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Terimakasih atas perhatiannya.

Hormat saya,

Numlil Khaira Rusdi

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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

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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]

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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

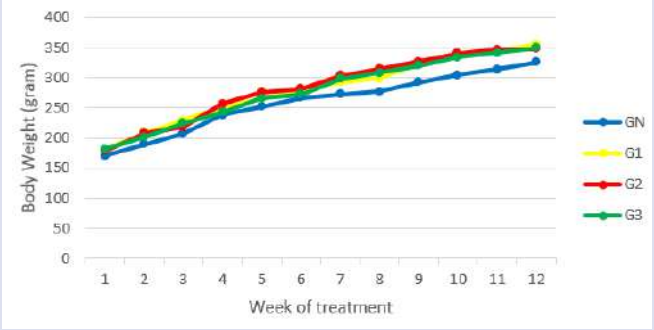

Figure 1. Female and male rats' body weights. Bodyweight in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

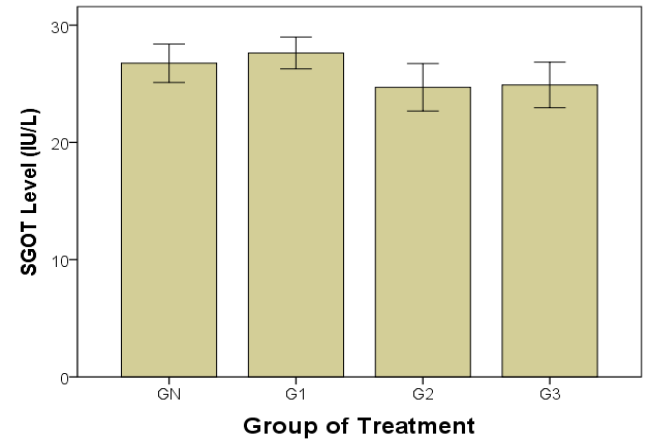

Figure 2. SGOT level in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

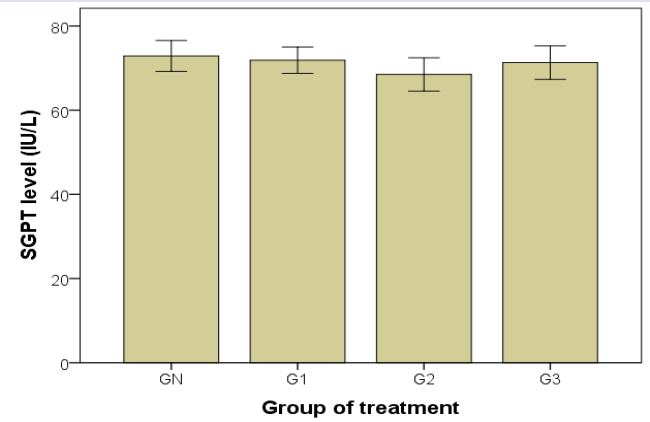

Figure 3. SGPT level in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

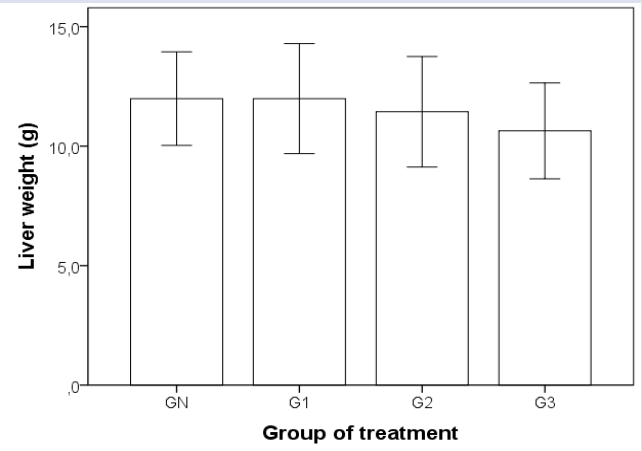

Figure 4. Liver weight in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

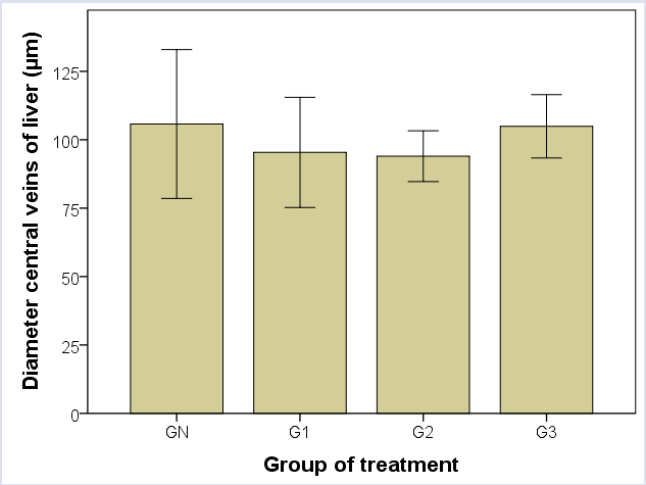

Figure 5. Diameter central vein in the treatment groups (G1, G2, and G3) was no different with control groups (GN) (p>0,05)

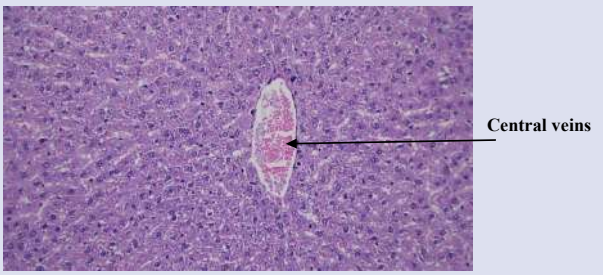

Figure 6. The Central Vein of Normal Group (GN).

