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Antihyperlipidemic activity of *Marchantia paleacea* herb and *Zingiber officinale* var. *Rubrum* rhizome ethanol extracts in Triton X-100-induced mice

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ABSTRACT: Non-communicable diseases cause 71% of deaths in the world, one of which is caused by dyslipidemia. Dyslipidemia has been established as a cause of various non-communicable diseases such as obesity and heart disease. This study aims to determine the antihyperlipidemic activity of the ethanol extract of the liverworts of *Marchantia paleacea* (EEMP) and the rhizome of *Zingiber officinale* var. rubrum (EEZOR) on male mice induced by Triton X-100. Grouping the number of test animals per group based on the Federer formula. Triton X-100 is used as an inducer of hyperlipidemic given intraperitoneally at a dose of 140 mg/kg body weight. Total cholesterol levels were measured by the colorimetric enzymatic method (CHOD-PAP) using a UV-Vis clinic photometer. Results from ethanol extract of the herb liverwort *Marchantia paleacea* (EEMP) and red ginger rhizomes *Zingiber officinale* var. *Rubrum* (EEZOR) can respectively reduce total hypercholesterol levels in male mice induced by Triton X-100 whose data were analyzed using the One-Way Anova test. The results of optimal dose of EEMP 200 mg/kg bw had the highest percentage of antihyperlipidemic activity and effectiveness (%) and also had a significant reduction in total cholesterol levels compared to the negative control group ($p<0.05$). While the results for the optimal dose of EEZOR 1000 mg/kg bw with the highest percentage of activity and effectiveness (%) and having a significant decrease compared to the negative control group ($p <0.05$). In conclusion, these findings suggest that both extracts have potential as natural antihyperlipidemic agents that can be further explored in the management of hyperlipidemic (dyslipidemic) and related non-communicable diseases.

KEYWORDS: Antihyperlipidemic; CHOD PAP method; *Marchantia paleacea;* total cholesterol levels; Triton X-100 induced; *Zingiber officinale* var. *Rubrum.*

INTRODUCTION

Non-communicable diseases are responsible for the mortality of around 41 million individuals annually, constituting approximately 71% of global deaths. Dyslipidemia has been identified as a contributing factor to a range of non-communicable disorders, including obesity. According to data provided by the *World Health Organisation* (WHO) in 2018, hypercholesterolemia affected a global population exceeding 160 million individuals, characterized by total cholesterol levels exceeding 200 mg/dL, a classification denoting a significantly elevated range. Furthermore, the data revealed that over 34 million adults residing in the United States exhibited total cholesterol levels surpassing 240 mg/dL, a threshold indicative of high cholesterol necessitating therapeutic intervention. Based on data from the American Heart Association in 2018 shows the prevalence of people with cholesterol levels ≥240 mg/dL of 31.9 million people (13.8%) of the population. Based on the results of Riskesdas (2018) which shows that the population aged 15 years and over for the national prevalence in Indonesia around 21.2% of the population in Indonesia suffers from cholesterol above 200 mg/dL, 36.5% had LDL levels above 100 mg/dL, 24.3% had HDL levels less than 40 mg/dL, and 13.3% had triglyceride levels above 150 mg/dL [1],[2].

Cholesterol has a crucial role in the regulation of both membrane fluidity and permeability. In addition, it is an amphipathic lipid as the outer layer of plasma lipoproteins which has a very important role in the body

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contained in the blood and produced by the liver. Elevated cholesterol levels in the blood are referred to as hypercholesterolemia. Hypercholesterolemia can occur due to abnormalities in lipoprotein levels in the blood. Pharmacological therapy by providing antihyperlipidemic drugs, one of which is a statin class drug, including simvastatin drugs. Statins, as a pharmacological class, are considered primary therapeutic agents that function through the inhibition of the enzyme known as 3-hydroxy 3-methyl-glutaryl-CoA reductase (often referred to as HMG-CoA reductase inhibitor). This enzyme is responsible for the conversion of HMG-CoA into mevalonic acid, ultimately leading to the production of cholesterol [3]. In rodent test animal models such as mices, Triton X-100 compound has a mechanism of action to increase lipid levels (including total cholesterol) by blocking the process of liver tissue absorbing lipoproteins from the bloodstream leads to elevated lipid levels in the blood and promotes cholesterol production by inhibiting lipase enzyme activity [4],[5],[6],[7],[8],[9],[10].

