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Original Article

SUB-ACUTE TOXICITY STUDY OF PEGAGAN EMBUN (*HYDROCOTYLE SIBTHORPIOIDES* LAM.) EXTRACT ON THE SGPT AND SGOT LEVEL OF WISTAR WHITE MALE RATS

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ABSTRACT

Objective: Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) is one of the herbs used in ethnomedicines as an immunostimulant during the COVID-19 pandemic. This present study aims to discover the potential toxicity effect of pegagan embun extract through sub-acute administration on the SGPT and SGOT levels of Wistar white male rats.

Methods: Thirty-six test animals were divided into four groups: the control group was given Na CMC 0.5%, and the treatment groups were treated with ethanol extract of pegagan embun at doses of 7, 35, and 150 mg/kgBW. All groups were treated orally for 7, 14, and 21 d once daily. On the 8th, 15th, and 22nd day, the SGPT and SGOT of the test animal level were measured. The data were analyzed by two-way ANOVA followed by Duncan's multiple range test ($p<0.05$).

Results: The study revealed that administration of pegagan embun extract did not cause any harmful effect on the liver but significantly decreased the level of SGPT and SGOT influenced by the variety of doses and duration of administration ($p<0.05$). Significant reductions in SGPT and SGOT levels are seen after extract administration at dosages of 7 mg/kgBW for 21 d.

Conclusion: This study showed that pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract sub-acute administration at doses of 7, 35, and 150 mg/kgBW is relatively non-toxic and safe to be used as an immunostimulant. There was no sign of damage showed in the liver of treated rats based on the levels of SGOT and SGPT.

Keywords: Pegagan embun, *Hydrocotyle sibthorpioides* Lam., Sub-acute toxicity, SGPT, SGOT

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INTRODUCTION

Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) is a creeping herb belonging to the Araliaceae family, which contains flavonoids [1], saponins [2], and triterpenoids [3]. Previous research has shown that pegagan embun is a significant medicinal plant with several pharmacological applications. Pegagan have a variety of pharmacological effects to treat a wide range of illnesses, including anti-inflammatory [4], antioxidant [5], and cytotoxic activity on tumors [6], breast cancer and anemia [7] and immunostimulant activity [8]. Based on a study by Afriwardi in 2021, pegagan embun herb extract can raise leukocyte counts and the proportion of leukocytes in the lymphocyte and neutrophil segments in male white mice. These effects are shown at dosages of 10, 50, and 200 mg/kgBW [8].

The rise of Coronavirus Disease 2019 (COVID-19) cases in Indonesia negatively impacts many aspects of life, including people's health [9]. The Indonesian Ministry of Health officially released HK.02.02/IV/2243/2020 notice on the use of traditional medicine for health maintenance, disease prevention, and health care included in Public Health Emergencies and/or National Disasters COVID-19. WHO acknowledges the many advantages of traditional or complementary medicine, and Indonesia has a rich history of the use of traditional medicine. The efficacy and adverse side effects of medicinal herbs like Pegagan embun, which are being examined as potential therapies for COVID-19, should be studied. Even though treatments are natural and drawn from conventional practice, it is crucial to demonstrate their efficacy and safety via thorough clinical studies [10].

Due to their frequent usage by humans, plants with therapeutic properties like pegagan embun should have minimal toxicity. Toxic effects have been recorded for medicinal plants used in traditional remedies [11]. Examination for subacute toxicity provides essential information on a chemical's cumulative toxicity as well as the effects of a substance on physiological systems, internal organs, and metabolism when exposed for an extended period at low doses. Numerous different negative effects can be found. These studies' findings may offer data to help decide on a dosing level [12].

