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By ENDANG DARMAWAN

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Subchronic toxicity test on combination of extracted *Phyllanthus niruri* and *Centella asiatica* on haematology in rats

Siti Fatmawati Fatimah[1], Endang Darmawan*[1], Iin Narwanti[1], Dzulhaifa[1], Ira Aprilia Wulandari[1], Ria Putri Salma[1]

[1] Department of Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

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Corresponding author:
endang.darmawan@pharm.uad.ac.id

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ABSTRACT

Background: Meniran (*Phyllathus niruri*) and Pegagan (*Centella asiatica*) are well-known medicinal plants in Indonesia. Uses of Meniran and Pegagan as herbal medicines need to be examined for their subchronic activity to assure their safety.

Objective: This study aimed to evaluate subchronic effects of a combination of extracted Meniran and Pegagan on Wistar rats through haematological parameters (erythrocytes, haemoglobin, haematocrit, MCV, MCH, and MCHC).

Methods: Animal models used were 56 male and female Wistar rats and were divided randomly into 4 groups. Group 1 received CMC-Na 1% (control group). Group 2 received extracts of Meniran and Pegagan (50:50 mg/kgBW). Group 3 received extracts of Meniran and Pegagan (250:250 mg/kgBW). Group 4 received extracts of Meniran and Pegagan (1250:1250 mg/kgBW). Subchronic test of Meniran and Pegagan was conducted by providing treatments for the Wistar rats for 28 days. Their haematological substances were analysed statistically by using one way ANOVA and Kruskal-Wallis method with confidence interval of 95% and post-hoc.

Results: This study found that the haematological substances in male Wistar rats were normal and did not significantly change ($p>0,05$). It also showed that haemoglobin and haematocrit substances in female rats were normal and did not significantly change ($p>0,05$). Erythrocytes, MCV, MCH, and MCHC in female rats indicated significant change ($p<0,05$), but they were still in normal ranges.

Conclusion: It could be concluded that administration of the combination of extracted Meniran and Pegagan was not toxic to haematology of the rats in all doses.

Latar Belakang: *Meniran (Phyllanthus niruri L.) dan Pegagan (Centella asiatica L.) merupakan salah satu tanaman obat yang dikenal baik di Indonesia. Pemanfaatan meniran dan pegagan perlu dilakukan evaluasi secara subkronis untuk melihat keamanannya.*

Tujuan: *Untuk mengevaluasi pengaruh pemberian subkronis kombinasi herba meniran dan herba pegagan pada tikus galur Wistar terhadap parameter hematologi (eritrosit, hemoglobin, hematocrit, MCV, MCH dan MCHC).*

Metode: *Studi ini menggunakan tikus galur Wistar jantan dan betina sebanyak 56 ekor yang dibagi secara acak menjadi 4 kelompok. Kelompok 1 diberi CMC-Na 1% (kelompok kontrol). Kelompok 2 diberi ekstrak*

meniran-pegagan 50:50 mg/kgBW. Kelompok 3 diberi ekstrak meniran-pegagan 250:250 mg/kgBW. Kelompok 4 diberi ekstrak meniran-pegagan 1250:1250 mg/kgBW. Pengujian subkronis dari kombinasi ekstrak meniran dan pegagan dilakukan dengan memberikan perlakuan selama 28 hari kepada tikus galur Wistar. Data hematologi dianalisis secara statistika menggunakan metode one way ANOVA dengan taraf kepercayaan 95% dan post-hoc.

Hasil: *Hasil penelitian menunjukan bahwa nilai hematologi pada tikus wistar jantan adalah normal dan tidak mengalami perubahan signifikan (p>0,05). Hasil penelitian nilai hemoglobin dan hematokrit pada tikus Wistar betina menunjukan normal dan tidak adanya perubahan signifikan (p>0,05). Nilai eritrosit, MCV, MCH dan MCHC pada tikus Wistar betina menunjukan perubahan signifikan (<0,05), namun kadar masih berada dalam rentang normal tikus.*

Kesimpulan: *Hasil penelitian dapat disimpulkan bahwa pemberian kombinasi ekstrak meniran dan pegagan pada seluruh dosis tidak toksik terhadap hematologi tikus.*