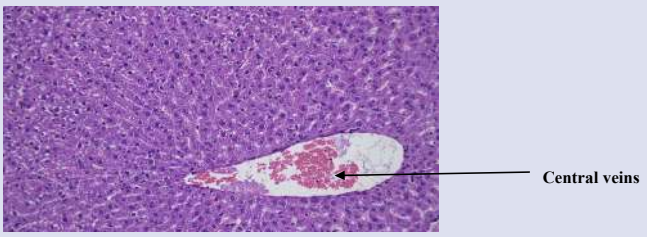

Figure 7. The Central Vein group of 250 mg/kg (G1).

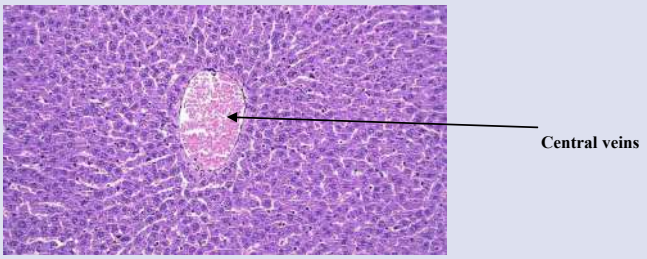

Figure 8. The Central Vein group of 500 mg/kg (G2).

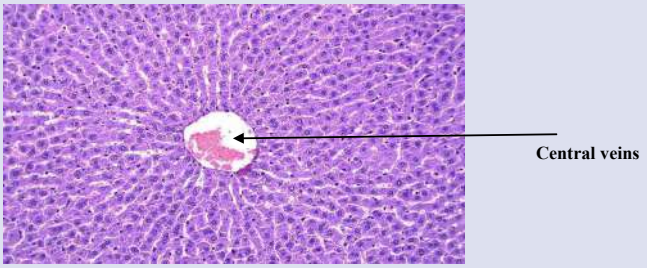

Figure 9. The Central Vein group of 750 mg/kg (G3).

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.

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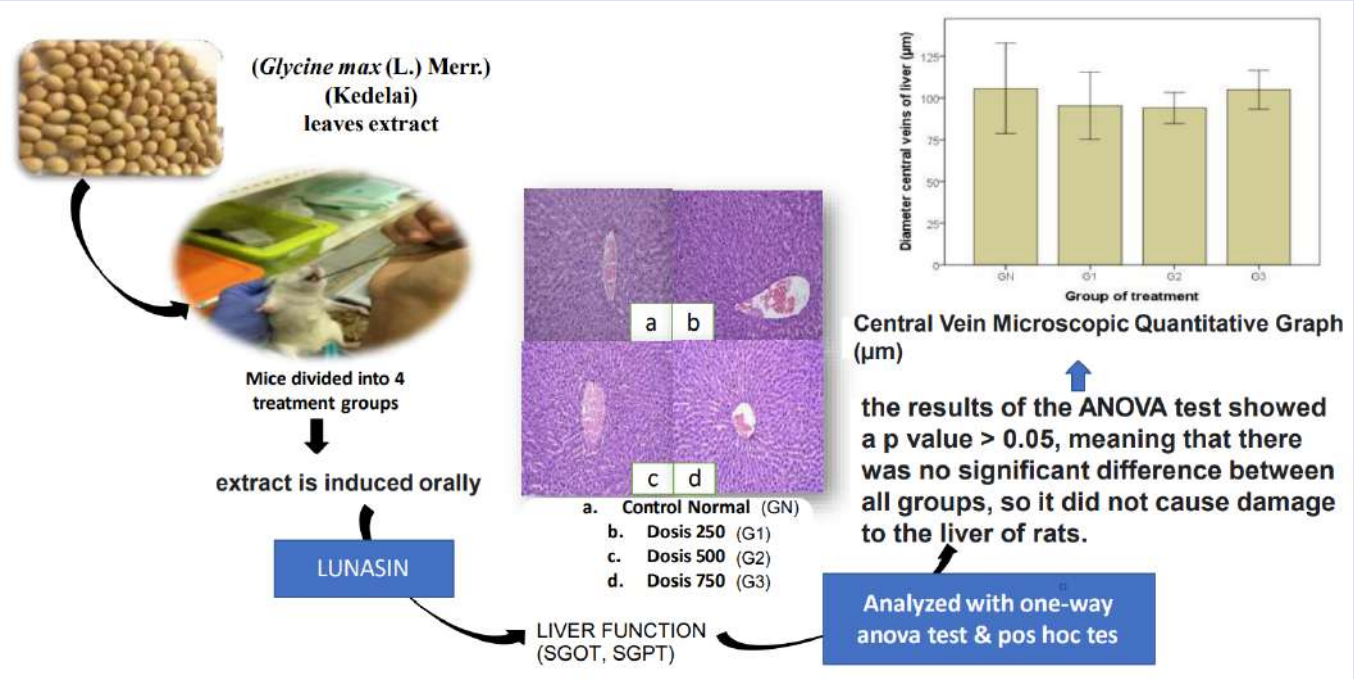
GRAPHICAL ABSTRACT



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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.