Liver is the target for toxins because of the gastrointestinal tract carries most toxins into the liver through the portal vein. Although the liver has enzymes that could metabolize the toxins into a less toxic substance, the high level of toxins can damage and kill the hepatocytes, marked by the increase of Serum Glutamic-pyruvic Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT) level [13]. The liver, cardiac muscle, kidney, brain, pancreas, skeletal muscle, lung, erythrocytes, and leukocytes are just a few of the organs that contain SGOT. However, the hepatic parenchyma has the largest concentration. SGPT, on the other hand, is a cytosolic enzyme that is mostly found in the liver. As a result, SGPT is a more accurate marker of liver damage than SGOT. However, the level of liver damage may not be correlated with the degree of serum transaminase increase [14].

Scientific reference on the safety of pegagan embun is limited, besides its activity as an immunostimulant during the COVID-19 pandemic. Based on the previous explanation, the study was conducted to determine the effect of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract in sub-acute administration on the SGPT and SGOT levels of white male rats.

MATERIALS AND METHODS

Tools

The equipment used were as follows: measuring cylinder (Pyrex), analytical balance (Ohaus®), lab animal scales, metabolic cages (Fengshi), oral gavage, watch glass, dropper, spatula, beaker glass, hot plate (Thermo®), test tubes, test tubes rack, gel and clot activator tube (GP VACUUM PET®), mortar and pestle, rotary evaporator (Buchi®), grinder (Lingling Brand®), glass funnel, vials, 500 ml glass bottle, volumetric flask, volumetric pipette, oven, (MEMMERT®), furnace (JINHE®), freeze dryer, micro tube, micropipette, capillary glass, centrifuge (Rotofix 32®), vortex (IKA VORTEX GENIUS 3®), photometer 5010 v5+(Riele®).

Materials

The materials used were as follows: pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract, ethanol 70%, Na CMC 0.05%, aquadest, filter paper, aluminium foil, TLC Silica gel 60 F_{254} plate (Merck®), rutin standard, animal standard foods, SGPT analysis reagent (Greiner®), SGOT analysis reagent (Greiner®).

Preparation of extract

An amount of 650 g dried and finely grinded pegagan embun was macerated with ethanol 70% (one part of the sample macerated with ten pieces of solvent). After soaking the sample for the first six hours with periodic stirring and letting it for 18 h, filter it through filter paper to get the macerate. The maceration process was repeated twice with the same amount of solvent. Collect all macerate and evaporate them with a rotary evaporator until a thick extract is obtained. The extract was weighed to calculate the rendement. The extract was then characterized using both specific and nonspecific parameters. Particular parameters were as follows: organoleptic, phytochemical screening, thin layer chromatography (TLC) profile, and total flavonoid content. The following nonspecific characteristics were used: acid-insoluble ash content, total ash content, and loss on drying [15].

Sub-acute toxicity study

A total of 36 test animal with the requirement of male wistar rats aged 2-3 mo weighed 200-250 g was used in this research. The test animals were acclimatized for 7 d before the study. The test animals must be in good health, did not experience 10% changes in body weight, and show normal behaviour during the acclimatization [16]. This study was approved by the Research Ethical Committee of Medical Faculty Andalas University No. 402/UN.16.2/KEP-FK/2021. The test animal were divided into four groups. The rats were treated, group 1: Na CMC 0.5% as a control; group 2-4: extract of pegagan embun with doses of 7, 35, and 150 mg/kgBW orally once daily for 7, 14, and 21 consecutive days. Blood sample was collected on the 8th,15th, and 22nd day to measure the SGPT and SGOT level. The test animal's condition must be closely monitored and protected since it will have an impact on the study result.

Measurement of SGPT and SGOT level

Mix 5 parts of reagent 1 with 1 part of reagent 2 (1000 µl reagent 1+200 µl reagent 2) and called as monoreagent. Then, 100 µl serum and 1000 µl monoreagent were added into each test tubes and homogenized for 1 minute. Its absorbance was recorded as a blank solution absorbance. Then, the absorbance of the serum and monoreagent mixture was measured by photometer 5010 v5+(Riele®) on the first minute at the wavelength of 340 nm. Record the measured SGPT and SGOT level in U/l.