INTRODUCTION

Meniran (*Phyllanthus niruri L.*) and Pegagan (*Centella asiatica L.*) are plants that could be developed as traditional medicines. Substances that has been known in Meniran are compounds like flavonoid, quercetin, quercitrin, astragalin, catechins, terpenes, lignans, lupeols, coumarin, tannins, alkaloids, and saponins.[1,2] Then Pegagan contains terpenes (monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes), phenolics (flavonoids, phenylpropapoids, and tannins), polyacetyl, alkaloids, carbohydrates, vitamins, minerals, and amino acids.[3] Meniran and Pegagan have pharmacological activities functioning as immune stimulators.[4,5] Therefore, if both of them are combined, they can produce a higher total of potential activity as immune stimulator. Such traditional medicines need further research; as a result, their uses could be scientifically verified, especially regarding its efficacy, safety, and quality standards. Both traditional medicines contain tannin and saponin. Effects of tannin and saponin may arise if their use exceeds the safe limit. Tannin and saponin can reduced erythropoietin production.[6] Toxicity test is important to determine safety issues of substances that can damage humans.[7] In single administration, Pegagan and Meniran are considered safe to use. Research about acute toxicity of extracted Pegagan had been conducted by determining LD_{50} in Balb/c rat models. Research using post-test only control group design method in various doses of 5 mg/kgBW, 50 mg/kgBW, 500 mg/kgBW, and 2000 mg/kgBW found no death of the rat models in the highest dose, therefore; it was included in pracical non-toxic criteria.[8] A research related to subchronic toxicity of ethanol extracts of turmeric and Meniran by a dose of 90 mg/kgBW, 180 mg/kgBW, and 360 mg/kgBW in female Wistar rats on haematological parameters. Another research stated that administration of a combination of extracted turmeric and Meniran by a dose of 90-360 mg/kgBW for 90 days did not show any subchronic toxicity to haematological profile of female Wistar rats.[9] Hidayati (2018) had conducted a research related to the combined activity of dried-extracted Meniran and Pegagan by a dose of 100:100 mg/kgBW orally for 30 days on gentamicin-induced SD rats. Her research reported that the combination by a dose of 100:100 mg/kgBW could increase values of erythrocytes, haemoglobin, haematocrit.[10] Thus, those existing researches indicated that there has been less research regarding safety of the combination of Pegagan and Meniran.

Subchronic toxicity test aims to obtain information of toxic effects of substances that are not detected in acute toxicity test. In addition, it is to determine the toxic effects after repeated exposure within a certain period. Researches related to the subchronic toxicity of the combination of extracted Meniran and Pegagan in parameters of liver and kidney function showed no subchronic toxicity.[11,12] Another subchronic toxicity test parameter used is haematology. Analysis of haematological parameters include numbers of erythrocytes, haematocrit, haemoglobin, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin cells).

This study aims to evaluate effects of subchronic combination of extracted Meniran and Pegagan on haematological parameters in male and female Wistar rats for 28 days.

METHODS

Research Methods

This study was conducted at the Laboratory of the Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta. This research procedure has received ethical clearance from Ethics Committee of Research, Ahmad Dahlan University, No. 011803043. Its research method was an experimental study using a post-test control group design.

Samples

Samples used in this study were a combination of extracted Meniran (Batch Number: 049PP02.2) and Pegagan (Batch Number: 056PP01.2) obtained from PT. Borobudur Herbal Medicine Industry, Central Java, Indonesia. The extracted herbs of Meniran and Pegagan that agreed to quality requirements and had no contamination were approved by measurements of water content, microbial contamination, mold, yeast, heavy metals Pb and As.

Test Subjec

Subjects used in this study were male and female Wistar strain rats with a weight range between 100-300 grams, age between 2-3 months and origin from the Integrated Research Laboratory of Gadjah Mada University. Their specific characteristics are clear eyes, active, non-standing hair, and no anatomical defect. The rats were treated in cages, and AD2 pellets and enough water were given.

Interventions to Animal Models

The animal models of rats were grouped randomly into 4 groups, and each group consisted of 7 male and 7 female rats. This study was conducted for 28 days. Determination of dosages referred to guidelines of BPOM regarding oral toxicity tests on rodensia. The highest doses of this test preparation had a toxic effect but caused no death or severe symptoms of toxicity; medium doses caused milder toxic symptoms; and the lowest doses did not cause toxic symptoms.[7] Group 1 was a group of healthy rats and received 0.5% CMC-Na orally. Groups 2,3 and 4 received a suspension of the extracted Meniran and Pegagan by a dose of 50:50 mg/kgBW, 250:250 mg/kgBW, 1250:1250 mg/kgBW in 0.5% CMC-Na once each day and orally.