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Research Article

In Vivo Antimammary Tumor Effects of Soybean Extract with Targeted Lunasin (ET-Lun)

Numlil Khaira Rusdi[1,2], Erni Hernawati Purwaningsih[3,7], Andon Hestiantoro[4], Berna Elya[5], Kusmardi Kusmardi[6-8,*]

ABSTRACT

Background/Objective: Lunasin is a peptide, consist of 44 amino acids which have anti-cancer, antioxidant, and anti-inflammatory activity. The price of commercial Lunasin was very expensive due to the high cost of lunasin synthesis and the lack of methods to obtain the pure lunasin weight from plant sources, involving time-consuming analytical instruments. To overcome these problems, the soybean extract with targeted Lunasin (ET-Lun) was made. The aim of this study was to investigate anti-cancer properties of ET-Lun in breast cancer models *in vivo*. **Methods:** Effect of ET-Lun was evaluated in 7,12-*Dimetilbenz[a]antrasen* (DMBA) induced breast cancer rat model. Tumor Mass, volume, and number were measured. The expression of HER2 and EGFR from each treatment group in DMBA-induced rat was evaluated using immunohistochemistry. **Results:** The results shown that ET-Lun could reduced tumor volume (p=0,021). ET-Lun decreased EGFR expression compared to negative control DMBA (p=0,012). **Conclusions**: These results indicated that the ET-Lun has anti-breast cancer activity *in vivo*.
Key words: *In-vivo*, Soybean, Breast cancer, HER2, EGFR.

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INTRODUCTION

Breast cancer (BC) is the most common malignancy occurred in women worldwide.[1] In 2018, Globocan data shown that the BC's prevalence was 11.6 %.[2] Breast cancer is also one of the most frequent cancers in Indonesia.[3] According to the Riskesdas in 2018, breast cancer prevalent was increased from 1.4% in 2013 to 1.8% in 2018.[4]

Cancer therapy is still developing, one way is by natural compound exploration as sources of therapy to reduce side effects which may cause due to undesirable outcomes of chemotherapy. One of the medicinal plants that has anti-cancer activity has and being developed as an anticancer drug is soybeans (Glycine max (L.) Merr.). Consumption of soy products has been known to decrease mortality and incidence of breast cancer[5], prostate cancer[6], colon cancer[7], and lung cancer[8,9]. The active compounds in soybeans were isoflavonoids (genistein, daidzein and glycitein), Bowman-Birk protease inhibitor, Kunitz trypsin inhibitor, sitosterol, saponin, lectins, and lunasin.[9-10] Isoflavones were compound of flavonoid in soybeans has known as a strong antioxidant. Soybean has many advantages to health may be obtained from isoflavonoid. While many research has been extended to understand the anti-cancer potential of isoflavonoid, not every anti-cancer effect related to soybean consumption was from isoflavonoids.[9] Recent studies shown that a significant anti-cancer compound in soybean was a bioactive peptide : lunasin.[10],[11]

Lunasin is a peptide, consist of 44 amino acids,[12] which have anti-cancer,[9,13] antioxidant and anti-inflammatory activity[14]. Galves et al. [15] demonstrated that lunasin can inhibit the mitosis of MCF-7 cancer cells, *murine hepatoma* (Hepa 1c1c7), and embryonic fibroblast murine cells (C3H 10T1/2), resulted cell death due to binding chromatin in the kinetochore area in the centromere and blocking the microtubule attachment. Lunasin can also increase apoptosis by inducing PTEN and demonstrated to inhibit caspase-3 *in vitro* and in *vivo*.[16] Previously, lunasin also found to inhibit metastasis by suppressing cellular migration, invasion, and expression of matrix metalloproteinases (MMP)-2 and MMP-9.[17]

However, commercial price of Lunasin was very expensive due to the high cost of lunasin synthesis and the lack of methods to obtain the pure lunasin weight from plant sources, involving time-consuming analytical instruments.[18-19] The concentration of Lunasin may be influenced by cultivar, environmental factors, in particular temperature and conditions during processing.[20] To overcome such limitations, the soybean extract with targeted Lunasin (ET-Lun) was made. This extraction method was obtained from several combinations of research that conducted by Vuyyuri, et al[9], and Serra, et al[19]. ET-Lun was lunasin that extracted from soybean seed, which that has been undergoing fat removal, followed by PBS solvent under a pH of 7.4. The lunasin content in the extract was 0.86 mg/g extract of soybean.[21]

Several studies related to ET-Lun activity, including the potential of ET-Lun to reduced COX-2 expression in a dose of 150 mg/kg BW and 200 mg/kg BW of mice ($p < 0.05$). ET-Lun were also shown to decrease iNOS expression in a dose of 150 mg/kg BW of mice.[22] Moreover, ET-Lun can inhibit Goblet cell counts and micro blood density,[23] inhibit colon cancer by increased apoptosis in a dose of 150 mg/kg BW in mice, and reduced dysplasia at a dose of 200 mg/kg BW mice.[24].

The purpose of this study was to analyze the anti-breast cancer activity of ET-Lun in vivo assay. *In vivo* assay was evaluated the expression of HER2, and EGFR of cancer mammae from the treatment group in DMBA-induced rat by immunohistochemistry.

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METHODS

Plant material collection, identification and extraction

Soybean seeds (Glycine max (L.) Merr) of Grobogan variety were purchased and identified from Indonesian Legumens and Tubers Crops Research Institude, (Balitkabi) Malang, East Java. The first extraction process was oil removal of soybean seed by pressing the seeds at 100-150 atm for 30 minutes at a temperature of 120°F (48.89°C). The process was followed by a blending process to produce a powder. Soybean powder was macerated in Phosphate Buffered Saline (PBS) solvent at pH 7.4 by volume as much as 5 times of weight of the powder for 60 minutes, followed by filtration using Whatman™ 54. The filtrate was then evaporated using a vacuum rotary evaporator until thick extract was obtained.

