatment group and the control group (p > 0.05). On microscopic observation, there was no change or damage to the liver of rats in each group. The diameter of the central vein of the rat liver shows no significant difference between the control and treatment groups. **Conclusion:** The ET-Lun does not produce adverse effects in liver rats after subchronic treatment.

Key words: Soybean, Lunasin, liver, SGOT, SGPT, Subchronic toxicity.

INTRODUCTION

Indonesia is a subtropical country that is rich in natural resources and biodiversity, including various potential medicinal plants. Recently, applications of natural medicine for medications have become a trend in modern society, this then causes an increasing number of various studies in explorations and applications of plants that are believed to have medicinal properties. One of the plants that are used in traditional medicine is soybean seeds (*Glycine max* (L.) Merr.).[1] Soybean seed has several pharmacological effects including antioxidant, estrogenic, anti-diabetes, anti-hyper cholesterol, and anti-cancer.[2,3]

Several studies to ensure the safety of natural medicine for medications should be performed. For instance, by conducting a toxicity assay. Toxicity is the potency of xenobiotics to cause damage to an organism either during use or in the environment. Toxicity assay can be divided into two types, which general toxicity (acute, sub-acute/sub-chronic, chronic) and specific (teratogenic, mutagenic, and carcinogenic).[4,5] The acute toxicity assay is an assay that detects toxicity effect, which may appear in a short time after 24 hours of single or repeated dose administrations of the test solution.[4,6] Sub-chronic toxicity is an assay that is performed to detect toxicity effect after repeated doses of oral administration in animal models for part of the life of the animal, but no more than 10 % of the entire life of the animal.[4]

A previous study by Wijiasih (2017)[7] showed the extract of soybean seed contained 0.823 mg/g of lunasin. This extract significantly reduced COX-2 and iNOS expression in colon preneoplasia of mice at doses of 150 mg/kg BW and 200 mg/kg BW (p <0.05).[7] Another study proved the soybean extract was able to increase apoptosis (p = 0,001) at a dose of 150 mg/kg BW, and reduced dysplasia (p = 0,024) at a dose of 200 mg/kg BW.[8] The soybean extract have also inhibitory activity in colitis-associated colon carcinogenesis through inhibiting reduction in the number of goblet cell and microvessel density.[9] Moreover the soybean extract with targeted Lunasin (ET-Lun) could reduced tumor volume (p=0,021) and decreased EGFR expression in DMBA induced breast cancer rat model.[10]

Previously, acute toxicity has been performed using doses of 500, 2000, 5000 mg/kg BW The acute toxicity showed ET-Lun has LD50 more than 5000 mg/kg BW and was practically non-toxic (unpublished data). The aim of this study was to evaluate the subchronic toxicity of ET-Lun with targeted organs and observed changes in the liver using histopathology examinations, Serum Glutamine Oxaloacetate Transaminase (SGOT), and Serum Glutamine Pyruvate Transaminase (SGPT) test.

METHODS

Preparation of Simplisia, extraction and Phytochemical Screening

The soybean seeds (*Glycine max* (L.) Merr) of Grobogan variety, the extraction procedure and phytochemical screening were in accordance with previous studies.[10]

Cite this article: Rusdi NK, Yuliana WL, Purwaningsih EH, Hestiantoro A, Kusmardi K. Subchronic Toxicity of Lunasin Targeted Extract (ET-Lun) from Soybean Seed (*Glycine max* (L.) Merr.): Perspective from Liver Histopathology, SGOT, and SGPT Levels in Sprague Dawley Rats. Pharmacogn J. 2021; 13(6): 1384-1388.

Animal Models preparations

The study using animal models has been approved by ethical committee from Faculty of Medicine, Universitas Indonesia, Protocol ID : 20-09-1077. The animal models was weighed and underwent aclimizations for 7 days by standard feeding and drink. The animal models were placed in a cage with a husk, with 5 animals per cage. The animal was maintained in a clean room.