Blood sampling was conducted after 24 hours of administration of the combination of the both herbs on the 29th day. Their blood was drawn in the rats' eye vein (sinus orbitalis) which was then collected in tubes containing EDTA (ethylene diamin tetra acetic acid) to prevent blood clots. A volume of blood taken was approximately 1 mL. Tubes filled with blood were stored in containers containing ice cubes with a temperature of ± 4°C. The sampling results were then measured at the Integrated Clinical Laboratory of Parahita in Yogyakarta.

Data Analysis

Data in this stud ere analysed by using SPSS. Anova test was used to analyse significant differences between groups in eryt cyte, haemoglobin, haematocrit, and MCH. Kruskal-Wallis test was used to analyse MCV and MCHC data. Post hoc tests with 95% of confidence interval were to examine differences among the treatment groups.

RESULTS

Blood samples from the rats were analysed statistically by using SPSS to determine differences among the groups after treated for 28 days. The results of toxicity test of the combination of extracted Meniran and Pegagan on haematology in the rat models are below .

Based on Table 1, the results of the combination of both herbs in male ra indicated a lower mean value of erythrocytes compared to the control group. The lower value of erythrocytes could be caused by bleeding, erythrocyte damage, or lack of erythrocyte production due to folic acid deficiency.[14] Erythrocyte values in all groups showed results that did not agree to theoretical

Table 1. Values of Erythrocyte in male and female rats after receiving combination of extracted Meniran and Pegagan for 28 days with ANOVA analysis

Intervention	N	Erythrocyte count (x10^6/µl) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	7.06 ± 1.04	
K2. MP (50:50)	5	4.58 ± 0.70	0.286 (p>0.05)
K3. MP (250:250)	6	5.82 ± 0.56	
K4. MP (1250:1250)	5	5.79 ± 1.06	
Female Rats			
K1. Control (Healthy)	7	7.22 ± 0.37	
K2. MP (50:50)	7	6.33 ± 0.43*	0.048 (p<0.05)
K3. MP (250:250)	7	7.60 ± 0.22*	
K4. MP (1250:1250)	7	7.83 ± 0.42*	

Note
MP : combination of Meniran and Pegagan
p : significance between control and intervention group
* : significant difference (p<0,05) between intervention group

values of erythrocytes in male Wistar strain rats (7.2-9.6 x10^6/µL15). The erythrocyte values compared to ranges of reference values could be slightly different due to influences of environmental conditions, cages, feed treatment methods, and maintenance, as well as study area influencing climates and temperatures during their treatment process.[16] Based on these results, the combination of both herbs could not be considered to cause toxicity to erythrocytes in male rats even though there were decreasing values in the erythrocyte compared to normal group, but based on statistical analysis there were no significant differences.

In addition, based on Table 1, average values of erythrocytes in the normal group of female rats and the treatment group showed results that did agree to theory of erythrocyte values in female Wistar strain rats (5.7-9.0 x10^6/ µL15). This indicated that influences of the environment did not significantly affect the erythrocyte profile in female Wistar rats. The intervention group by a dose of 50:50 mg/kgBW showed a decreased value in erythrocyte values compared to the control group. The decrease of average values in the treatment group by a dose of 50:50 mg/kgBW could be caused by bleeding, damage to erythrocytes, or lack of erythrocyte production due to folic acid deficiency.[14] The intervention group by a dose of 250:250 mg/ kgBW and 1250:1250 mg/kgBW illustrated an increase of erythrocyte values compared to the control group. Based on these results, although the combination of both herbs decreased and increased the values of erythrocytes compared to the normal group, they were still in normal ranges. The combination of both herbs could not be considered to cause toxicity to erythrocytes of female rats.