Data analysis

The data obtained from the measured SGPT and SGOT results were analyzed statistically by two-way ANOVA and significant results were further analyzed by Duncan's multiple range test ($P<0.05$).

RESULTS

In vivo study examined the effects of several dosages and exposure times on the SGOT and SGPT levels of Wistar white male rats to assess the safety of administering pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract in everyday life. The photometer 5010 v5+ was used to test the SGOT and SGPT level. The pegagan embun sample was collected in Padang City, West Sumatra, at Bukit Ngalau Street, Indarung, Lubuk Kilangan. It was documented in the Andalas University Herbarium (ANDA), Padang City, West Sumatra, Faculty of Mathematics and Natural Sciences, Department of Biology.

A total of five kilograms of fresh pegagan embun were harvested, dried, and finely crushed to yield a 650g fine sample and macerated with ethanol 70%. The maceration procedure results in a 119.3 g thick extract with a rendement of 18.35%. Then, the extract was characterized to ensure the parameters matched the requirement stated by the Indonesia Herbal Pharmacopoeia.

The organoleptic test results show that pegagan embun extract is thick, dark brown colored, has a distinct smell, and has a bitter taste. For nonspecific parameters, the study showed that the loss on drying, total ash content, and acid insoluble ash content of the extract was 9.18%±0.39%, 8.82%±0.73%, and 0.5%. The results from loss on drying, total ash content, and acid insoluble ash content show that pegagan embun extract qualifies the standard stated by the Indonesian Herbal Pharmacopoeia.

The phytochemical screening showed that pegagan embun contains flavonoid, phenolic, saponin, and terpenoid. Thin Layer Chromatography (TLC) profile test was carried out to identify the flavonoid compound in the extract by using rutin as the marker. The stationary phase used was TLC Silica gel 60 F_{254} plate. The mobile phase used was n-butanol, acetic acid, and water (4:1:5). The TLC profile was examined under 254 nm UV light. The retention factor (Rf) of rutin and pegagan embun extract was 0.6. It can be concluded that the pegagan embun extract contains rutin (fig. 1).

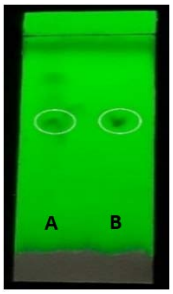

Fig. 1: Thin layer chromatography profile of (A) Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract (Rf=0.6); and (B) rutin (Rf=0.6)

The total flavonoid content was assessed to quantify the flavonoid content in the pegagan embun extract. The assessment was done based on method one stated in the Indonesian Herbal Pharmacopoeia. Rutin was used as the standard solution to plot the standard curve, while the extract solution (test solution) was used to obtain the absorbance. The result shows that pegagan embun extract has total flavonoid content of 13,37±0,13 mgRE/g at the wavelength of 415 nm.

The sub-acute toxicity study of pegagan embun in the liver was determined by measuring the SGOT and SGPT levels in the group treatment on days 8th, 15th, and 22nd. Based on this research, the mean SGPT value of control and extract-treated groups with doses of 7, 35, and 150 mg/kgBW were 50.11±1.45 U/l; 45±3.5 U/l; 48.44±4.72 U/l; 50.89±2.52 U/l respectively; while mean SGPT value on the 8th, 15th and 22nd day were 50.33±2.49 U/l; 49.42±3.96 U/l; 46.08±3.89 U/l respectively (table 1 and fig. 2). The mean SGPT value of white male rats in this study is still in the normal range for all groups' treatment (25-55 U/l) [17] and is relatively nontoxic.