Preliminary phytochemical screening

Preliminary phytochemical screening of ET-Lun was performed on various phytoconstituents such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids and glycosides, using various reagents.[25]

Quantitative estimation of phytochemicals

Extract's Phytochemical compounds were analyzed using several methods as follows; water value and ash value determined by Thermal Volumetric Analysis (aufhauser) and Gravimetric analysis, the microbial contaminations and were evaluated using direct inoculation, and heavy metal contaminant was examined using Atomic Absorption Spectrophotometer (AAS)

Ethics and Study Design

Female Sprague Dawley (SD) rat aged 6 weeks, divided into 5 groups of four animals in each. The groups were normal control (NOR), rats induced by 7,12-Dimethylbenz[a]anthracene (DMBA) that received corn oil as vehicle as negative control. Rats that induced by DMBA were divided into several treatments such as Tamoxifen (TAM), Lunasin (ET-Lun), and combination of ET-Lun and tamoxifen (Adjuvant group). This experiment has been approved by the Ethics Commission of the Faculty of Medicine, the University of Indonesia (the number certificate was KET-647 / UN2.F1 / ETIK/ PPM.00.02 / 2019

Animal Experimental

All rats in negative control groups were firstly induced by DMBA that has been dissolved in 2 mg/ml of corn oil. The DMBA induction was given intra-gastric at a dose of 20 mg/kg BW, carried out 11 times, twice a week.[26] Treatment was given to rats with a tumor volume of 1-2 cm^3. The ET-Lun group was given ET-Lun at doses 500 mg/kg BW, TAM was treated with Tamoxifen 10 mg/kg BW, and adjuvant group was treated with combination ET-Lun and Tamoxifen. Treatment was conducted in 8 weeks. For negative controls, tumor growth in rats was observed for 8 weeks. After following treatment, rats were terminated and the tumor tissue was removed for analysis. Tumor tissue was processed and embedded in paraffin blocks for Immunohistochemical (IHC) testing.

Immunohistochemistry of HER2 and EGFR

The IHC stain were performed according to the IHC-Paraffin Protocol from Abcam®. Assessment of HER2, and EGFR expression was performed using weighted histoscore/ H-Score, which was based on the percentage of stained cells and the intensity of the streaks. The intensity measurement was given a score of 0 - 3 (0 = none; 1 = weak; 2 = moderate; and 3 = strong). Cell calculations were performed using ImageJ and Image Profiler software, using a histoscore (H-score). The H-score was calculated by multiplying the percentage value of the intensity score. HER2 and EGFR H-Score[27,28] = 1 x (cell membrane and cytoplasm of tumor cells that stained with weak intensity, 1+) + 2 x (cell membrane and cytoplasm of tumor cells were stained with moderate intensity, 2+) + 3 x (cell membrane and cytoplasm of tumor cells that stained with with strong intensity, 3+).

RESULTS

Plant material collection, identification and extraction

Soybean plant certification was issued by the Indonesian Legumens and Tubers Crops Research Institute, (Balitkabi) Malang, East Java. The variety of soybean seed was Grobogan. (Certificate number is 0310/009. KD. Gro. BS. Kp. 19/08.19-LA).

Physicochemical evaluation

The standardization of the extract obtained was a water value of 29.82%, ash value of 2.75% and the percentage of extraction yield was 12.34%, heavy metals; lead (Pb) was 1,05 ppm and cadmium (Cd) was negative. Phytochemical screening found that soybean extracts contained flavonoids, alkaloids, saponins, triterpenoids, and glycosides.

Effect on mammary tumors

During treatment, tumor diameter and volume were evaluated once a week. The results of tumor volume for 8 weeks of treatment shown that treatment with tamoxifen, ET-Lun, and a combination of ET-Lun and tamoxifen/ adjuvant group, could reduce tumor volume when compared to negative controls (DMBA). The reduction in tumor volume was shown by the adjuvant group (Figure 1). There were differences in the tumor volume of the DMBA group and the ET-Lun and Adjuvant group starting at week 3 to 8 of treatment ($p < 0.05$). At week 3, tumor volume of ET-Lun and Adjuvan group were different compared to the negative control group, while but tumor volume of the tamoxifen group did not differ with the negative control group ($p = 0.149$). The tumor volume in the tamoxifen group was different from the DMBA group at week 4 to week 7 ($p < 0.05$). At week 8, the tumor volume in the tamoxifen group did not differ from the tumor volume in the DMBA group, whereas the tumor volume in the adjuvant group and the group given ET-Lun shown differences in the DMBA group.

The results of tumor weight (g/kg BW) after treatment, the group given the ET-Lun, tamoxifen, and the adjuvant group, could reduce tumor weight when compared to negative controls DMBA (Figure 2A). There was no difference in the weight of the DMBA tumor group with the tamoxifen, ET-Lun, and Adjuvant groups ($p > 0.05$). The tumor volume in the tamoxifen group, the ET-Lun group, and the adjuvant group was shown decreased when compared to DMBA negative control group (Figure 2B). Statistical analysis has shown that tumor volume in the DMBA group were differed from the tumor volume in the ET-Lun group ($p = 0.021$), while no difference in tumor volume of tamoxifen and adjuvant groups ($p > 0.05$).

Immunohistochemistry analysis for EGFR and HER2

The expression of EGFR (Figure 3A) showed a significant difference between the negative control DMBA and the group given ET-Lun (p=0,012) and adjuvant (p=0,021). The results of microscopic observation of EGFR expression also showed that the ET-Lun and adjuvant group was able to reduce EGFR expression compared to the DMBA group (Figure 4). EGFR expression is indicated by the presence of brown-stained cells (red arrow) on the cell membrane and cytoplasm. HER2 expression is indicated by the presence of brown-stained cells (red arrow) on the cell cytoplasm (Figure 5). In the DMBA group, almost all cell membranes were brown with moderate to strong intensity covering the membrane and cytoplasm. In contrast, in the ET-Lun group, the presence of brown-stained cells were not as much as in the DMBA group with weak intensity. Some epithelial cells were negative stained (blue), with the nucleus still clearly visible.