Based on Table 2, average values of the control group indicated suitability to values of normal haemoglobin of male Wistar rats (11-17 g/dL).[15] Meanwhile, the treatment group showed decreased values of haemoglobin compared to the control group. The decreased values of haemoglobin pointed out a sign of anaemia (mainly anaemia due to lack of iron) due to decreased values of erythrocytes.[17] Values of haemoglobin in the treatment group did not fit to normal ranges of haemoglobin in the rats. Ranges of reference value could be slightly different due to influences of environmental conditions, cages, feed treatment methods, and maintenance, as well as study area influencing climates and

Table 2. Values of haemoglobin of male and female rats after receiving combinations of extracted Meniran and Pegagan for 28 days with ANOVA analysis

Intervention	N	Haemoglobin (g/dL) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	12.27 ± 1.77	
K2. MP (50:50)	5	8.02 ± 1.02	0.263 (p>0.05)
K3. MP (250:250)	6	9.75 ± 0.80	
K4. MP (1250:1250)	5	10.02 ± 1.94	
Female Rats			
K1. Control (Healthy)	7	13.00 ± 0.65	
K2. MP (50:50)	7	11.71 ± 0.79*	0.111 (p>0.05)
K3. MP (250:250)	7	13.43 ± 0.37	
K4. MP (1250:1250)	7	13.90 ± 0.65*	

Note
MP : combination of Meniran and Pegagan
p : significance between control and intervention group
* : significant difference (p<0.05) between intervention group

temperatures during their treatment. The average values of haemoglobin in the treatment group were decreased compared to the values of the control group and were in normal ranges of haemoglobin, but SPSS did not illustrated any significant difference between the control and the intervention group. As a result, the combination of both herbs could not be considered to cause toxicity to haemoglobin in male rats.

Table 2 demonstrated normal group of female rats having average values of haemoglobin by13.00 ± 0.56 g / dL. The groups with doses of 250:250mg/kgBW and 1250:1250mg/kgBW showed a significant increase compared to the control group. Values of Haemoglobinin in the three groups fitted to normal values of haemoglobin (13.2-14.8 g/dL). The treatment group by a dose of 50:50 mg / kgBW showed a decrease in the average values of haemoglobin compared to the control group. Decreased haemoglobin values were a sign of anaemia caused by a decreased number of erythrocytes. The treatment group by a dose of 50:50 mg / kgBW explained results that did not agree to the reference values. Abnormal conditions such as lack of haemoglobin could cause anaemia, especially anaemia due to lack of iron, bleeding, hyperthyroidism, and cirrhosis. Based on these results, the treatment group did not accord to the range values of theoretical haemoglobin, but the results of statistical analysis showed no significant differences, so the combination of both herbs in female rats had no effects on the values of haemoglobin.

Values of haematocrit in male rats in all groups showed results that did not agree to the theoretically normal values (42.5-49.4%). The values of haematocrit, compared to the range in the reference values, would be significantly different because of the influences of environmental conditions, cages, feed, care and maintenance methods as well as the study area influencing climates and temperatures during the treatment process. Based on the values of haematocrit shown in Table 3, the mean values of haematocrit in the intervention group experienced a decrease compared to the control group. Decreased haematocrit was an indicator of anaemia, leukaemia, cirrhosis, loss of blood and hyperthyroidism. Haematocrit values increased in erythrocytosis, polycythemia, dehydration and shock. Statistical analysis illustrated that there were no significant differences in each group so that the combination of both herbs could not be

Table 3. Values of haematocrit of male and female rats after receiving combination of Meniran and Pegagan for 28 days with ANOVA analysis

Intervention	N	Haematocrit (%) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	37.85 ± 4.58	
K2. MP (50:50)	5	26.90 ± 2.02	0.315 (p>0.05)
K3. MP (250:250)	6	33.78 ± 3.96	
K4. MP (1250:1250)	5	30.40 ± 5.11	
Female Rats			
K1. Control (Healthy)	7	39.86 ± 1.64	
K2. MP (50:50)	7	39.76 ± 1.08	0.882 (p>0.05)
K3. MP (250:250)	7	40.74 ± 1.16	
K4. MP (1250:1250)	7	41.13 ± 1.73	

Note
MP : combination of Meniran and Pegagan
p : significance between control and intervention group

considered to cause toxicity to the haematocrit of male rats.

Based on Table 3, the statistical test results of haematocrit values in female rats showed a significance value of 0.882 (p> 0.05) meaning that there were no significant differences in each group. The values of haematocrit were in the range of reference values (39-55%). This indicated that the values were not affected by the existing environment. Based on these results, the combination of both herbs could not be considered to affect haematocrit values in female rats.