Determinations of Subchonic toxocity test

In this study, 40 Sprague Dawley (SD) rats aged 6 weeks (twenty female and twenty male) were divided into four groups and each group consisted of 5 male and female rats respectively. Animals were randomly assigned to a control (GN) and three treatment groups ; dose 250 mg/kg BW (G1), 500 mg/kg BW (G2), and 750 mg/kg BW (G3). The dose that used in this study was based on the previous study.[7,10]

The treatment group was orally administered with ET-Lun extract for 90 days. The weight of the rat was measured one times a week for 3 months. On the 91st day, the rats were anesthetized using ketamine and xylazine, and blood samples were collected from each animal by cardiac puncture. After the collection of blood samples, the rats were sacrificed by cervical dislocation and the liver was dissected for histopathology analysis.

Determination of SGOT SGPT Level

The blood was put into a centrifuge tube and allowed to stand at room temperature for 10 minutes, then transferred to an ice bath for no more than 20 minutes and immediately centrifuged for 10 minutes at 3000 rpm. Furthermore, the serum was separated and stored in a freezer at -20°C. The SGOT and SGPT levels were measured according to DiaSys® protocol.

Histopathology Examinations

The liver of male and female rats was immediately fixed in 10% neutral buffered formalin (NBF), fixation and, the paraffin block was made afterward. The tissue blocks were sectioned in ribbons at a thickness of 5 µm with Leica microtome (Leica DM 750, Germany). Slide preparations and Hematoxylin Eosin staining were conducted.

Data Analysis

Data were presented as mean ± SEM with 95% confidence interval and analyzed by SPSS version 24. One-way ANOVA and continued with the Tukey HSD test with a 95% confidence level to determine the differences between each group.

RESULT

In this study, defatted soybean seeds were extracted using PBS. The macerate was dried to obtain a thick extract with specific characteristics (Tables 1 and 2). Standardization of ET-Lun in the form of water value, ash value, and phytochemical screening was shown in Tables 3 and 4.

Giving ET-Lun to the treatment groups for 3 months did not show a significant difference in body weight when compared to the control group (Figure 1). Likewise, the results of the SGOT and SGPT level, and the liver weight examinations (Figure 2-4). A microscopic study of the liver was also showed no damage on the liver and the diameter central vein showed no difference between the treatment group and the control group (Figure 5-9).

Table 1. Extraction Results of Soybean Seed.

No	Type	Result
1	Soybean seed	5 kg
2	Powder	2 kg
3	Thick Extract	413 g

Table 2. Soybean Characterization.

No	Organoleptic	Powder	Extract
1	Form	Fine Powder	Thick Extract
2	Color	Chocolate	Chocolate
3	Smell	Distinctive	Distinctive
4	Taste	Plain	Plain

Table 3. Water and Ash Content of Soybean Seed Extract.

No	Type	Result
1	Water content	28.26 %
2	Ash content	5.57 %

Table 4. Phytochemical Screening.

Active Compound	Reagents	Result
Alkaloid	Dragendorf	+
	Bouchardat	+
Flavonoid	H2SO4(p)	+
Saponin	Reaksi Busa	+
Tannin	Uji Gelatin	-
Triterpenoid	Eter, AAA, H2S04	+
Phenolic	NaOH	+
Steroid	Eter, AAA, H2SO4	-
Glycosides	FeCl3	+

(+) = Positive (-) = Negative

Table 5. Observations Results of Color and forms of Liver Organ.