The use of plants that have the potential as medicine is traditionally widely used by the people of Indonesia as a complementary therapy. Liverwort plants of the genus *Marchantia* including the species *Marchantia paleacea* is a plant that has been extensively utilized in empirical applications for therapeutic purposes. *Marchantia paleacea* is a species of liverwort that belongs to the Marchantiaceae family. *Marchantia paleacea* Bertol. is a liverwort species of to the genus *Marchantia*, which has historically been employed as an empirical remedy in China, Europe, North America, and Indonesia. Nevertheless, the investigation and application of pharmacological actions remain inadequately investigated [11], [12], [13], [14]. Secondary metabolite compounds such as: terpenoids, flavonoids, saponins and phenolic compounds are antioxidants that play a role in the mechanism of improving lipid profiles. Flavonoid compounds have been observed to decrease cholesterol synthesis through the inhibition of acyl-CoA cholesterol acyl transferase (ACAT) enzyme activity in HepG2 cells. This inhibition is associated with a reduction in cholesterol esterification in both the intestine and liver. Additionally, the compounds also inhibit the activity of 3-hydroxy-3-methyl-glutaryl-CoA enzyme, leading to the inhibition of cholesterol synthesis [15]. Flavonoid group compounds are also contained in the herb of liverwort *Marchantin paleacea*, namely marchantin compounds and in the rhizomes of *Zingiber officinale* var. *Rubrum* like gingerol. Rhizome *Zingiber officinale* var. *Rubrum* also contains more 10-gingerol, 6-gingerol, and its acetate derivatives [13],[16],[17].

However, there has been no data on preclinical antihyperlipidemic pharmacological activity in a test animal model of mice induced by Triton X-100 from ethanol extract of the liverwort herb *Marchantia paleacea*. Likewise, there is still little data on the antihyperlipidemic activity of ethanol extract of *Zingiber officinale* var. *Rubrum* rhizome preclinically (in-vivo). Based on this, researchers are interested in conducting research on the potential antihyperlipidemic activity of ethanol extract of the herb liverwort *Marchantia paleacea* and rhizomes *of Zingiber officinale* var. *Rubrum* in a test animal model of male mice of *the Swiss Webster* strain induced by Triton X-100.

▪ MATERIALS AND METHODS

Material

The ingredients used during the study were: herb of the liverwort plant *Marchantia paleacea*, rhizomes of red ginger *Zingiber officinale* var. *Rubrum,* technical 96% ethanol, simvastatin tablets 10 mg (Selvim®, PT. IFARS Pharmaceutical Laboratories, Indonesia), Na-CMC, Triton X-100, reagents and total cholesterol standards (Reiged Diagnostic®, Saudi Arabian Arabic), physiological NaCl 0.9%, Wagner reagent, Dragendorff reagent, Mayer reagent, concentrated HCl, HCl 2N, concentrated H_2SO_4, chloroform, ethanol, sodium chloride, anhydrous acetic acid, concentrated ammonia, n-hexane, gelatin, magnesium powder (Mg), ferric chloride ($FeCl_3$), KOH 5N, and aquadest.

Animal test

The test animals used were male mice with *Swiss Webster* strains aged the specimen under consideration is estimated to be between 2 and 3 months old, exhibiting a body mass ranging from 20 to 30 g. Test animals are acclimatized for 7 days which are placed in standard laboratory cages of test animals at room temperature.

Equipment

The tools used during this study were analytical balances, test animal analytical balances, oral sonde mice, disposable syringes, disposable tips, centrifuges, centrifugation tubes, UV-Vis clinical photometers (Biolabo® Chemical Analyzer, Maizy City, France), micropipettes, microtubes, rotary vacuum evaporators (Rotavapor R-3 BUCHI®, Flawil City, Swiss), beaker glass (Pyrex®, New York, USA), measuring cups (Pyrex®, New York, USA), measuring flasks (Pyrex®, New York, USA), glass funnels, stirring rods, drip pipettes,

spatels, steam cups, waterbath, scissors, mortar, stamper, blender, glass jar, filter paper, and other laboratory tools.

Population and test samples

The population in this study was the herb of the liverwort plant *Marchantia paleacea* was collected from Padajaya Village and Sindangjaya Village, located in the Cipanas District of the Cianjur Regency in West Java, Indonesia and the red ginger rhizomes, scientifically known as *Zingiber officinale* var. Rubrum, were acquired from the Manoko Experimental Garden located in Lembang, West Bandung Regency, West Java, Indonesia.

The test extract samples in this study were 96% ethanol extract from the herb of liverwort *Marchantia paleacea* (EEMP) and rhizomes of *Zingiber officinale* var. *Rubrum* (EEZOR). While the rodent test samples used were male mice of the *Swiss Webster* strain with the number of uses of test animals using the Federer formula [11], [12], [18].