Based on Table 4, male rats in the control

Table 4. Values of MCV in male and female rats after receiving combination of Meniran and Pegagan for 28 days with Kruskal-Wallis analysis

Intervention	N	MCV (fL) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	54.88 ± 2.21	
K2. MP (50:50)	5	62.12 ± 6.17	0.566 (p>0.05)
K3. MP (250:250)	6	57.98 ± 3.36	
K4. MP (1250:1250)	5	54.08 ± 2.24	
Female Rats			
K1. Control (Healthy)	7	55.39 ± 0.88	
K2. MP (50:50)	7	64.13 ± 3.87*	0.007 (p<0.05)
K3. MP (250:250)	7	53.64 ± 0.74*	
K4. MP (1250:1250)	7	52.73 ± 0.92*	

Note
MP : combination of Meniran and Pegagan
p : significance between control and intervention group
* : significant difference (p<0,05) between intervention group

group had average values of MCV of 54.88±2.21 fL, and these results were similar to the average values of the intervention group by a dose of 1250: 1250 mg/kgBW. The control group and the intervention group by a dose of 1250: 1250 mg/kgBW had values that were not in accordance with the normal values of MCV in male Wistar rats (57-65 fL). The below normal values of MCV could indicate anaemia due to iron deficiency, thalassemia and secondary anaemia. The treatment group by a dose of 50:50 mg/kgBW and 250:250 mg/kgBW had values of MCV that were in accordance with the normal value. The group by a dose of 50:50 mg/kgBW and 250:250 mg/kgBW showed an increase of the value of MCV compared to the control group. The increased values of MCV could be caused by erythrocyte agglutination. The increase of the values could indicate macrocytic anaemia. Statistical analysis pointed out that there were no significant differences in each group, so the combination of both herbs could not be considered to cause toxicity.

Based on the average values of MCV in female rats, it implied that female rats in the control group had average values of MCV of 55.39±0.88 fL. The MP treatment group by a dose of 50:50 mg/kg showed an increased mean of MCV compared to the control group. The treatment group by a dose 250: 250 mg / kgBW and 1250: 1250 mg / kgBW illustrated a decrease in the values of MCV compared to the control group. The increased values of MCV could be caused by erythrocyte agglutination, vitamin B12 and folic acid deficiency, while the decreased values of MCV could be caused by iron deficiency anemia, thalassemia and inflammatory anaemia. The values of MCV compared with the range of reference values in the female Wistar (55-65%) revealed that the intervention group of 1250: 1250 mg / kg had lower results. The results of statistical analysis illustrated significant differences that could be caused by a test of compounds. The average values of MCV in rats by extract given by a dose of 1250 mg/kgBW did not agree to the normal range, so a dose of 1250:1250 mg/kgBW could influence the values of MCV in female Wistar rats.

Based on Table 5, male rats receiving a dose of 50:50 mg / kgBW demonstrated a significant increase in the values of MCH compared to the control group. Meanwhile, Values of MCH in the

Table 5. Values of MCH in Male and Female Rats After Receiving Combination of Meniran and Pegagan for 28 Days with ANOVA Analysis

Intervention	N	MCV (fL) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	17,38 ± 0,25	
K2. MP (50:50)	5	17,82 ± 0,52	0,389
K3. MP (250:250)	6	16,85 ± 0,37	(p>0,05)
K4. MP (1250:1250)	5	16,98 ± 0,52	
Female Rats			
K1. Control (Healthy)	7	18,00 ± 0,18	
K2. MP (50:50)	7	18,48 ± 0,21*	0,046
K3. MP (250:250)	7	17,67 ± 0,18*	(p<0,05)
K4. MP (1250:1250)	7	17,81 ± 0,23*	

Note
MP : Combination of Meniran Pegagan
p : significance between control and intervention group
* : significant difference (p<0,05) between intervention group

intervention group receiving a dose 250:250 mg / kgBW and 1250: 1250 mg / kgBW decreased. An increased value of MCH indicated macrocytic anaemia, whereas a decreased values of MCH meant microcytic anaemia. Values of MCH in all groups were in accordance with the normal range (14.6-21.3 pg). The combination of both herbs did not seem to affect MCH parameters in male Wistar rats.

Based on Table 5, female rats in the intervention group by a dose of 50:50 mg / kgBW demonstrated a significant increase in the average values of MCH compared with the healthy group. The intervention group by a dose 250: 250 mg / kgBW and 1250: 1250 mg / kgBW showed a decrease in the values of MCH compared to the normal group, although there was a decrease and an increase value compared to the control group, but all groups showed average values in accordance to the reference range (17-22). Based on these results, the combination of both herbs could not be considered to cause toxicity to the MCH of female rats, although the analysis results pointed out significant differences but they were still in the normal range.