Group	Macroscopic Examination	Liver organ
Normal	Color	Red
	Form	Normal
Dose of 250 mg/KgBW	Color	Red
	Form	Normal
Dose of 500 mg/KgBW	Color	Red
	Form	Normal
Dose of 750 mg/KgBW	Color	Red
	Form	Normal

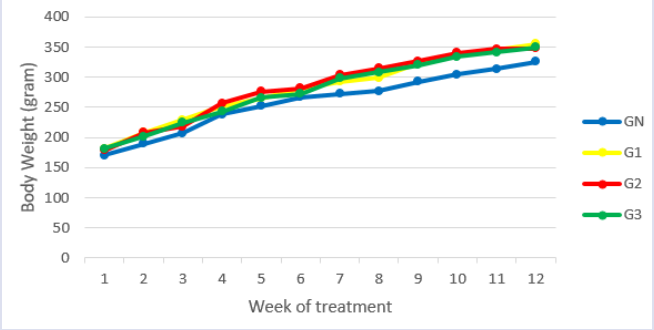

Figure 1. Female and male rats' body weights. Bodyweight in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

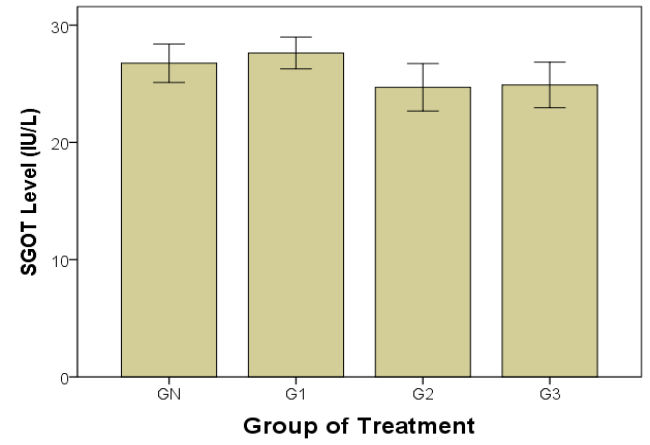

Figure 2. SGOT level in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

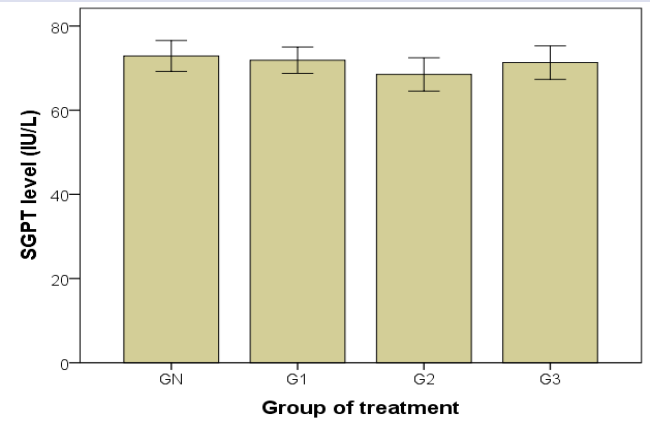

Figure 3. SGPT level in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

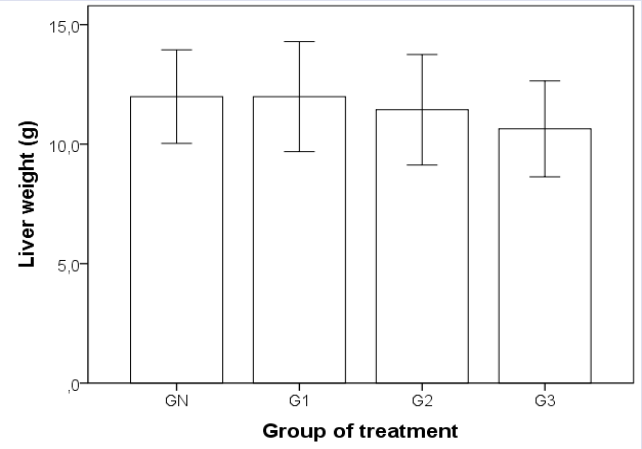

Figure 4. Liver weight in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

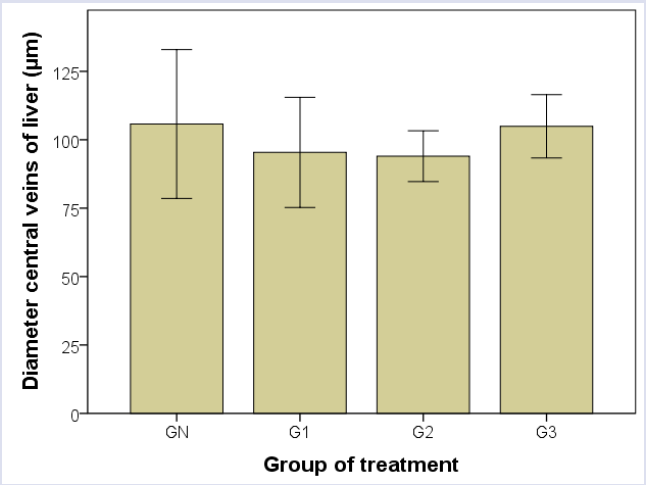

Figure 5. Diameter central vein in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

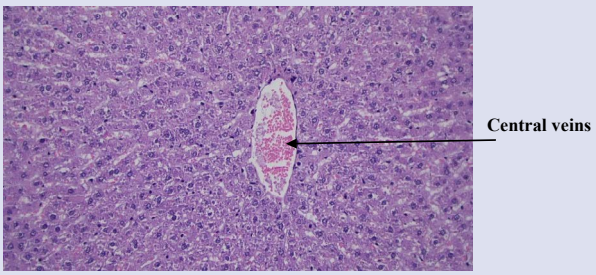