Time and place of research

The research was carried out at the Phytochemistry Laboratory, Pharmacology Laboratory, and Pharmaceutical Analysis Chemistry Laboratory on the campus of the Department of Pharmacy, Poltekkes Kemenkes Bandung and Integrated Laboratory Poltekkes Kemenkes Bandung in 2023.

Plant determination and making of *herbaceous simplisia of liverworts Marchantia paleacea* and *Rhizome of Zingiber officinale var. Rubrum*

Both plants were carried out a process of determination of test plant samples carried out at the Plant Taxonomy Laboratory of the Department of Biology, FMIPA Universitas Padjadjaran. Both samples undergo a process of rinsing with flowing water in order to eliminate impurities such as soil, gravel, and grass. The specimen was subjected to ambient conditions for a duration of seven days in order to facilitate the drying process. Then the sample is sorted and ground using a blender into simplisia powder [19], [20], [21].

Determination of drying shrinkage

The gravimetric approach is employed for the determination of drying shrinkage in simplisia. The two simplisia that have been dried are then chopped into smaller sizes. The porcelain dish is cleaned and heated using an oven at 105°C for 2 hours. Then cooled on a desiccator for 15 minutes, then weighed as the weight of an empty cup. Simplisia is put into a porcelain dish of approximately 1 g and then weighed as the weight of simplisia before heating. The porcelain cup containing simplisia was reheated using an oven at 105 °C for 2 hours with the lid of the porcelain cup open. Then the porcelain dish is cooled on a desiccator for 15 minutes, then weighed as a simplisia weight after heating [22],[23].

Preparation of ethanol extracts of *Marchantia paleacea (EEMP)* and *Zingiber officinale var. Rubrum Rhizome (EEZOR)* and phytochemical screening in both test extracts

A total of 600-grams of both types of simplisia were then the extraction process employed the maceration method, utilizing a solvent consisting of 96% ethanol. The maceration process is carried out for 3 x 24 hours with a periodic stirring process. The liquid extract is then evaporated with a solvent with a rotary vacuum evaporator at 45°C and then thickened by evaporation using a waterbath at 50°C until a viscous extract with a constant weight is obtained then calculated the yield results of the two test extracts (%). Phytochemical screening on the two test extracts was also carried out to test the class of alkaloids, flavonoids, tannins, and polyphenols, saponins, and steroids/triterpenoids in accordance with standardized methods from WHO, Ministry of Health, and several scientific journals [11], [22], [23], [24], [25], [26].

Antihyperlipidemic activity testing in a test animal model of male mice of Swiss Webster strain induced by Triton X-100

This preclinical (in-vivo) research was carried out through approval from the Research Ethics Committee for the Use of Test Animals KEPK Poltekkes Kemenkes Bandung with number: 24/KEPK/EC/V/2023. The test was done by reduction of total cholesterol levels in male mice of the *Swiss Webster* strain from the two test extracts with the following procedure:

1. The test animals underwent a period of acclimatization lasting seven days, after which they were subsequently separated into nine distinct treatment groups, namely: normal control group (Na-CMC 0.5%), negative control (Triton X-100), positive control (simvastatin dose 13 mg/kg bw); EEMP 50, 100, and 200 mg/kg bw; and EEZOR groups 250, 500, and 1000 mg/kg bw. Furthermore, all test animals were satisfied for 18 hours. Then all groups of test animals (except the normal control group) were induced with Triton X-100 at a dose of 140 mg/kg bw intraperitoneally until day 3 (day 1, 2, and 3).

2. After 24 hours of Triton X-100 induction, the first dose of each test solution was administered in each treatment group except in the normal control group orally from day 2 to day 8.
3. Blood sampling of 0.5–1.0 mL from each test animal was taken intracardiac after 24 hours of the last dose.
4. Blood samples are then centrifuged, and blood serum is taken from each test animal to test total cholesterol levels by the enzymatic colorimetric method (CHOD-PAP) by pipetting into a microtube.
5. A serum sample of 10 µL was then mixed with a 1000 µL CHOD-PAP working reagent (as well as the standard reagent provided), subsequently, the samples were incubated for a duration of 10 minutes under ambient conditions (room temperature) or 5 minutes at 37^{o}C. Absorbance readings were performed on a clinical UV-Vis photometer at a wavelength of 500 nm. The calculation of total cholesterol levels was obtained using absorbance data as conducted by Parwin, et al. (2019) and Ajayi, A., et al. (2019) [4], [27], [28], [29], [30].

