ICOPMES2020_paper_34.pdf

by Icopmes 2020

Submission date: 10-Dec-2020 03:18AM (UTC-0500)
Submission ID: 1470780527
File name: ICOPMES2020_paper_34.pdf (792.27K)
Word count: 5274
Character count: 25421

Study of Acute and Sub-acute toxicity of *Boehmeria virgata* (Forst) Guill leaf ethanol extract in Wistar Rats

Marianti Manggau[1], Husni Cangara[2], Maghfirah[1], Endang Kusuma Intan[1], Wira Bahari Nurdin[3], and Dong-Seok Kim[4]

[1]Faculty of Pharmacy, Hasanuddin University, [2]Faculty of Medicine, Hasanuddin University, [3]Faculty of Mathematic and Natural Sciences, Hasanuddin University, [4]Faculty of Medicine, Chung Ang University, Seoul.

Email : wbnurdin@gmail.com

A B S T R A C T

Objective: This study evaluates acute and sub-acute toxicity of a standardized *Boehmeria virgata* leaf extract (BVLE). Plant *Boehmeria virgata* is a traditional herb used by the Makassar tribe to treat inflammation and cancer. In a previous study, we showed that BVLE is very potent in inhibiting the growth of cervical cancer cells. However, information concerning its safety remained unknown.

Material and Methods: In the current study thirty Wistar rats were distributed into 6 groups (3 male and 3 female) for acute treatments. Similarly for investigating sub-acute treatments 40 Wistar rats were split amongst into 8 groups (4 males and 4 female). The rats received BVLE by oral administration once 2000 and 5000 mg/kg for acute treatment, and everyday in 28 days with 250, 500 and 1000 mg/kg for sub-acute treatment. During a 14 days period in which the rats were treated they were monitored for any form of behavioral change, weight, food and water intake, and histopathology. The treated animals underwent hematological, biochemical, histopathological, and organ weight analysis after 28 days. Histopathological study revealed a general hydropic degeneration of the liver after acute treatment with 5000 mg/kg; such a degenerate did not occur in the kidney and kidney glomerulus of BVLE treated rats.

Results: There were no significant differences of hematological, biochemical, organ weight and histopathology data in sub-acute BVLE treated groups compared with those in the control group.

Conclusion: BVLE is *not toxic* at doses up to 1000 mg/kg. BVLE can therefore fulfill a preclinical criterion necessary for its further establishment as a clinically useful extract.

Keywords: *Boehmeria virgate* leaf extract, acute toxicity, sub-acute toxicity, hematology parameters, biochemical parameters, histopathology parameters

1. Introduction

Boehmeria virgata (Forst) Guill (*Boehmeria virgata*) belongs to the Urticaceae family and has been traditionally used by the Makassar tribe to treat inflammation and cancer (Manggau et al, 2011). Ethanol extract of *Boehmeria virgata* and 3 other plants traditionally used by the Makassar tribe as anticancer and anti-inflammation agents, namely *Acanthus ilicifolius Linn*, *Acalyphaindica* L. dan *Eupatorium odoratum*, have been studied for their activity against HeLa cervix cancer cells. In addition, the anti-proliferative activity of BVI03 compound on HeLa cell lines occurred through the inhibition of p53 and Caspase-3 (Manggau et al, 2013). Wardihan et al. (2013) investigated the selective cytotoxicity of BVLE on some cancer cell lines, namely HeLa, T47D, WiDr, Vero *cells*. It was demonstrated that the respective IC_{50} of *Boehmeria virgata* of these cancer lines 8.991±0.234, 12.732±0.945, 18.925±1.277, and 16.022±0.663 µg/ml. The corresponding selectivity indices of these cancer line were 0.844, 1.258, 0.847, and 1.000. The BVI03 compound from the *Boehmeria virgata* leaf extract (BVLE) was characterized as an alkaloid compound, namely 10- (6,6-dihydroxy-hexyl)-2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide. This was reported as an anti-proliferative active ingredient in *Boehmeria virgata* against HeLa cell line (Manggau et al, 2018). Other study showed, that *Boehmeria virgata* compound in term of BVI03, NBB (nano-encapsulated BVI03) and NBVG (nano-encapsulated vaginal bioadhesive gels) had the anti-proliferation activities with an IC_{50} of 2.88, 59.26 and 725.46 µg/ml, respectively (Lukman *et al*., 2014). Not without extensive studies, there is limited toxiological information concerning the safety of BLVE owing to its vulnerability. Indonesian authorities are currently concerned with the safety and toxicity of medical and consumable plants (BPOM, 2014).

Presently, there is no research work that creates a toxicological reaction of BVLE. Hence, this study aims at performing an astute toxicological study with sub-sections to conform a preclinical benchmark necessary for additional establishment, by evaluating biochemical, hematological and histopathological analysis of wistar rats evaluated to BVLE. What's more, advancement in behavior, skin, body and organ weight, daily water and food consumption level were also taken into consideration.

2. Materials and methods

2.1. Plant material and extract process

The plant *Boehmeria virgata* is native to Malino Town, Gowa City, South Sulawesi Province, Indonesia. A specimen of the plant was identified at Indonesian Institute of Sciences (LIPI), Jakarta, Indonesia. After being collected, the leaves were dried and chopped into small pieces. For the ethanol extract preparation, about 150 g of leaf were macerated for 4 days using 70% ethanol as solvent.

2.2 *Characterization of active isolate as marker*

The active isolate of BVLE (BVI03) as marker were characterized by physicochemical properties including UV of wavelength 254 and 366 nm and IR spectra.