Figure 6. The Central Vein of Normal Group (GN).

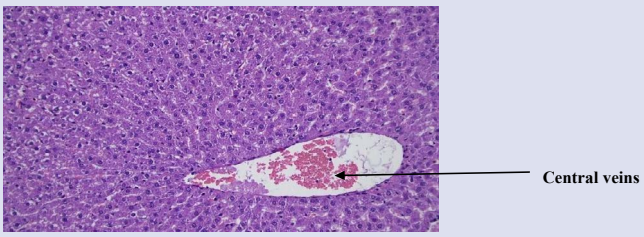

Figure 7. The Central Vein group of 250 mg/kg (G1).

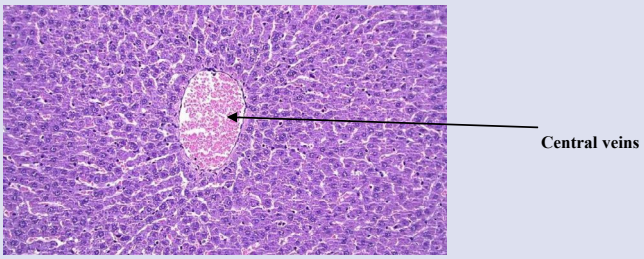

Figure 8. The Central Vein group of 500 mg/kg (G2).

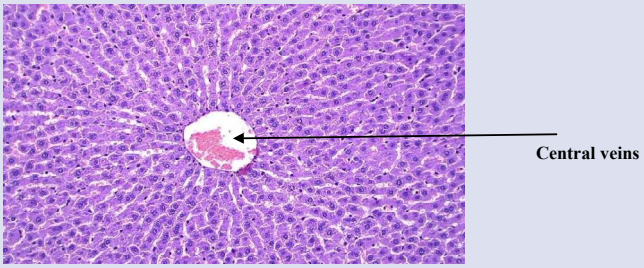

Figure 9. The Central Vein group of 750 mg/kg (G3).

DISCUSSION

Sub-chronic ET-Lun toxicity for soybean seeds was carried out with the aim of obtaining information on the presence of symptoms of toxic effects that were not detected in the acute toxicity test. A subchronic toxicity test can be done if the plant has passed the acute toxicity test. The acute toxicity test on soybean extract obtained the results of giving soybean seed extract given in a single dose and observed death within 24 hours and continued observation for 14 days showed no death in experimental animals with doses of 500 mg/kg BW, 2000 mg/kg BW and 5000 mg/kg (unpublished data).