Data processing and analysis

The data from the study in the form of total cholesterol levels were analyzed statistically. The first test is the normality test and homogeneity test as a condition for the next test. If the data is normal and homogeneous, then proceed to use the One-Way Anova test and to find out which group has a significant difference, a post hoc test is used, namely LSD. If the data is abnormal or inhomogeneous, then an alternative test is carried out, namely the Kruskal-Wallis test with post hoc analysis used is the Mann-Whitney test [4], [28], [29], [31].

▪ RESULTS AND DISCUSSION

Results of organoleptic examination, drying loss rate, and extract yield percentage (% w/w)

The test samples used for antihyperlipidemic activity research in Triton X-100-induced mice test animal models are the herb *of Marchantia paleacea* liverwort and red ginger rhizomes (*Zingiber officinale* var. *Rubrum*). The determination on the liverwort herb plant *Marchantia paleacea* was carried out at the Plant Taxonomy Laboratory of the Department of Biology, FMIPA Universitas Padjadjaran which has a certificate with No. 30/HB/05/2023. Samples of liverwort herb *Marchantia paleacea* were obtained by harvesting in Padajaya Village, Sindangjaya Village, Cipanas District, Cianjur Regency, West Java, Indonesia. While the rhizome of *Zingiber officinale* var. *Rubrum* was obtained at Manoko Experimental Garden, Lembang, West Java. Both fresh ingredients are dried by aerating until dry simplisia weights are obtained on the herb of *Marchantia paleacea* liverwort and rhizomes of *Zingiber officinale* var. *Rubrum*. The two simplisia were then tested for drying shrinkage which was obtained respectively by 9.44% (*M. paleacea* liverwort herbs) and 7.21% (*Z. officinale* var. *Rubrum* rhizomes).

Herbaceous simplisia of liverworts *M. paleacea* and rhizomes of *Z. officinale* var. *Rubrum* is carried out a cold extraction process of maceration was conducted employing a solvent consisting of 96% ethanol. The extraction process is carried out for 3 x 24 hours which is periodically carried out by the stirring process. The maserat is subsequently subjected to concentration using a rotary vacuum evaporator maintained at a temperature of 40°C and then evaporated on a waterbath at 40°C to become a thick extract. The yield of *M. paleacea* liverwort herb extract obtained was 4.18% and *Z. officinale* var. *Rubrum* rhizome extract obtained was 23.13% as seen in Table 1. Based on observations from Table 1, ethanol extract of liverwort herb of *M. paleacea* has the appearance of a thick brownish-green liquid, tasteless but over time feels chelate and has a characteristic aromatic odor. While ethanol extract of rhizomes of *Z. officinale* var. *Rubrum* is a thick reddish-brown extract, spicy taste, and distinctive aroma of ginger.

Table 1. Results of organoleptic examination, drying loss rate, and percentage of extract yield (% w/w) on 96% ethanol extract of *Marchantia paleacea* Bertol. Liverwort Herbace. (EEMP) and Rhizome of *Zingiber officinale* var. *Rubrum* (EEZOR).

Plant parts and plant types tested	Types of solvents used	Extraction method	Organoleptic observation of test extracts	Extract yield percentage (% w/w)
Liverwort Herb of *Marchantia paleacea* Bertol. (EEMP)	Ethanol 96%	Maceration	a. Form: Viscous liquid (extract) b. Color: Brownish-green c. Taste: Tasteless but over time it feels chelate d. Smell: Smelly with a characteristic aroma	4.18
Rhizome *Zingiber officinale* var. *Rubrum* (EEZOR)	Ethanol 96%	Maceration	a. Form: Viscous liquid (extract) b. Color: Reddish brown c. Taste: Spicy d. Smell: Smelly with the characteristic aroma of the rhizomes of *Zingiber officinale*	23.13

This study was conducted to determine the antihyperlipidemic activity of ethanol extract of the herb liverwort *Marchantia paleacea* and rhizomes of *Zingiber officinalis* var. *Rubrum* in a test animal model of male mice of the *Swiss Webster* strain induced by Triton X-100. *Marchantia paleacea* is an empirical plant species that has been widely used as part of traditional medicine (ethnomedicinal), such as in China, Latin America, India, and Indonesia. Related to the results of pharmacological activity tests that have been carried out to date from various scientific literature, namely as antioxidants, antibacterials, vasorelactan, immunostimulants (immunomodulators), and hepatoprotectors [12], [13], [14], [32], [33]. While research on red ginger rhizomes (*Zingiber officinale* var. *Rubrum*) because there is still little data on preclinical (in-vivo) antihyperlipidemic studies in rodent test models (including: mice) induced by Triton X-100 compounds [34], [35].