2.3 Animals

Both genders of adult Wistar rats in the current study had an average weight of 150 ± 10 g. They were purchased from Central Rodent Cultivation, Airlangga University, Surabaya. These test animals, were randomly collected into (i) 6 groups for acute and (ii) 8 groups for sub-acute toxicity tests with each group containing five Wistar rats. Polypropylene cages were used to house the rats with the range of temperature and relative humidity were 24 - 26°C and 45–55%, respectively. The time cycle was 12:12 h. Water and food pellets were the major food nutrients given to these animals, unless stated otherwise. They were also given a week to adapt to their new environment before being used for the experiments. The experimental protocol of these animal had been approved by Indonesian committee and it had been carried out in accordance with the National Institutes of Health guidelines on animal care.

2.4 Experimental Design

In the acute toxicity tests the rats were administered orally using gavage needle once with BVLE and evaluated daily within 14 days. In sub-acute toxicity test the rats were given BLVLE daily for 28 days; these rats were

evaluated on the 14th and 28th day, following the recommendations by Indonesian National Agency of Drug and Food Control (Sparingga, 2014). Six groups for acute treatments (3 groups of male and 3 groups of female Wistar rats) received vehicle, 2000 and 5000 mg/kg BLVE, respectively. Eight groups undergoing sub-acute treatments (4 groups of male and 4 groups of female Wistar rats) received vehicle, 250, 500 and 1000 mg/kg BVLE, respectively (Sparingga, 2014). The vehicle was given to the control group for both acute and sub-acute treatments. Observations were carried out every day, to ascertain the causes of death and behavioral changes. Weekly body weight analysis was also carried out and the various dose administered to the rats were weekly adjusted to enable the body weight control the target dose level for all rats. Furthermore, there was a daily examination of the detailed preclinical analysis and measurement of the food and water consumed.

2.5 Hematology analysis

On the last day of the sub-acute study, chloroform was used to anesthetize the animals. Plastic test tubes, which are used to collect the samples, contained EDTA an anticoagulant. Hematology analyses were carried out using a Hematology Analyzer (Sysmex XS-8000i). This was used to determine the mean corpuscular hemoglobin (MCH), erythrocyte count (RBC), white blood cells (WBC), MCH concentration (MCHC), red blood cell distribution width (RDW), RDW-standard deviation (RDW-SD), RDW coefficient of variation (RDW-CV), mean corpuscular volume (MCV), hemoglobin, hematocrit, neutrophil, lymphocyte, eosinophil, monocytes, and platelet counts.

2.6 Biochemical Analysis

Following to the analysis of the sub-acute toxicity, chloroform was used to anesthetize all the animals used in the study. They subsequently bled through an inferior vena cava. After all specimens were gathered in sample tubes, they were placed in a standing positing for outright clotting. A total of 15 minutes was used to centrifuge the blood clot at 3000 rpm after that, the serum specimen was kept frozen at -80° Celsius. Samples of serum were also used to analyze and determine the total creatinine, serum, Aspartate-Aminotransferase (AST) and Alanine-Aminotransferase (ALT) concentration.

2.7 Histopathological Observations

Kidney, liver, and kidney glomerulus were tested with the aid of a microscope after each one of them undergoing eosin and hematoxylin staining. Dissection of the liver, kidney glomerulus and kidney was performed to test for signs of toxicity. Any inherent found irregularities the slides were tagged “mild”, “cautious” and “uncompromising” in a manner identified to that described by Jain et al (2008) and Chi et al. (2014).

2.8 Statistical analysis

Kruskal–Wallis one-way and two-way analysis of variance (ANOVA) were applied to subjugate data in the form of Mean ± SEM (Standard Error of Mean) Mann–Whitney-U-test (two-tailed) was applied to make inter group comparisons for responses that produced huge treatment effects in the ANOVA test with $p<0.05$.

3. Results

3.1. Characterization of active isolate as marker

Characterization measurements were performed with an ultraviolet spectrophotometer by using dichloromethane. The solvent displayed maximum absorption for BVI03 isolate at wavelengths 262, 287 and 369 nm. This enable us to detect the presence of conjugated dienes. In addition, the UV spectrum also showed maximum absorption at a wavelength of 285 nm. The latter absorption peak indicates which gives an indicates a transition to the state π^* at the solitary n electrons in N atoms occuring at wavelengths greater than 270 nm (Figure 1).

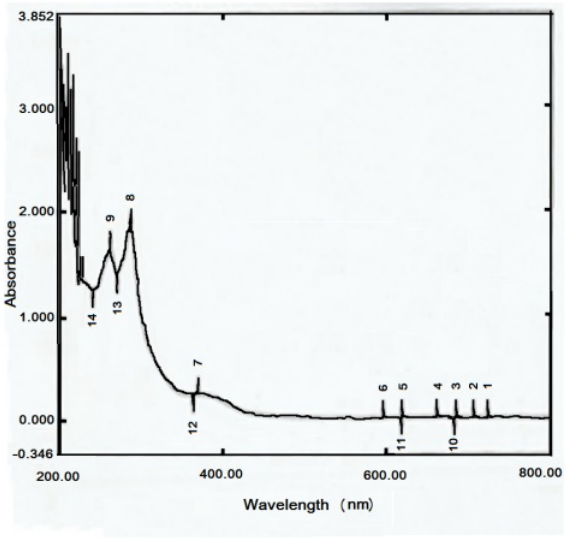

Figure 1. UV spectra data of BVI03 isolate as marker of BVLE

The infra-red spectrum data of BVI03 isolates at a wave number of 3372 cm^{-1}, indicates the presence of OH bound and an amine group. The medium absorption at a wave number of 927 cm^{-1}, and strong absorption at a wave number of 1556 cm^{-1} indicates the possibility of primary amine. Moreover, the absorption at a wave number of 2926 cm^{-1} indicates the presence of an aliphatic CH group. The presence of a band that absorbs strongly and sharply at wave numbers 1413 cm^{-1} indicates the presence of an aromatic group. Strong absorbtion at 647 cm^{-1}, 620 cm^{-1} and weak for 1129 cm^{-1} indicates the presence of alkenes (Figure 2).