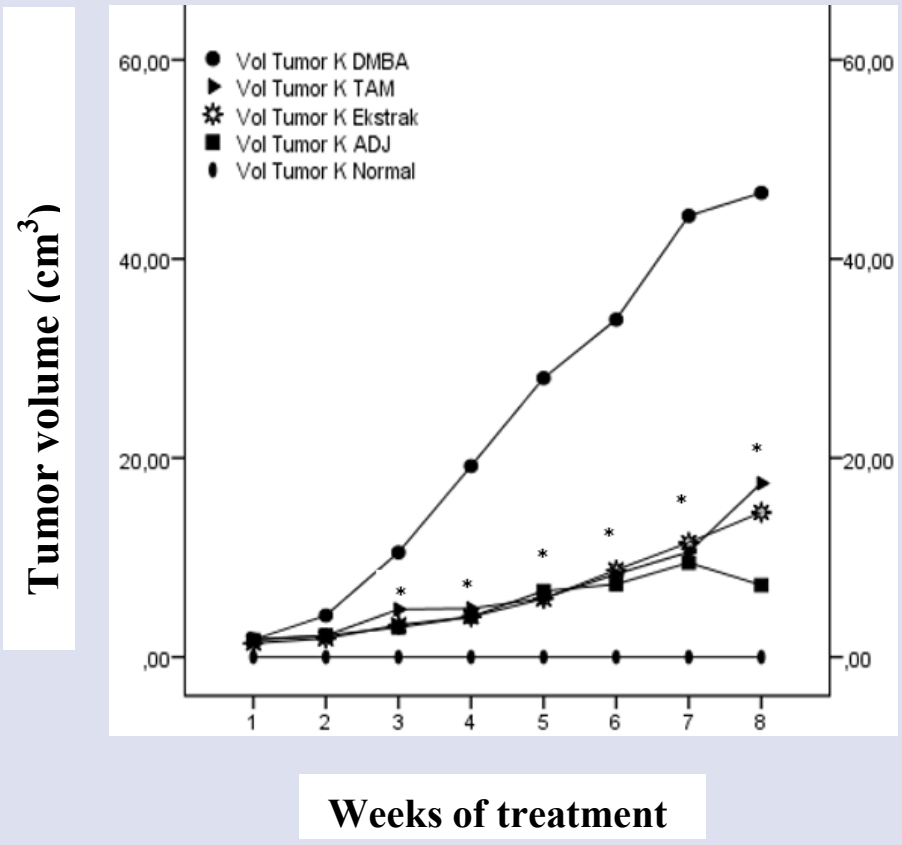
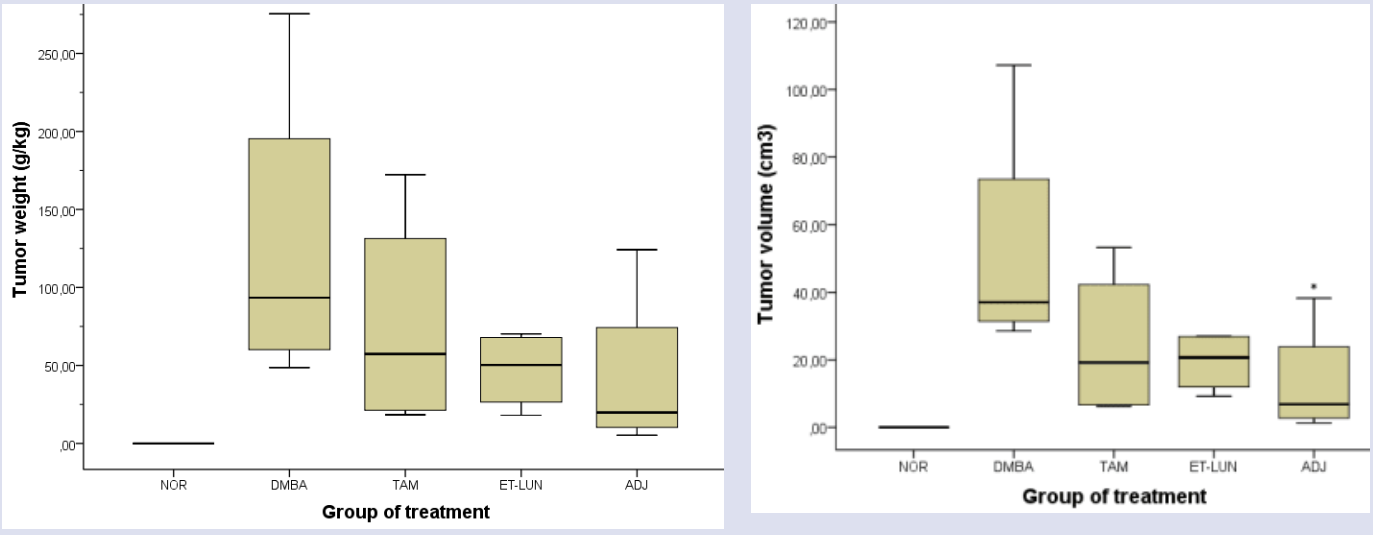


Figure 1: The tumor volume (cm3) for 8 weeks of each group treatment. Normal = normal control treated with corn oil as vehicle. DMBA = negative control treated with DMBA dissolved in corn oil; Tam = positive control treated with tamoxifen 10 mg/kg BW. K extract = treated with ET-Lun 500 mg/kg BW. Adj = treated with tamoxifen and ET-Lun. *p<0,05 compare negative group (DMBA).



(A) **(B)**

Figure 2: (A) The weight of tumor after treatment (g/kg). (B) The volume of tumor after treatment (cm^3). Nor = normal control treated with corn oil as vehicle. DMBA = negative control treated with DMBA dissolved in corn oil; Tam = positive control treated with tamoxifen 10 mg/kg BW. ET-Lun = treated with ET-Lun 500 mg/kg BW. Adj = treated with tamoxifen and ET-Lun. *p<0,05 compare negative group (DMBA).