Phytochemical screening results on 96% ethanol extract of *Marchantia paleacea* Bertol. Liverwort Herb (EEMP) and Rhizome of *Zingiber officinale* var. *Rubrum* (EEZOR)

Results of phytochemical screening from ethanol extracts of liverwort herb *M. paleacea* and rhizomes of *Z. officinale* var. *Rubrum* contains several secondary metabolite groups as shown in Table 2. Ethanol extract of liverwort herb of *M. paleacea* contains secondary metabolites of steroids/triterpenoids, flavonoids, saponins, and polyphenols/tannins. While ethanol extract of rhizomes of *Z. officinale* var. *Rubrum* contains secondary metabolites such as triterpenoids (leukosanthines), flavonoids (flavanones), alkaloids, saponins, and polyphenols.

Table 2. Phytochemical screening results on 96% ethanol extract of *Marchantia paleacea* Bertol. Liverwort Herba (EEMP) and Rhizome of *Zingiber officinale* var. *Rubrum* (EEZOR).

Class of secondary metabolite compounds	Types of reagents (reagents)	Results from EEMP	Results from EEZOR
Steroid and Triterpenoid	Lieberman Burchard	+	+
	Salkowski	+	n/a
	Bate Smith-Metcalf (2 mL concentrated HCl)	+	+ (Leukosanthines)
Flavonoid	Wilstater (2 mL HCl concentrated + 4 Magnesium powder tape)	+	+ (Flavanon)
Alkaloid	Mayer	-	+

Class of secondary metabolite compounds	Types of reagents (reagents)	Results from EEMP	Results from EEZOR
	Wagner	-	+
Antraquinone	Borntrager		-
Saponin	Froth test	+	+
Polyphenol dan Tannin	Gelatin 1% Test	+	-
	1% $FeCl_3$ Test	+	+ (Polyphenol)

Information:
EEMP = Ethanol extract of *Marchantia paleacea* herb
EEZOR = Ethanol extract of *Zingiber officinale* var. *Rubrum*

Some secondary metabolite content in liverwort herb *M. paleacea* as in the research of Fadhilla, et al. (2012) and Asakawa, Y. (2017) namely bis-bibenzyl (marchantin) compounds, terpenoids, flavonoids, saponins, and phenolic compounds [36], [37]. While on the rhizomes of red ginger (*Zingiber officinale* var. *Rubrum*) has a higher gingerol and shogaol content than empirrit ginger and elephant ginger, namely the average gingerol (23-25%) and shogaol (18-25%) levels [38].

The process of determining the test plant is carried out to determine the correctness of the identity of the test sample and avoid errors in sample collection [39]. The process of drying fresh ingredients into simplisia by aeration, namely by drying indoors so as not to be exposed to direct sunlight to avoid damage to the active compounds contained in the sample which is a group of flavonoids and phenolic compounds that have the characteristics of not being resistant to heat and easily oxidized at high temperatures [11], [12], [40]. Simplisia that has been dried is then tested for drying loss using gravimetric method using an oven at 105°C. The outcome of drying loss can establish an upper bound on the quantity of compounds that are lost during the drying procedure (in general, the drying loss requirement is less than 10%). The amount of water contained after the drying process in simplisia which is less than 10% can stop the enzymatic reaction in simplisia to prevent damage to the secondary metabolite compounds it contains and can be stored for a longer time [22], [41].

Size reduction in liverwort herb *M. paleacea* and rhizomes of *Z. officinale* var. *Rubrum* is also carried out to expand the surface of the simplisia in contact with the solvent so that the extraction process will be easier [42], [43]. The maceration method was chosen by considering the characteristics of the compounds contained in simplisia, which are not resistant to heat and easily oxidized at high temperatures. In addition, this method of maceration requires only relatively simple equipment [44], [45]. 96% ethanol solvent was chosen as the solvent of the extraction process with this maceration method because it is a type of solvent that has the ability to extract with a wide level of polarity (universal solvent) ranging from non-polar to polar compounds. So, it is expected to extract flavonoid compounds such as bis-bibenzyl compounds (marchantin) from ethanol extracts of liverwort herb *M. paleacea* which has semi-polar properties. In addition, gingerol compounds in EEZOR have a good degree of polarity in 96% ethanol solvents compared to 30% and 70% ethanol solvents [46], [47], [48]. The calculation of the percentage of extract yield (%) is done to find out how much compound can be extracted from a material such as: plants or other natural materials after a certain extraction method process [11].