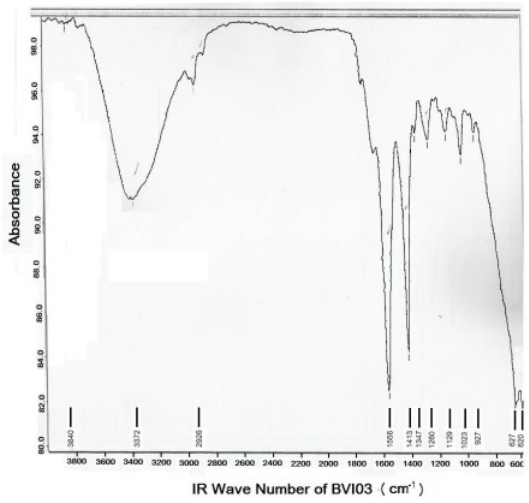

Figure 2 IR spectra data of BVI03 isolate as marker of BVLE

3.2 Acute toxicity study

3.2.1. Animal observation

The oral administration of 2000 and 5000 mg/kg of BVLE produced no changes in skin color, behavior, loss of hair, defecation, postural abnormalities, breathing, urination, breakage in water or food intake. When assessing their sense of touch and hearing, it was found that the rats were more highly sensitive with a lower activity level. Furthermore, no death was observed in the various BVLE treated groups.

3.2.2. Body weight measurement

For 14 successive days, the average weight of the rat was measured. The control group displayed an upsurge in body weight as opposed to those administered with BVLE as displayed in Table 1. The minimum change in body weight was seen on rats administered with 2000 mg/kg.

Table 1. Body weights of rats following acute treatment with BVLE. Data indicate mean ± SEM, n = 5. These data showed no statistically significant difference from control, $p < 0.05$

	Body Weight (g)		
	Day 0	**Day 7**	**Day 14**
Female			
Control	170 ± 2.57	171 ± 1.63	171 ± 2.74
2000 mg/kg	171 ± 1.50	173 ± 0.76	174 ± 0.47
5000 mg/kg	169 ± 4.47	170 ± 2.73	170 ±2.18
Male			
Control	182 ± 1.43	184 ± 0.56	186 ±4.65
2000 mg/kg	183 ± 4.65	186 ± 3.07	1.85 ± 3.61
5000 mg/kg	182 ± 4.18	184 ± 3.46	185 ± 2.54

3.2.3 Intake of food and water

Calculation of the quantity of water and food used up on a daily basis, was defined as the difference between the initial quantity, and the total quantity left sub-sequential to 24 hours. The calculated values obtained

depended on the total quality of food consumed and the average physical critical weight. The obtained data indicated no statistically meaningful difference (CMC), $p > 0.05$.

3.2.4 Organ weight

On the 14th day, the organs underwent acute toxicity tests. For each organ type we computed the average over 5 samples for each group. Using Kruskal–Wallis analysis, the significant difference was obtained with $p > 0.05$ as shown in Table 2 and 3.

Table 2. Histology and weight of female Wistar rat liver and renal cells after single dose (acute) treatment of BVLE. Values are expressed as mean ± SEM., $n = 5$. Significantly different from control, $p<0.05$. - (normal): 0-25%, + (mild): 25-49%, ++ (moderate): 50-74%, +++ (severe): 75-100% . (Ghufron. 2011)

Treatment	Liver			Renal		
	Weight (g)	Hydropic Degeneration (%)	Degree	Weight (g)	Hydropic Degeneration (%)	Degree
Control	3.51± 0.03	20.72± 3.54	-	0.61 ±0.001	19.65 ± 0,50	-
2000 mg/kg	3.65 ± 0.02	39.97± 5.89	+	0.69 ±0.002	34.02 ± 3,94	+
5000 mg/kg	3.84 ± 0.02	52.39± 8.28	++	0.73 ±0.002	47.13 ± 10,90	++

Table 3. Histology of renal glomerular after single dose treatment of BVLE and CMC as control. Data indicate mean ± SEM. - (normal): 0-25%, + (mild): 25-49%, ++ (moderate): 50-74%, +++ (severe): 75- 100% (Ghufron. 2011).

No.	Treatment	Enlargement of renal glomerular (%)	Degree
1	Control	22.8667 ± 3.33933	-
2	2000 mg/kg	22.3867 ± 6.20238	-
3	5000 mg/kg	28.2133 ± 4.68740	+

3.2.5 Histology of liver, renal and renal glomerular after single dose treatment of BVLE

Histological tests of the organs indicated that acute BVLE treatment by an oral administration of up to 2000 mg/kg yields mild hydropic degeneration. At a dose of 5000 mg/kg moderate hydropic degeneration appeared as compared with the control group (Figure 3 and 4, Table 2 and 3).

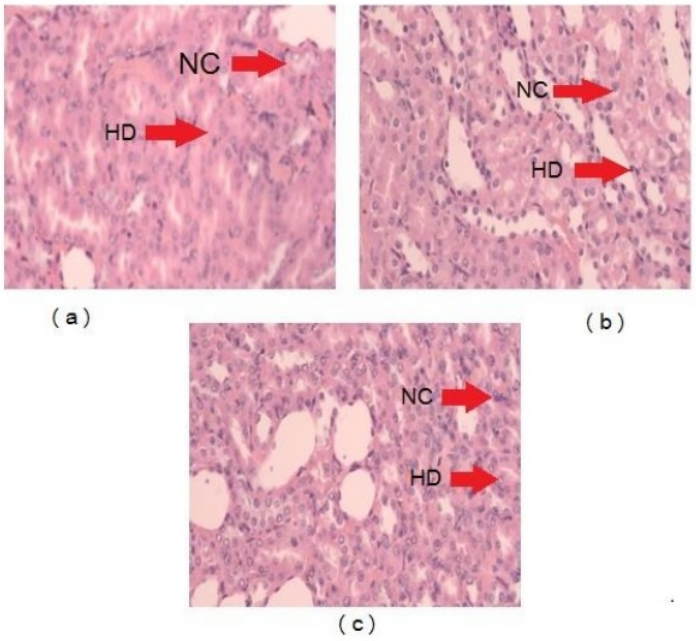

Figure 3 Renal histology of female white rats in (a) control group (CMC) or after a single (acute) dose of Boehmeria virgata Linn ethanol extract in (b) 2000 mg/kg or (c) 5000 mg/kg (HE. 100x). Description: NC: normal cells, HD: hydropic degeneration