The results of the extraction and standardization of the extract in this study showed that ET-Lun met the quality standard of the extract.[11,12] The administration of a targeted extract of soybean seed lunasin at each different dose did not affect the bodyweight of the rats. Parameters of SGOT and SGPT levels are indicators that can indicate liver damage.[13] The presence of damage to liver cells can be characterized by increased levels of SGOT and SGPT enzymes. SGOT-SGPT are two transaminase enzymes produced by liver cells. An increase in SGOT and SGPT indicates damage to liver cells.[14] SGOT serves as an indicator and evaluation of the function of the liver and heart muscle and monitors the effects of hepatotoxic and nephrotoxic drugs, while SGPT functions as an indicator of liver function, monitoring the effects of hepatotoxic drugs. SGOT is found mainly in red blood cells, in the heart and skeletal muscle, and in the kidneys. SGPT is an enzyme found in the liver and is the most sensitive marker for liver cell damage.[13,14]

The result of this study showed that there was no significant difference in the levels of SGOT and SGPT between the control group (GN) and the ET-Lun group at doses of 250 (G1), 500 (G2), and 750 mg/kg (G3). These results were supported by microscopic examination. The general architecture of the liver, the appearance of the hepatocytes, the hepatic sinusoids, portal triads, and central veins are normal as compared with controls.

CONCLUSION

The results of the sub-chronic toxicity can be concluded that ET-Lun from soybean seeds (*Glycine max* (L) Merr.) with a dose of 250 mg/kg BW, 500 mg/kg BW, and a dose of 750 mg/kg BW is proven not to be toxic. There was no significant difference between control and treatment groups to SGOT and SGPT levels ($p > 0.05$). On microscopic observation of the central vein of the liver, there was no change or damage in each dose group. Meanwhile, the microscopic quantitative measurement of the diameter of the central vein of the rat liver showed no difference between groups ($p > 0.05$). The results showed that ET-Lun from soybean seeds did not cause toxicity to rats.

ACKNOWLEDGEMENT

The authors are grateful to the Directorate of Research and Development, University of Indonesia for funding the 2020/2021 doctoral grant with the contract NKB-590/UN2.RST/HKP.05.00/2020 and BA-076/UN2.RST/PPM.00.03.01/2021.