Results of average total cholesterol levels (mg/dl), percentage of antihyperlipidemic activity (%), and percentage of antihyperlipidemic effectiveness (%) of the entire test group in male mice of the *swiss webster* strain after triton-x induced antihyperlipidemic activity testing

Test animals before being given test treatment are acclimatized first for 7 days so that test animals can adapt to environmental conditions during research. The number of male mice of the *Swiss Webster* strain used for both tests in each test group was used as many as 5 replicates of test animals (n = 5). The feed of each test group used uniform laboratory standard feed (the same), as well as the drinking given.

The results of testing antihyperlipidemic activity in a test animal model of male mice of the *Swiss Webster* strain were carried out during 8 days of testing. Total cholesterol levels on day 8 (post-test) can be seen in Table 3. The results showed that the average total cholesterol levels in ethanol extract of *M. paleacea* herb ranging from the lowest to the highest respectively were 59 mg/dL (negative control), 59.25 mg/dL (EEMP 200 mg/kg bw), 63.75 mg/dL (positive control/simvastatin), 70.50 mg/dL (EEMP 100 mg/kg bw), 88.75 mg/dL (EEMP 50 mg/kg bw), and 125.25 mg/dL (negative control). While the average result of total cholesterol levels in ethanol extract of rhizomes of *Z. officinale* var. *Rubrum* from lowest to highest total cholesterol levels respectively amounted to 15.10 mg/dL (EEZOR 1000 mg/kg bw), 42.73 mg/dL (EEZOR 500

mg/kg bw), 47.72 mg/dL (EEZOR 250 mg/kg bw), 59.00 mg/dL (normal control), 63.75 mg/dL (positive control/simvastatin), and 125.25 mg/dL (negative control).

Table 3. Average total cholesterol levels (mg/dL) of the entire test group in male mice of the *Swiss Webster* Strain after Triton-X induced antihyperlipidemic activity testing at day 8 (n=5).

Test group	Average total cholesterol levels (mg/dL; $\bar{x}$ ± SD)
Control Normal[a]	59,00 ± 11,9722**
Negative Control[b]	125,25 ± 11,7580
Positive Control (Simvastatin)[c]	63,75 ± 2,5000**
EEMP 50 mg/kg bw[d]	88,75 ± 7,6322**
EEMP 100 mg/kg bw[e]	70,50 ± 10,1490**
EEMP 200 mg/kg bw[f]	59,25 ± 10,8743**
EEZOR 250 mg/kg bw[g]	47,72 ± 8,5496**
EEZOR 500 mg/kg bw[h]	42,73 ± 6,3092**
EEZOR 1000 mg/kg bw[i]	15,10 ± 4,3403**

Information:
[a]Group given 0.5% Na-CMC suspension preparation orally
[b]The Triton X-100 induced group of 140 mg/kg bw (2.8 mg/20 g bw) mice intraperitonial and administered 0.5% Na-CMC suspension orally
[c]The Triton X-100-induced group of 140 mg/kg bw (2.8 mg/20 g bw) mice intraperitonial and given simvastatin 1.3 mg/kg bw (0.026 mg/20 g bw) orally
[d]The Triton X-100 induced group of 140 mg/kg bw (2.8 mg/20 g bw) mice intraperitonial and given ethanol extract of the liverwort herb of *M. paleacea* 50 mg/kg bw orally
[e]The Triton X-100 induced group of 140 mg/kg bw (2.8 mg/20 g bw) mice intraperitonial and given ethanol extract of the liverwort herb of *M. paleacea* 100 mg/kg bw orally
[f]The Triton X-100 induced group of 140 mg/kg bw (2.8 mg/20 g bw) mice intraperitonial and given ethanol extract of the liverwort herb of *M. paleacea* 200 mg/kg bw orally
[g]The Triton X-100-induced group was then given ethanol extract of rhizomes of *Zingiber officinale* var. *Rubrum* 250 mg/kg bw
[h]The Triton X-100-induced group was then given ethanol extract of rhizomes of *Zingiber officinale* var. *Rubrom* 500 mg/kg bw
[i]The group induced Triton X-100 which was then given ethanol extract of rhizomes of *Zingiber officinale* var. *Rubrum* 1000 mg/kg bw
*There was a significant decrease in the negative control group ($p<0.05$)
**There was a significant decrease in the negative control group ($p<0.01$)