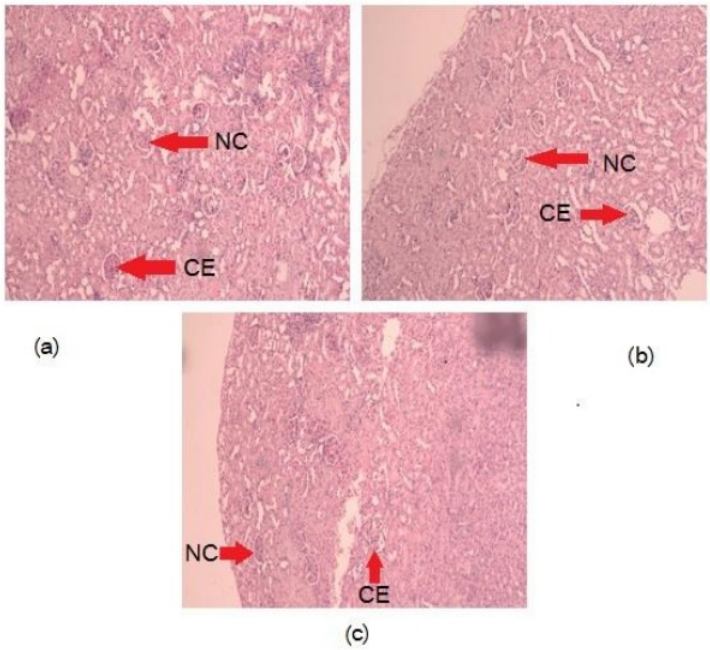

Figure 4 Renal glomerular histology of female white rats in (a) control group (CMC) or after a single (acute) dose of (b) 2000 mg /kg or (c) 5000 mg/kg BVLE (HE. 100x). Description: NC: normal cells, CE: glomerular cell enlargement

3.3 Sub-acute toxicity study

3.3.1 Physical weights and food intake

The physical weights and food intake of rats were not altered by the BVLE administration. As shown in Table 4 and 5, after 28th day of treatment of wistar rats of both gender with doses up to 1000 mg/kg, no significant changes in body weights and food intake were found.remained the same before and after treatment of BVLE

Table 4. Body weights of rats following sub-acute treatment with BVLE Data indicate mean ± SEM, $n = 5$. These data showed no statistically significant difference from negative control (CMC), $p < 0.05$

	Body weight (g)				
Treatment	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Female					
Control	162 ± 4.47	165 ± 3.54	167 ± 2.74	167 ± 2.86	171 ± 2.23
250 mg/kg	162 ± 3.56	167 ± 2.73	162 ± 4.47	167 ± 2.73	172 ± 2.74
500 mg/kg	162 ± 4.47	167 ± 3.47	166 ± 4.18	168 ± 2.74	171 ± 2.24
1000 mg/kg	163 ± 4.47	168 ± 2.73	166 ± 4.18	167 ± 2.74	172 ± 2.74
Male					
Control	186 ± 3.53	188 ± 4.56	192 ±3.71	197 ± 2.74	198 ± 4.16
250 mg/kg	187 ± 4.37	190 ± 3.07	194 ± 4.61	197 ± 5.58	202 ± 5.70
500 mg/kg	186 ± 4.18	189 ± 6.52	195 ± 3.54	193 ± 5.71	199 ± 5.41
1000 mg/kg	190 ± 5.09	193 ± 4.70	195 ± 4.18	193 ± 2.24	200 ± 4.18

Table 5 Food intake of rats following oral administration of the ethanol extract from BVLE. Values are expressed as mean ± S.E.M., $n=5$, these data showed no statistically significant difference from control (CMC), $p<0.05$.

Treatment	**Food Intake (g)**				
	Sex	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	Male	64.12 ± 1.25	74.21±0.899	83.14±1.07	82.12 ± 1.74
	Female	49.41 ±1.13	62.71±1.18	62.03±1.17	67.85 ±0.73
250 mg/Kg	Male	64.71± 1.08	74.42±0.64	81.28±0.96	83.71 ± 0.68
	Female	49.14 ±1.03	63 ± 1.02	58.28 ± 0.68	68.42 ±0.57
500 mg/Kg	Male	64.71 ±1.01	73.42±1.23	80.36 ± 1.24	83.57 ± 0.78
	Female	49.14 ±1.01	60.01±2.34	60.15 ± 3.14	68.42 ± 0.52
1000mg/Kg	Male	65.85 ±1.10	74.85±1.42	80.24 ± 3.27	81.42± 1.42
	Female	49.57 ±0.64	60.34±3.24	60.17 ± 2.43	68.42 ± 0.36

3.3.2 Biochemical Analysis

After the administration of BVLE for the pre-treatment of AST (Fig 5), ALT (Fig. 6), ureum (Fig. 7), and

creatinine levels (Fig. 8) no significant changes were found after the 28th day treatment.