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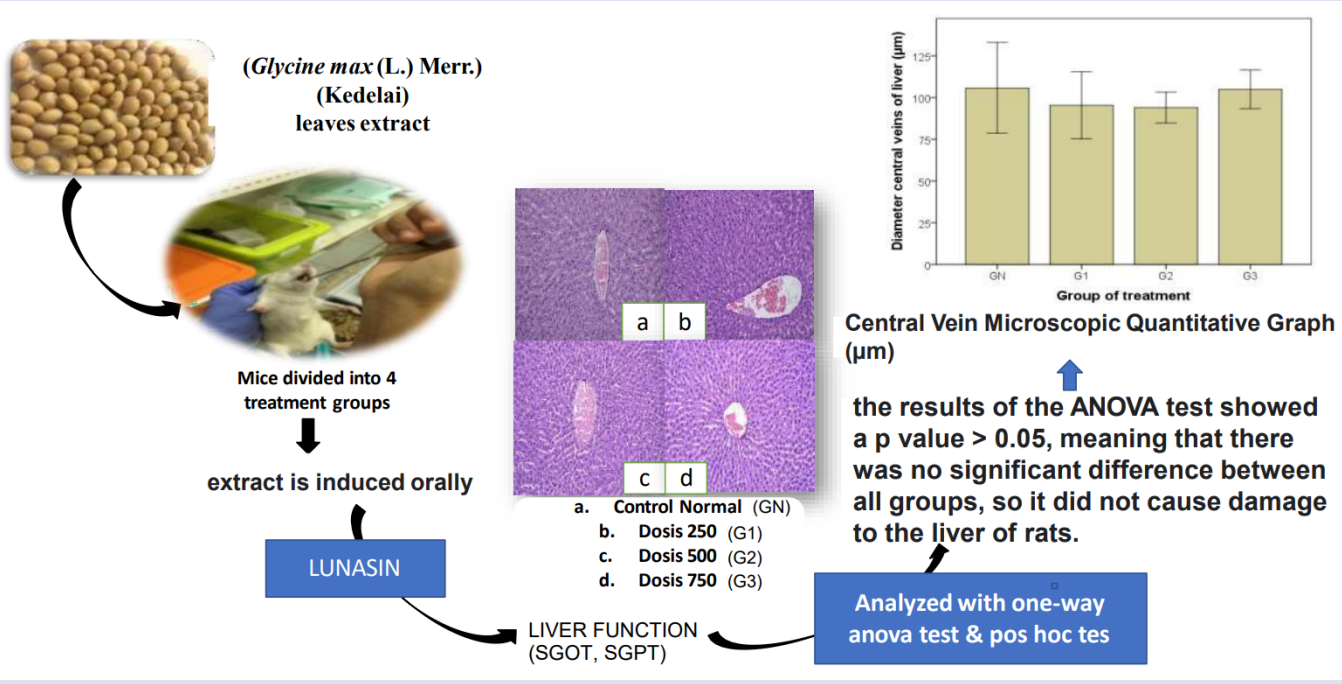
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Numlil Khaira Rusdi. Lecturer in the Department of Pharmacy, University of Muhammadiyah Prof. Dr. HAMKA Jakarta. Focuses on Pharmacology and Community Pharmacy, and Doctoral student at Biomedical Sciences, Faculty of Medicine, Universitas Indonesia. She is currently conducting research on anti-breast cancer in medical plants.

Weri Lia Yuliana. Student in the Department of Pharmacy, University of Muhammadiyah Prof. Dr. HAMKA Jakarta.

Erni H. Purwaningsih is a Professor in Medical Pharmacy, Faculty of Medicine Universitas Indonesia, and as a vice coordinator of Drug Development Cluster, Institute of Medical Education and Research in Indonesia (IMERI) Universitas Indonesia. She achieved her MD, Magister and Doctor degrees from Faculty of Medicine, Universitas Indonesia. She conducts research activities especially on Herbal Medicine, and as a supervisor for several candidates on Biomedical Magister and/or Doctorate Program Faculty of Medicine, Universitas Indonesia. She is consultant and/or reviewer on Traditional Herbal Medicine that conducted by the Director of Traditional Health Services, the Ministry of Health and for the National Agency of Drug and Food Control of Indonesia.

Andon Hestiantoro is a Professor in obstetrics and gynecology, and works as consultant in reproductive endocrinology and infertility. He achieved his MD and PhD degrees from University of Indonesia, Jakarta, and obtained his MPH degree from University of Gajah Mada, Yogyakarta. He is the head of Human Reproduction, Infertility, and Family Planning Cluster, Indonesia Medical and Research Center, Universitas Indonesia. He conducts research activities at the Yasmin Fertility Clinic, Cipto Mangunkusumo Hospital, Jakarta, Indonesia. He is interested in research in female endocrinology.

Kusmardi is Associate professor at Anatomic Pathology, Faculty of Medicine, Universitas Indonesia. The major research focus on colorectal and breast cancer, include the potential inhibition of some Indonesian natural medicine on the both carcinogenesis, the indentification of normal tissue vs cancer development using some molecular marker and computational model. He wrote the mouse model for breast cancer book, the mouse model for colorectal adjuvant chemopreventive book, and Lunasin: a soybean polypeptide as chemopreventive adjuvant for colon cancer.