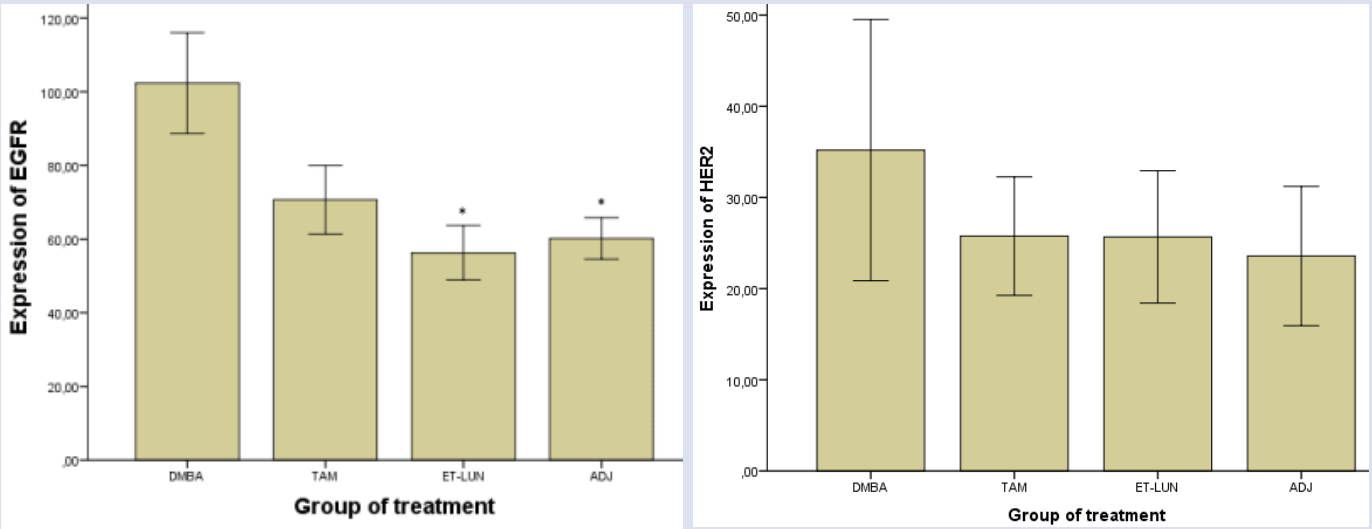


Figure 3: (A) EGFR Expression Score of the treatment group. (B) HER2 Expression Score of the treatment group. DMBA = negative control treated with DMBA dissolved in corn oil; Tam = positive control treated with tamoxifen 10 mg/kg BW. ET-Lun = treated with ET-Lun 500 mg/kg BW. Adj = treated with tamoxifen and ET-Lun. Data are presented as means±SEM (n=4). ** $p < 0,05$ compare DMBA.