The initial stage carried out is a normality test with the Shapiro Wilk test for a sample count of less than 50. Then obtained normal data results ($p > 0.05$). Following the completion of the normality test, a subsequent homogeneity test was conducted using the Levene test. The acquired result indicated a p-value greater than 0.05, leading to the conclusion that the data exhibits equal variance, thus confirming its homogeneity. The data obtained have a normal distribution of data and have the same data variance. Furthermore, the One-Way Anova test was carried out with the LSD post hoc test. The test results can be seen in Table 3. The statistical analysis procedure was conducted in order to ascertain the disparity in total cholesterol levels among each treatment group. From the average results of cholesterol levels in both types of test extracts, the percentage of antihyperlipidemic activity (%) and the percentage of effectiveness of antihyperlipimidemic (%) were calculated as can be seen in Figure 1. The highest and second highest percentages of antihyperlipidemic activity (%) were obtained in ethanol extracts of rhizomes of *Z. officinale* var. *Rubrum* (EEZOR) 1000 and 500 mg/kg bw which was 166.26% and 124.56% respectively and the highest and second highest percentage of antihyperlipidemic (%) effectiveness was obtained at EEZOR 1000 and 500 mg/kg bb respectively at 179.11% and 134.18%, respectively. While the highest percentage of antihyperlipidemic activity and effectiveness (%) in ethanol extract of liverwort herb of *M. paleacea* was respectively 99.62% and 107.31% compared to positive controls (simvastatin) which were respectively 92.83% (percentage of activity) and 100.00% (percentage of effectiveness).

As seen in Table 3, it appears that all treatment groups showed a reduction in total cholesterol levels compared to the negative control group ($p<0.05$). This suggests that both EEMP and EEZOR have some level of antihyperlipidemic activity in this model. In particular, the group given the highest dose of EEZOR (1000 mg/kg body weight) showed the most substantial reduction in cholesterol levels. The use of simvastatin, a known cholesterol-lowering medication, as a positive control validates the effectiveness of the treatment since it also showed a significant reduction in cholesterol levels. The negative control group had an increase in cholesterol levels indicating the successful induction of hyperlipidemia by Triton X-100. Treatment groups receiving EEMP at various doses all showed a reduction in cholesterol levels, with the 200 mg/kg bw dose

showing the most significant effect, nearly matching the levels of the normal control group. The groups treated with EEZOR also displayed reduced cholesterol levels, with the most pronounced effect observed at the 1000 mg/kg bw dose. It is also important to consider the pharmacological relevance of these findings to humans and the potential for these extracts to be developed into therapeutic agents [49], [50], [51], [52], [53].

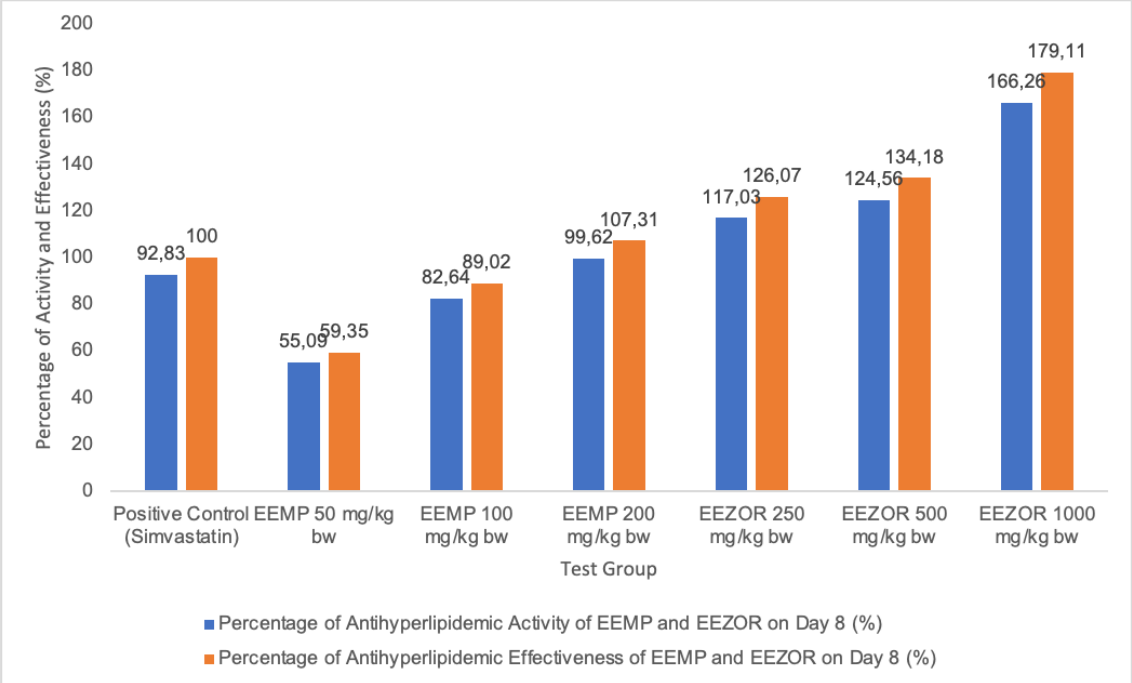

Figure 1. Bar chart of antihyperlipidemic activity and effectiveness percentage (%) in the entire test group. (EEMP and EEZOR) at day 8 in Triton X-100-induced test animal models (n=5)