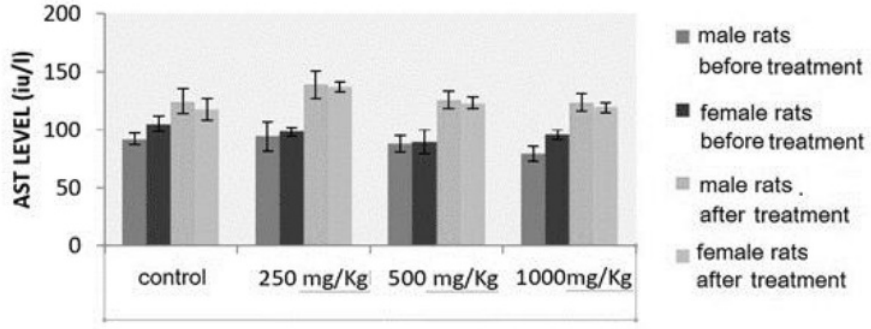

Figure 5. AST levels before and after sub-acute administration of the BVLE for 28 days. Data: mean ± SEM. n = 5 (5 males, 5 females), $p<0.05$.

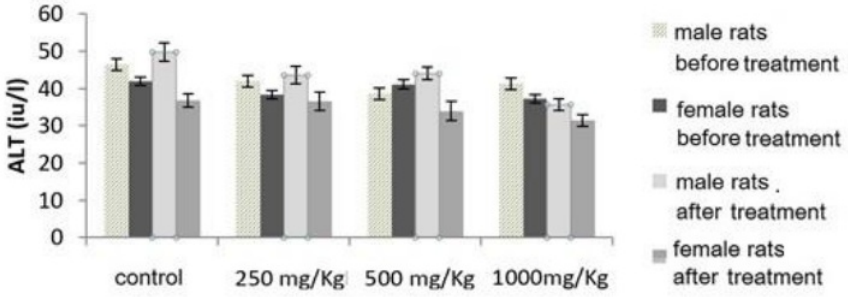

Figure 6. ALT levels before and after sub-acute administration of *Boehmeria virgata* Linn leaves ethanol extract for 28 days. Data: mean ± SEM. n = 5 (5 males, 5 females), $p<0.05$.

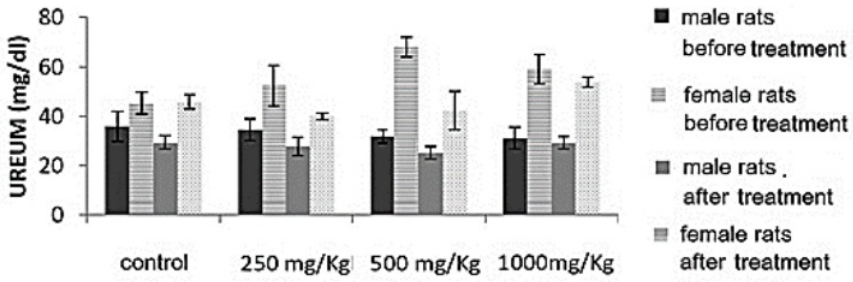

Figure 7. Ureum levels before and after sub-acute administration of BVLE for 28 days. Data: mean ± SEM. n = 5 (5 males, 5 females), $p<0.05$.

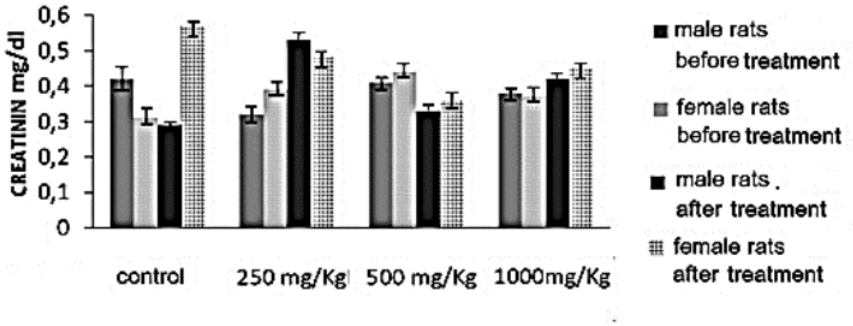

Figure 8. Creatinine levels before and after sub-acute administration of ethanol extract of *Boehmeria virgata* Linn leaves for 28 days. Data: mean ± SEM. n = 5 (5 males, 5 females), $p<0.05$.

3.3.3 Hematological Analysis

Tables 6-9 display the hematological profile of both groups (control and treated), which are not different significantly in the various hematological parameters examined.

Table 6 Hematology analysis data of erythrocyte rats following treatment with BVLE Data indicate mean ± SEM, $n = 5$. These data showed no statistically significant difference from control (CMC), $p<0.05$.

Hematology	Sex	Treatments			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Erithrocyte ($x10^6/\mu l$)	Male	8.56± 0.33	8.17± 0.29	7.13± 0.15	6.90± 0.99
	Female	6.70 ± 0.69	6.80± 0.47	7.60± 0.24	7.40±0.24
MCV (fl)	Male	49.40± 0.77	48.43±1.07	52.33± 1.02	53.26 ±2.86
	Female	56.50 ± 2.91	56.80± 3.83	51.46± 0.66	54.16± 0.26
MCH (pg)	Male	17.60± 0.15	17.16±0.26	18.00± 0.30	17.73 ±0.17
	Female	18.80 ± 0.17	19.50±0.90	18.30± 0.20	18.96± 0.08
MCHC (g/dl)	Male	35.63± 0.50	35.43±0.26	34.46± 0.18	33.50 ± 1.70
	Female	33.40 ± 1.36	34.40±0.79	34.73± 0.28	35.00± 0.11

Table 7 Hematology analysis data of leucocyte rats following treatment with BVLE ta indicate mean ± SEM, n = 5. These data showed no statistically significant difference from control,p<0.05.