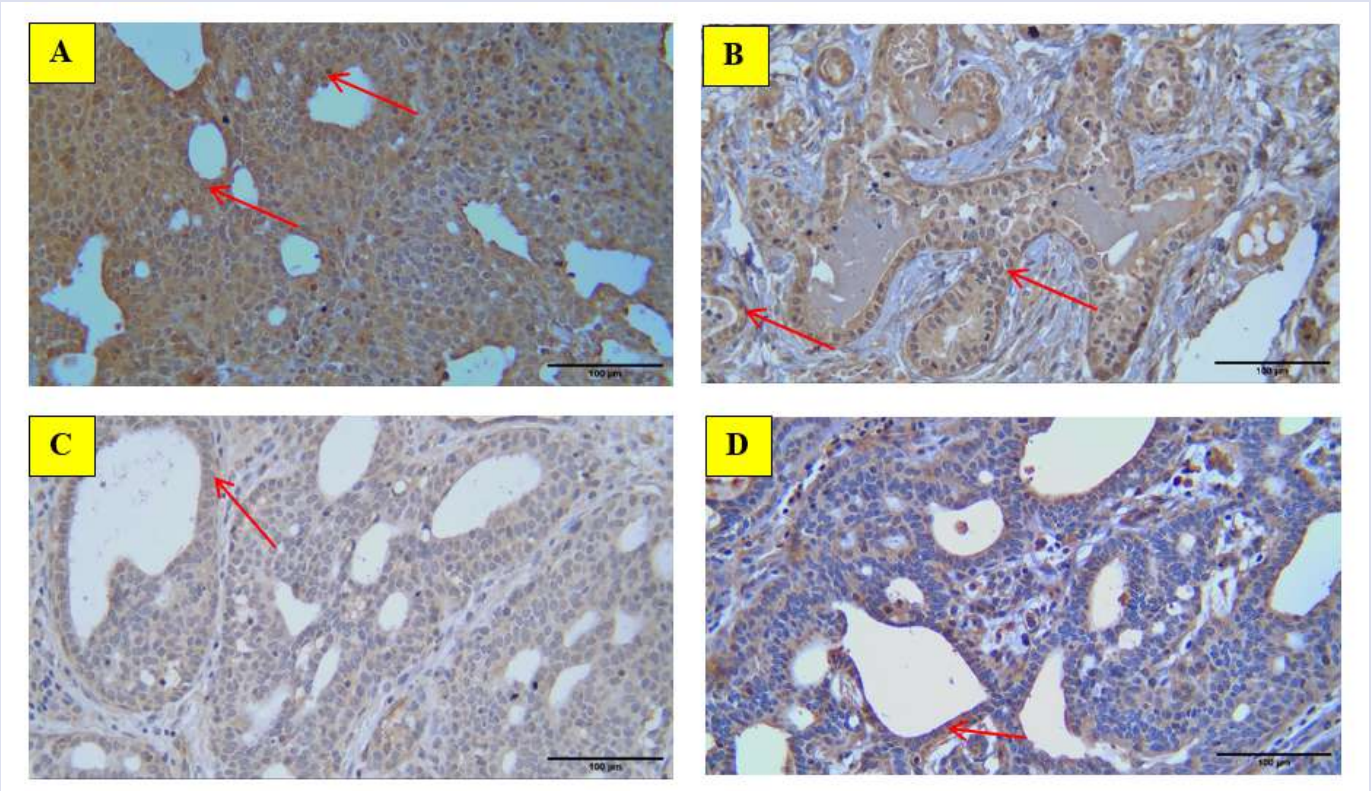


Figure 4: EGFR expression from breast cancer tissue with immunohistochemical staining in the treatment group (400X). (A) DMBA group, (B) Tamoxifen group, (C) ET-Lun, and (D) Adjuvan group.The red arrow indicates the expression of EGFR in membrane and cytoplasm of tumor cells.

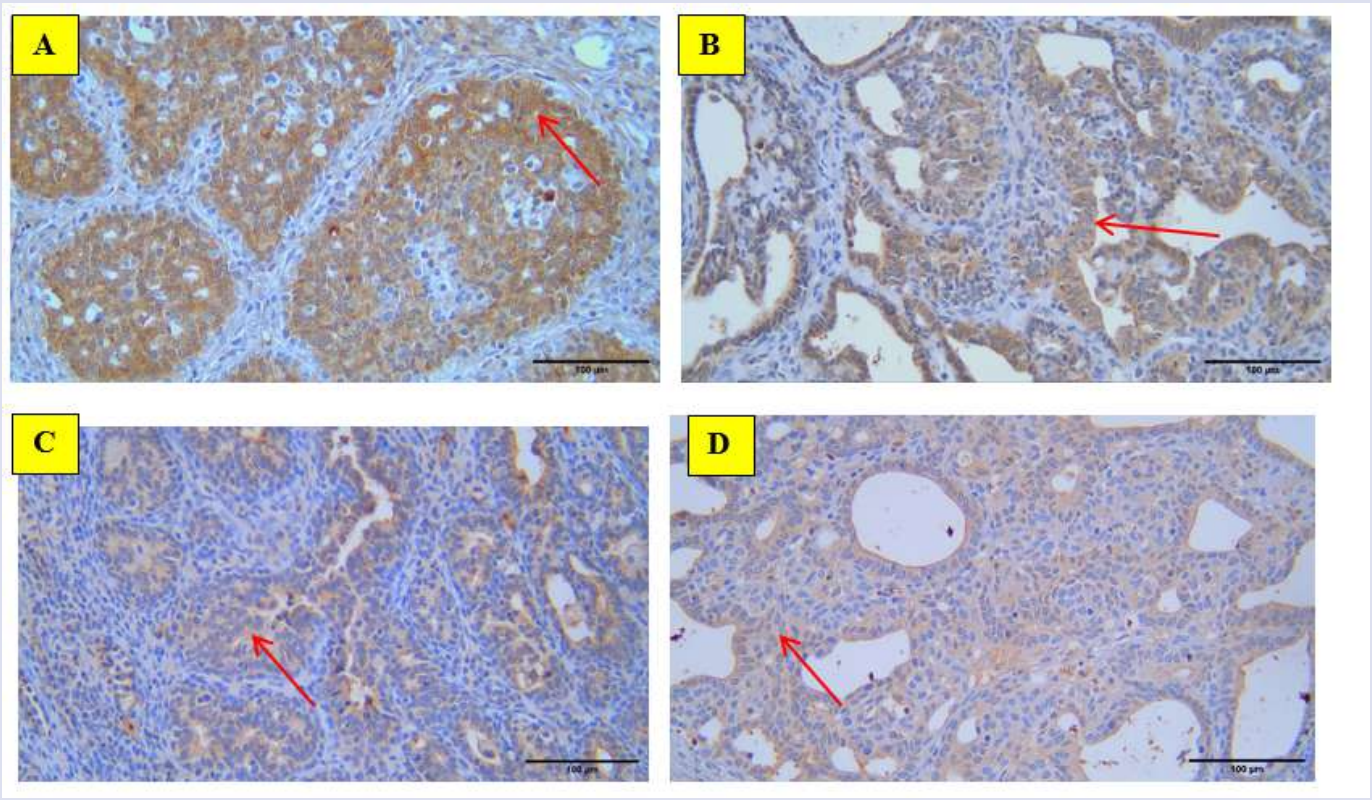


Figure 5: HER2 expression from breast cancer tissue with immunohistochemical staining in the treatment group (400x). (A) DMBA group, (B) Tamoxifen group, (C) ET-Lun, and (D) Adjuvan group. The red arrow indicates the expression of HER2 in cytoplasm of tumor cells.

DISCUSSION

Authentication and standardization are prerequisite steps while considering the source materials for herbal formulation in any system of medicine. The standardization of medical plant was necessary to ensure the effectiveness, safety, stability, and quality of phytoconstituents in medicinal plant.[29] The soybean plant is one of the plants whose their activity is being developed as a medical plant.[30] In this study, the extraction of soybean seed powder followed the previous research procedure.[22,24]

The variation of natural product composition in extract may have the pharmacological effects synergically, so that the characterization of the extract is needed for quality assurance.[29] The water value is needed to maintain the extract stability. The results of this study shown that the water value of thick extract was 29,82%. This result was in accordance with the requirements for thick extract content to that 5-30%[31] , while the ash value was 2,75%. The determination of the ash value is an indication of certain medicinal plant species because each plant has specific remains. In additions, the results of microbial contamination and heavy metal contamination are in accordance with the requirements and safety to be used.