The experimental subjects employed in this preclinical investigation of antihyperlipidemic activity were male *Swiss Webster* mice. Mice have physiological similarities with humans. Mice are used in research because of advantages such as relatively short life cycles, large number of children per birth, easy to handle, have reproductive characteristics similar to mammals, anatomical, physiological, and genetic structures similar to humans and have a calmer nature and easier to intervene, making it easier to provide test treatment. The selection of male sex is therefore only slightly affected by hormonal. In male mice, estrogen hormone levels are less than in female mice. In addition, female mice also have a higher level of stress so it is feared that it can interfere with the testing process [49], [50], [51], [52], [53].

In this study, positive control/comparison of simvastatin compounds was used. Simvastatin is a first-line drug class that works by inhibiting the enzyme 3-hydroxy 3-methyl-glutaryl-CoA reductase (HMG-CoA reductase inhibitor) which converts HMG-CoA into mevalonic acid with the product, cholesterol. The choice of simvastatin is based on similarities with the mechanism of action of one of the compounds contained in the herb marchantin liverwort *Marchantia paleacea*, namely marchantin compounds [3], [13], [54], [55], [56]. Another effect of using simvastatin is that it can reduce oxidative stress so that there is a decrease in lipid peroxidation. In addition to lowering total cholesterol levels, statin antihyperlipidemic drugs can also reduce LDL and triglyceride levels and increase HDL levels in the blood [3]. Marchantin compounds which are flavonoids (phenolics) can act as antioxidants that can prevent lipid peroxidation. In addition, compounds from this group are thought to have a mechanism of action to reduce the activity of the enzyme HMG-CoA reductase [15], [16], [17].

The process of inducing hyperlipidemic is carried out by intraperitonial administration with Triton X-100 at a dose of 140 mg/kg bw (2.8 mg/20 g bb). Triton X-100 is classified as a non-ionic surfactant compound that has a mechanism of action that can increase the synthesis of cholesterol and triglycerides in the liver and can suppress the activity of lipoprotein lipase impedes the uptake of lipoproteins from the peripheral circulation, leading to elevated lipid levels in the bloodstream [29]. When lipoprotein lipase which is a lipolytic enzyme is inhibited, VLDL cannot be converted into free fatty acids in the extrahepatic circulation. So that it can stimulate lipid synthesis resulting in hyperlipidemic. In previous studies, Triton X-100 can significantly increase total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, and lower HDL cholesterol [4].

Testing of total cholesterol levels from blood serum samples of mice test animals was carried out using the colorimetric enzymatic reaction method using a clinical UV-Vis photometer. The use of the enzymatic reaction method was chosen because it is a method that is in accordance with World Health Organization (WHO) or International Federation of Clinical Chemistry (IFCC) standards for preclinical (in-vivo) determination of total cholesterol levels. In the colorimetric enzymatic reaction method, blood serum samples are reacted using reagents containing certain enzymes as biocatalysts so that the reaction occurs more specifically. The principle of checking the colorimetric enzymatic approach is utilized to measure the total cholesterol levels in samples, including the various components of total cholesterol are hydrolyzed into free cholesterol then oxidized into cholestenone and hydrogen peroxide. The hydrogen peroxide produced reacts with 4-aminoantipyrine and N,N-bis(4-sulfobutyl)-m-toluodine to form a pink 4-(p-benzoquinone-monoamino)-phenazone compound, then this compound is measured for absorption at a wavelength of 500 nm so that total cholesterol levels are obtained [57].

▪ CONCLUSION

The action of the ethanol extract of the herb liverwort *Marchantia paleacea* (EEMP) and red ginger rhizomes *Zingiber officinale* var. *Rubrum* (EEZOR) has been observed in lowering total hypercholesterol levels in male mice of the *Swiss Webster* strain induced Triton X-100. The optimal dose as an antihyperlipidemic in EEMP is a dose of 200 mg/kg bw and the optimal dose as an antihyperlipidemic in ethanol extract of rhizomes of *Zingiber officinale* var. *Rubrum* is a dose of 1000 mg/kg bw.

Further research needs to be tested related to other antihyperlipidemic testing methods preclinically with different lipid parameters. Histopathological testing on antihyperlipidemic activity test can also be performed on these two test extracts.

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