Hematology Profile	Sex	Treatments			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Leucocyte (x $10^3/\mu l$)	Male	22.88±3.52	22.90±1.26	23.90±0.49	24.56±4.14
	Female	22.76±2.97	14.46± 2.30	16.79±1.90	14.58±1.79
Neutrophil (%)	Male	15.76±1.07	29.33±1.20	23.7±2.07	22.1±11.75
	Female	11.26±0.98	18.66±2.19	13.46±1.47	17.33±2.45
Lymphocyte (%)	Male	43.6±2.99	55.73±0.88	45.06 ±2.74	37.03±19.7
	Female	49.93±2.00	61.93±1.35	64.7±2.00	51.53±2.77
Monocyte (%)	Male	6.86±0.55	5.76±1.44	5.9±0.95	7.83 ±1.21
	Female	3.76±1.93	5.96±0.88	8.56±0.811	6.7 ± 0.43
Eosinophil (%)	Male	4.2 ±1.68	2.53±0.96	5.3±1.21	2.8 ± 0.81
	Female	3.36±0.85	3.43±0.58	6.26±1.13	4.43±0.17

Table 8 Hematology analysis data of thrombocyte rats following treatment with BVLE Data indicate mean ± SEM, n = 5. These data showed no statistically significant difference from control (CMC), p<0.05.

Hematology Profile	Sex	Treatments			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Trombocyte (X $10^3/\mu$L)	Male	1122.6±53.07	1099± 58.62	1129.6±63.18	1229±71.27
	Female	805.33±2.16	873.33± 2.81	1112.3±58.32	1196± 1.08
MPV (fl)	Male	7.66±0.12	7.23±0.08	7.76±0.03	7.7±0.05
	Female	8.03±0.47	7.76±0.29	7.56±0.17	7.5±0.15
PDW (fl)	Male	7.93± 0.17	7.63± 0.03	8.31± 0.33	8.33± 0.27
	Female	8.9± 0.77	8.33± 0.32	8.06± 0.14	8± 0.25
P-LCR (%)	Male	7.66± 0.90	6.2 ± 0.80	9.03± 0.51	8.9 ± 0.24
	Female	11.3± 3.19	9.13± 1.70	8.23± 1.47	7.36 ±0.97

Table 9 Hematology analysis data of hemoglobin, hematocrit, RDW-SD and RDW-CV rats following treatment with BVLE Data indicate mean ± SEM, n = 5. These data showed no statistically significant difference from control (CMC), p <0.05.

Hematology Profile	Sex	Treatments			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Hemoglobin (g/dl)	Male	15.06±0.57	14.01±0.28	12.83±0.14	12.26±1.81
	Female	12.56±1.18	13.23±0.76	13.9±0.32	13.53±0.28
Hematocrit (%)	Male	42.26±1.02	39.50± 0.62	37.23± 0.33	36.26±3.70
	Female	37.46±2.17	38.46±1.94	40.03±0.71	38.66±0.71
RDW-SD (fl)	Male	29.56±1.18	29.90±0.66	36.5±3.72	36.56±4.76
	Female	36.40±8.06	32.23±2.77	30.33±1.16	29.06±0.38
RDW-CV (%)	Male	20.06±0.80	20.20± 0.69	21.43± 1.31	21.33±0.92
	Female	19.93±3.14	17.80± 0.98	18.76± 0.29	17.13±0.32

4. Discussion and Conclusion

The threat of cervical cancer is still a serious problem for women's health. Anti cervical cancer drugs can cure cancer by killing cells. Unfortunately these drugs also destroy normal cells. But since the work mechanism of most anti-cancer drugs is to kill the fast growing cells, then it kills also fast growing normal cells that causes some unpleasant side effects. These side effects vary from one drug to another for many reasons. The development of effective chemotherapeutics with no toxic effect is urgently needed. Our previous study, showed that anti-cancer effect of Isolated Active Compound (BVI03) in Human Cancer Cervix HeLa Cells of BVLE was mediated by Activation of Caspase 3 and p53 Protein (Manggau et al, 2012).

Recently the putative of BVLE alkaloid compound 10-(6,6dihydroxyhexyl)-2,3,6-trimethoxyphenanthrene-9-carboxamide have been established as a potent anticancer agent (Manggau et al, 2018).

There are still no studies that establish the safe doses and possible toxicological reactions of BVLE which has been traditionally used by the Makassar tribe to treat inflammation and cancer. Therefore, the recent research was carried out to study the acute and sub-acute toxicity effects of BVLE using an *in vivo* model.

Intense administration of BVLE in rats of 5000 mg/kg doses led to no mortality nor signs of toxicity. This suggested that an LD50 higher than 5000 mg/kg may only be gained by oral administration. Since substances with LD50 larger than 5000 mg/kg by oral administration are non-toxic (Kennedy et al., 1986), the acute dispensation of BVLE is non-toxic. The treatment group of 2000 mg showed a degree of hydropic degeneration, 39.97% for liver and 34.02% for renal, that was categorized as the "mild damage" (<50%). Hydropic degeneration in hepatocyte cells also occurred in the control group (Fig. 1a). The group treated by 5000 mg/kg also showed the presence of hydropic degeneration (52.39% for liver and 47.13% renal) descriptively categorized as the "moderate damage" (Fig. 5c).

The process of hepatocyte damage starts from hydropic degeneration. Hydropic degeneration is mild damage and reversible. It can be a response to infection or exposure to toxicity. This toxicity causes a disruption in the mitochondrial organelle that produces ATP required for the gating of the cellular sodium via cell membrane (Na +) pumps. If there is no ATP, increasing the cell's osmotic potential and so its water intake. Sodium ions (Na^+) attract water, so water comes inside to the cell. Vacuoles containing water are clear and small in the cytoplasm. These unite to form larger vacuoles that occupy the cytoplasm and cover cell nuclei, and cause cell swelling.