Epidermal Growth Factor Receptor (EGFR) overexpression is phenotypes of an aggressive subtype of breast cancer and generally has a poor clinical prognosis. Sprague Dawley rat induced by DMBA, increased expression of ER, PR, EGFR, IGF1R, and activation of MAPK, JNK, and Akt signals, resulted in the development of carcinoma in breast tissue.[32,33] In this study there increased of EGFR expression was found in DMBA-induced.

Giving ET-Lun can reduce EGFR expression compared to DMBA negative control. This was also supported by a preliminary in silico test explained that lunasin can bind EGFR (data not shown). The binding of ET-Lun to EGFR resulted in decrease of EGFR expression and affects the signal propagations which are responsible for cell growth and apoptosis.

On the other hand, the EGFR expression of the tamoxifen group was not significantly different to the DMBA control group. Tamoxifen is a *selective estrogen receptor modulator* (SERM), suppress breast cancer growth by acting as an ER antagonist, by binding to the ER and inducing conformational changes that support the recruitment of corepressor proteins *nuclear receptor co-repressor* (NcoR) and *silencing mediator for retinoids and thyroid receptors* (SMRT) through the activity of histone deacetylation that plays a role in transcriptional repression.[34] In this case, tamoxifen acts only as an antiestrogen, but cannot inhibit the integration of signal transduction *growth factors*, such as EGFR.

The result of HER2 expression showed no difference between the DMBA group and ET-Lun, and Adjuvant groups. There was no difference in HER2 expression in the negative control group with the normal control group, indicated that DMBA induction in SD rats was not affected HER2 signaling. In this study, SD rats induced by DMBA increased the expression of the EGFR growth factor but did not alter HER2 expression.

CONCLUSIONS

This study demonstrated that ET-Lun could decrease EGFR expression compared to the negative control (DMBA) and might be used as a candidate for anti-breast cancer.

COMPETING OF INTEREST

None to declare.

ACKNOWLEDGEMENTS

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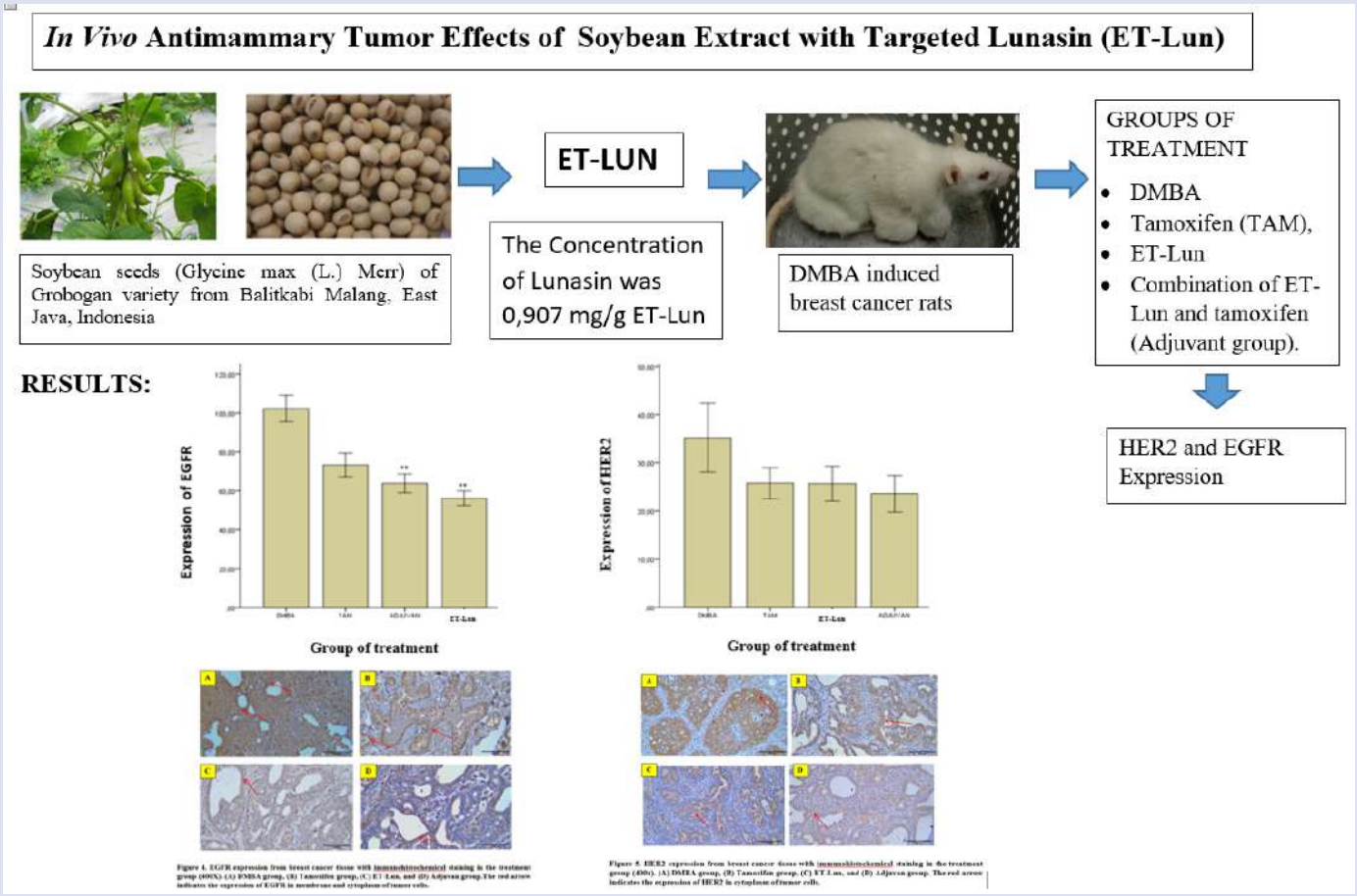
GRAPHICAL ABSTRACT



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