Based Table 6,Values of MCHC in male rats in the control group had average values of MCHC of 31.88±1.11g/dL. The intervention group by a dose of 50:50 mg/kgBW and 250:250 mg/ kgBW demonstrated a decreased value of MCHC compared to the control group. The intervention group by a dose 1250:1250 mg / kgBW showed values of MCHC similar to the control group. The values of all groups agreed to the normal MCHC (26-38g/dL). The combination of both herbs did not affect the MCHC parameters in male Wistar rats because the analysis results illustrated no significant difference between the groups, and the values were in the normal ranges of MCHC.

Table 6. Values of MCHC in male and female rats after receiving combination of meniran and pegagan for 28 days with Kruskal Wallis analysis

Intervention	N	MCHC (g/dl) Mean ± SE	*P*
Male Rats			
K1. Control (Healthy)	6	31,88 ± 1,11	
K2. MP (50:50)	5	29,22 ± 1,80	0,110
K3. MP (250:250)	6	29,47 ± 1,47	(p>0,05)
K4. MP (1250:1250)	5	31,70 ± 2,00	
Female Rats			
K1. Control (Healthy)	7	32,57 ± 0,37	
K2. MP (50:50)	7	29,44 ± 1,69*	0,024
K3. MP (250:250)	7	32,97 ± 0,19*	(p<0,05)
K4. MP (1250:1250)	7	33,77 ± 0,24*	

Note
MP : combination of Meniran Pegagan
p : significance between control and intervention group
* : significant difference (p<0,05) between intervention group

Table 6 demonstrated that female rats in the normal group had an average value of MCHC of 32.57±0.37g/dL, and this value was similar to the average value of MCHC in rats receiving a dose of 250: 250 mg / kgBW. The Intervention group by a dose of 50:50 mg / kg indicated a decrease in the average values of MCHC. The group by a dose of 1250:1250 mg / kg showed an increase in the average values of MCHC compared to the healthy group. The increased values of MCHC could be caused by haemolysis or

Heinz bodies. Heinz bodies were denatured and precipitated haemoglobin on the inner surface of erythrocytes. The cause of Heinz bodies was blood samples that were oxidized during the analysis or free radicals that cause oxidative damage. The values of MCHC in the four groups agreed to the reference values of MCHC (28-34 g/dL). Based on these results, the combination of both herbs could not be considered to cause toxicity, although the analysis showed significant differences, but the results were still in normal ranges.

DISCUSSION

Haematological examination included examination of erythrocytes, haemoglobin, haematocrit, MCV, MCH, and MCHC. Administering the combination of extracted Meniran and Pegagan in male rats did not demonstrate any significant differences to the haematological parameters. As a result, it could be concluded that the combination of both herbs did not cause toxic effects on the haematological parameters. Administering the combination of both herbs in female rats showed a significant difference of haematological parameters in each intervention group, but the post hoc test did not show any significant differences between the control group and the intervention group, so the administration of both herbs could not be considered to affect haematological values.

Erythrocyte examination of female rats showed significant differences between the groups with doses of 50:50 mg / kgBW, 250: 250 mg / kgBW, 1250: 1250 mg / kgBW, especially in the decrease of the average value of erythrocytes. The significant level of the combination in the control group (>0.05) showed no significant differences, so the administration of both herbs did not affect the values of erythrocytes.

Examination of MCV, MCH, and MCHC in female rats demonstrated differences between groups. In the MCV and MCH tests, there were significant differences in the groups by a dose of 50:50 mg / kgBW, 250: 250 mg / kgBW and 1250: 1250 mg / kgBW, especially in the increase of the average values of the MCV and MCH. In the MCHC test, there were significant differences in the groups by a dose of 1250: 1250 mg / kgBW, 50:50 mg / kgBW, and 250: 250 mg / kgBW, especially in the increase of the average values of MCHC. The level of significance of the combination in the control group in female rats (> 0.05) showed no significant differences , so the administration of both herbs did not affect the value of MCV, MCH, and MCHC.

Based on theory, erythrocyte, haemoglobin, haematocrit, MCV, MCH, and MCHC level in the male rats were higher than female rats in subtropical regions. Iheidioha et al. mentioned that varying haematological profiles could be influenced by geographical conditions and local environmental factors.[13] Results of measurements on Wistar rat blood profile in the tropic could have different results from the subtropical countries.[14,15] Based on this, the normal value could not be determined based on general references. Valid normal values could be determined locally, such as from a laboratory or animal house as a provider, keeper, and breeder of animal for research purposes.[16]

CONCLUSION

This study concluded that the administration of combination extracted Meniran and Pegagan subchronically for 28 days by a dose of 50 mg/kgBW, 250 mg/kgBW and 1250 mg/kgBW did not cause toxic changes in the haematological profile in male and female Wistar rats.