Furthermore, sub-acute treatment indicated that no death or toxic symptoms recorded after 28 days of daily administration of 250, 500 and 1000 mg/kg doses of BVLE. Physical weight as well as food intake were unaltered during this period. The doses of BVLE is ascertained to be approximately 50 (acute) and 15 (sub-acute) times stronger than other species of Boehmeria virgata, such as *Boehmeria nivea* var. Tenacissima and *Boehmeria nivea* var. nivea, used as hepatoprotective against liver injury triggered by carbon tetrachloride (CCl_4) (Chun-Ching et al, 1998). Other research has illustrated that daily extract administration of *Boehmeria nivea* of 32 g/kg does not lead to embryo or maternal toxicity in mice, notwithstanding the fact that it is likely to cause cytotoxicity in cultured ESCs when administered in large doses. *Boehmeria nivea* (L.) Gaud is another species of *Boehmeria virgate* which is commonly given to cure organ failure medically (Tian et al, 2011).

Treatment with BVLE led to no observable change to the biochemical parameters observed in this study. The

AST levels did not also differ from normally measured levels (Hall Robert L., 1992, Qili et al., 2017). All used standard hematological parameters were within the normal reference range. Normal value of erythrocytes and leucocytes also did not differ from normally measured levels (Harkness, *et al*., 2010). Compared to other study, platelet profile and platelet index levels for all sample groups are still in the normal range (Qili et al, 2017).

The study of effective chemotherapeutics with no toxic effect is urgently needed. This study showed no toxic effect of BVLE. Consistently with our previous study, that anti-cancer effect of Isolated Active Compound (BVI03) in Human Cancer Cervix HeLa Cells of BVLE was mediated by Activation of Caspase 3 and p53 Protein (Manggau et al, 2012).

No significant toxicity was shown in the biochemical, behavioral, histological and hematological parameters of Wistar rats after the acute and sub-acute administration of BVLE. However, the moderate hydropic degeneration can be seen in rat liver following acute administration of 5000 mg/kg BLVE. Further studies are needed, namely the mechanism of hydropic degeneration, measurement of microsomal enzymes induction parameters, and studies of chronic treatment effects. Therefore, BVLE fulfill a preclinical criterion necessary for being clinically useful extract for cervical cancer drugs.

Acknowledgement

The research was granted by WCP-B Program (Grant Number 123.48/D2.3/KP/2018), Ministry of Research, Technology and Higher Education, Republic of Indonesia. Authors thank to Dr. Guy Yealland for proofreading the manuscript.

References

Chi, MH., Hui, RC., Yang, CH., Lin, JY., Lin, YT., Ho, HC., Chung, WH., Kuo, TT(2014) Histopathological analysis and clinical correlation of drug reaction with eosinophilia and systemic symptoms (DRESS).J B Dermatol. 170, (4).

Chun-Ching, L., Ming-Hong, Y., Tsae-shiuan, L., Jer-Min, L (1998) Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria nivea* var *nivea* and *B. nivea* var *tenacissima*. J Ethnopharm. Volume 60, (1) : 9-17.

Mohamed, E.A.H, Lim, C.P., Ebrika, O.S., Asmawi, MZ., Sadikun, A., Yam, MF (2011) Toxicity evaluation of a standardised 50% ethanolic extract of *Orthosiphon stamineus*, J Ethnopharm. 133, 358-363.

Hall Robert L (1992) *Clinical pathology of laboratory animals*. In: Andress, J.M., (Eds.). Animal Models in Toxicology. New York. Marcell Dekker Inc. pp. 791.

Harkness, JE., Turner, PV., Van dewoude, S (2010) Harkness and Wagner's Biology and Medicine of Rabbits and Rodents. 5th ed. Ames : Willey-Blackwell.

Jain, M., Soni, L., Deb, A., Jain, S.P., Rout, VB., Gupta, KL., Krishna (2008) Antioxidant and hepatoprotective activity of ethanolic and aqueous extract of *Momordica dioica* Roxb leaves. J Ethnopharm. 115, 61-66.

Kennedy, GL., Ferenz, RL., Burgess, BA (1986) Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD_{50}. J App Toxicol. 145–148.

Lukman, M., Rusdi, M., Hatta, M., Rahman, L., Subehan., Manggau, M (2014) Anti proliferation activity of Nanoencapsulated bioadhesive vaginal gel of isolated active Compound (BVI03) from Boehmeria virgata (Forst) guill leaves against human cancer cervix Hela cells. Int J of Pharm Sci Res. Volume 6. 836-839.

Manggau, M., Mufidah., Lindequist, U (2009) Antiproliferation Against Human Bladder Cancer 5637 Cell Line and Antioxidant Activity of Various Plant Extracts. The Indo J Nat Prod. 6, 247-250.

Gufron M (2011) Gambaran Struktur Histologi Hepar dan Ren Mencit Setelah Pemberian Perlakuan Infus Akar Rimpang Jahe (Zingiber officinale) dengan Dosis Bertingkat. J Ked Yarsi. 11, 15-20.

Kennedy, GL., Ferenz, RL., Burgess BA (1986) Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD_{50}. J Appl Toxicol, 145–148

Manggau, M., Hasan, H., Wahyudin, E., Haryono, K., Mufidah, Lukman (2011) Efek Farmakologi Tanaman Antikanker yang Digunakan oleh Masyarakat Sulawesi Selatan. Balitbangda Sulawesi Selatan. ISBN 978 602 8400 55 8.

Manggau, M., Lukman., Rusdi, M, Hatta, M., Sinrang, AW., Subehan. (2012) Effect of an Isolated Active Compound (BVI03) of Boehmeria virgata (Forst) Guill leaves on Anti-Proliferation in Human Cancer Cervix HeLa Cells through Activation of Caspase 3 and p53 Protein. Trop Med & Surg. 1:3. 1-4.