CONFLICT OF INTEREST

None declare.

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REFERENCES

1. Alegantina S, Setyorini HA, Triwahyuni. Pengujian mutu dan penetapan kadar filantin dada ekstrak etanol herba meniran (*Phyllanthus niruri L.*). Buletin Penelitian

Kesehatan. 2015; 43 (1): 11-16.
2. Rivai H, Septika R, Boestari A. Karakteristik ekstrak herba meniran (*Phyllantus niruri Linn*) dengan analisa fluoresensi. Jurnal Farmasi Higea. 2013; 5(2): 15-21.
3. Chong NJ dan Aziz Z. A systematic review on the chemical constituents of Centella asiatica. Research Journal of Pharmaceutical, Biological, and Chemical Science. 2011; 2(3): 445-59.
4. Aldi Y, Artika D, Aria M, Pengaruh pemberian ekstrak etanol meniran (*Phyllantus niruri L.*) terhadap jumlah eritrosit, retikulosit, kadar hemoglobin dan nilai hematokrit pada mencit putih jantan. Prosiding Seminar Nasional dan Workshop Perkembangan Terkini Sains Farmasi dan Klinik IV, Padang, 2014.
5. Ningsih S dan Wibowo. Efek imunostimulan ekstrak meniran (*Phyllanthus niruri L.*) secara in vivo pada tikus. Jurnal Bahan Alam Indonesia. 2011; 7(1): 15-8.
6. Adedapo AA, Adegdayibi AY, Emikpe BO. Some clinico-phatological changes asociated with the aqueous extract of the leaves of Phyllanthus Amarus in rats. Phytotherapy research. 2005; 19: 971-6
7. Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia. Pedoman uji toksisitas nonklinik secara in vivo. BPOM RI. 2014.
8. Sulastry F. Uji toksisitas akut yang diukur dengan penentuan LD50 ekstrak daun pegagan (*Centella asiatica* (L.) urban) terhadap mencit Balb/c (Skripsi). Semarang: Universitas Dipenogoro, 2009.
9. Kusumawardani KC. Uji toksisitas subkronis produk kombinasi eksrtrak rimpang kunyit dan herba meniran pada tikus betina galur wistar kajian terhadap parameter fungsi ginjal (Skripsi). Yogyakarta: Universitas Gadjah Mada, 2016.
10. Hidayati N. Aktifitas kombinasi seledri, meniran, pegagan dan kunyit terhadap kadar eritrosit, hemoglobin, hematokrit dan trombosit pada tikus yang diinjeksi gentamisin (Skripsi). Yogyakarta: Fakultas Farmasi Universitas Ahmad Dahlan, 2018.
11. Salma RP. Efek kombinasi serbuk ekstrak meniran (*Phyllanthus niruri L.*) dan pegagan (*Centella asiatica L.*) terhadap SGOT dan SGPT pada tikus galur wistar (Skripsi). Yogyakarta: Fakultas Farmasi Universitas Ahmad Dahlan, 2019.
12. Wulandari IA. Uji toksisitas subkronik kombinasi ekstrak terstandarisasi meniran dan pegagan terhadap parameter fungsi ginjal pada tikus galur wistar (skripsi). Yogyakarta: Fakultas Farmasi Universitas Ahmad Dahlan, 2019.
13. Ihedioha JI, Ugwuja JI, Noeluneke OA, Udeani IJ, Danieligwe G. Reference values for the haematology profile of conventional grade outbred albino mice (Mus musculus) in Nsukka. Eastern Nigeria: Animal Research International. 2012; 9(2):1601-12.
14. Provan D. ABC of Clinical Haematology 2nd. London: BMJ, 2003.
15. Gad SC. Animal models in toxicology 3rd Ed. Press Boca raton: CRC, 2016.
16. Fitria L dan Sarto M. Profil Hematologi Tikus (Rattus novergicus Berkenhout, 1769) Galur Wistar Jantan dan Betina Umur 4,6 dan 8 Minggu. Jurnal Ilmiah Biologi Biogenesis. 2014; 2(2): 94-100.

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