Manggau, M., Andriani, F., Mufidah., Nurdin, WB., Yulianti, R., Usmar., Sutriadi, S., (2018) The in vitro study of the Alkaloid Anti Cancer Compound from Makassar Medicinal Plants Boehmeria virgata Linn. Int J of Pharma Sci Rev and Res. 49(1), 77-81.

Qili, H., Guoming, S., Keliang, L., Fangcheng, Z., Yong, J., Jun, G., Lida, L., Zhongren, J., Minwu, J., Huiping, X.(2017) Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. Journal Plos One.1-18.

Sparingga, Roy A (2014) BPOM-RI (Indonesian National Agency of Drug and Food Control, Republic of Indonesia), Non-Clinical In Vivo Toxicity Test Guidelines and Regulation, http://jdih.pom.go.id. (accessed 13 December 2018).

Tian, XY., Xu, M., Deng, B., Leung, KS., Cheng, KF., Zhao, ZZ., Zhang, SP., Yang, ZJ., Deng, PX., Xu, DY., Xu, XP., Koo, I., Wong, M (2011) The effects of Boehmeria nivea (L.) Gaud. on embryonic development: in vivo and in vitro studies. J Ethnopharm. 24, 134 (2):393-8.

Wardihan., Rusdi, M., Alam, G., Lukman., Manggau, M (2013) Selective Cytotoxicity Evaluation in Anticancer Drug Screening of *Boehmeria virgata* (Forst) Guill Leaves to Several Human Cell Lines: HeLa, WiDr, T47D and Vero. Dhaka Univ J Pharm Sci. 12(2): 87-90.

ICOPMES2020_paper_34.pdf

ORIGINALITY REPORT

13%	10%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	S.N. Harizal, S.M. Mansor, J. Hasnan, J.K.J. Tharakan, J. Abdullah. "Acute toxicity study of the standardized methanolic extract of Mitragyna speciosa Korth in Rodent", Journal of Ethnopharmacology, 2010 Publication	2%
2	repository.unand.ac.id Internet Source	2%
3	www.hindawi.com Internet Source	1%
4	www.mitrariset.com Internet Source	1%
5	www.longdom.org Internet Source	1%
6	academic.oup.com Internet Source	<1%
7	e-sciencecentral.org Internet Source	<1%
	downloads.hindawi.com	

8	Internet Source	<1%
9	Dammika Gunawardena, Sasini Jayaweera, Gayathri Madhubhashini, Dilani D. Lokumarakkala, Sameera J. Senanayake. "Reliability of Parameters of Complete Blood Count With Different Storage Conditions", Journal of Clinical Laboratory Analysis, 2017 Publication	<1%
10	Thamilvaani Manaharan, Srikumar Chakravarthi, Ammu Kutty Radhakrishnan, Uma Devi Palanisamy. "In vivo toxicity evaluation of a standardized extract of Syzygium aqueum leaf", Toxicology Reports, 2014 Publication	<1%
11	jjyyd.com Internet Source	<1%
12	Submitted to Jawaharlal Nehru Technological University Student Paper	<1%
13	Yu-Tang Tung, Jyh-Horng Wu, Chi-Chang Huang, Hsiang-Chi Peng, Ya-Ling Chen, Suh-Ching Yang, Shang-Tzen Chang. "Protective effect of Acacia confusa bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats", Food and Chemical Toxicology, 2009	<1%

	Publication	
14	repository.unib.ac.id Internet Source	<1%
15	scholarworks.uark.edu Internet Source	<1%
16	link.springer.com Internet Source	<1%
17	www.lib.kobe-u.ac.jp Internet Source	<1%
18	repositorio.ufpe.br Internet Source	<1%
19	www.omicsonline.org Internet Source	<1%
20	www.tandfonline.com Internet Source	<1%
21	repository-tnmgrmu.ac.in Internet Source	<1%
22	Salman A. A. Mohammed, Riaz A. Khan, Mahmoud Z. El-Readi, Abdul-Hamid Emwas et al. "Suaeda vermiculata Aqueous-Ethanolic Extract-Based Mitigation of CCl4-Induced Hepatotoxicity in Rats, and HepG-2 and HepG-2/ADR Cell-Lines-Based Cytotoxicity Evaluations", Plants, 2020 Publication	<1%

23	bestpractice.bmj.com Internet Source	<1%
24	etheses.saurashtrauniversity.edu Internet Source	<1%
25	Olusola B. Adewale, Amos Onasanya, Scholastica O. Anadozie, Miriam F. Abu et al. "Evaluation of acute and subacute toxicity of aqueous extract of Crassocephalum rubens leaves in rats", Journal of Ethnopharmacology, 2016 Publication	<1%
26	media.neliti.com Internet Source	<1%
27	www.toxicolres.org Internet Source	<1%
28	Kuriakose Jayesh, Lal Raisa Helen, A. Vysakh, Eldhose Binil, M.S. Latha. "In vivo toxicity evaluation of aqueous acetone extract of Terminalia bellirica (Gaertn.) Roxb. fruit", Regulatory Toxicology and Pharmacology, 2017 Publication	<1%
29	icnmnaturopathy.org Internet Source	<1%
30	Chuan-I Li. "Development and validation of molecular markers for characterization of	<1%

Boehmeria nivea var. nivea and Boehmeria nivea var. tenacissima", Chinese Medicine, 2010
Publication

31 Nizam Uddin, Md. Rakib Hasan, Md. Mahadi Hasan, Md. Monir Hossain et al. "Assessment of Toxic Effects of the Methanol Extract of Citrus macroptera Montr. Fruit via Biochemical and Hematological Evaluation in Female Sprague-Dawley Rats", PLoS ONE, 2014 <1%
Publication

32 Jolius Gimbun, Sook Fun Pang, Mashitah M. Yusoff. "Orthosiphon stamineus (Java Tea)", Elsevier BV, 2019 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		