Based on the data obtained from the study, the mean SGPT values were significantly influenced by doses and duration of administration of pegagan embun extract ($p<0.05$). Meanwhile, the interaction between doses and duration of administration did not significantly influence the mean SGPT value ($p>0.05$). Duncan's multiple range test on doses shows that extract treated group with a dose of 7 mg/kgBW significantly reduced the mean SGPT value compared to the control, 35, and 150 mg/kgBW groups. Otherwise, for the duration of administration, it can be seen that the mean SGPT value on the 22nd day significantly decreased compared to the mean SGPT value on the 8th and 15th days. It can be concluded that the SGPT level of the treatment group was within the expected value and relatively nontoxic (table 1).

Meanwhile, the mean SGOT value of control and extract-treated groups with doses of 7, 35, and 150 mg/kgBW were 70.67±2.74 U/l; 61.11±6.77 U/l; 65.78±8.59 U/l; 62.44±12.41 U/l respectively; while mean SGOT value on the 8th, 15th and 22nd days respectively were 69.25±4.14 U/l; 69.42±4.44 U/l; 56.33±9.45 U/l (table 2 and fig. 3). Based on the result, the mean SGOT value of white male rats is still in the normal range for the control and the extract-treated groups (45,7-80,8 U/l) [17] and is relatively nontoxic.

Table 1: The Effect of dosage and duration of administration of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract on the average SGPT levels

Dose (mg/kgBW)	The average of SGPT level (U/l) on day±SE			The average±SE
	8	15	22	
Control	51.33±1.53	50.00±1.00	49.00±1.00	50.11±1.45[b]
7	48,33±2.52	44.67±2.31	42.00±2.65	45.00±3.50[a]
35	49.67±2.52	51.33±5.13	44.33±4.04	48.44±4.72[ab]
150	52.00±2.65	51.67±2.52	49.00±2.00	50.89±2.52[b]
The average±SE	50.33±2.49[b]	49.42±3.96[a]	46.08±3.89[a]	

Notes: The mean data with different superscripts in the columns and rows showed a significant difference ($p<0.05$) based on duncan's multiple-range tests analysis

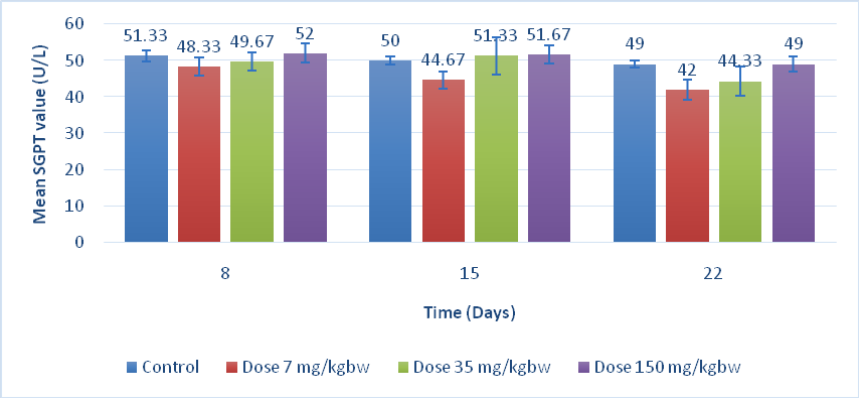

Fig. 2: Mean SGPT value (U/l) of treatment group and duration of administration of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract graph

Table 2: The effect of dosage and duration of administration of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract on the average SGOT levels

Dose (mg/kgBW)	The average of SGOT level (U/l) on day±SE			The average±SE
	8	15	22	
Control	71.00±1.00	71.33±1.15	69.67±5.03	70.67±2.74[c]
7	65.33±4.51	65.33±1.53	52.67±0.57	61.11±6.77[a]
35	70.00±5.00	71.67±3.79	55.67±4.93	65.78±8.59[b]
150	70.67±5.04	69.33±7.23	47.33±5.69	62.44±12.41[ab]
The average±SE	69.25±4.14[b]	69.42±4.44[b]	56.33±9.45[a]	

Notes: The mean data with different superscripts in the columns and rows showed a significant difference ($p<0.05$) based on duncan's multiple-range tests analysis

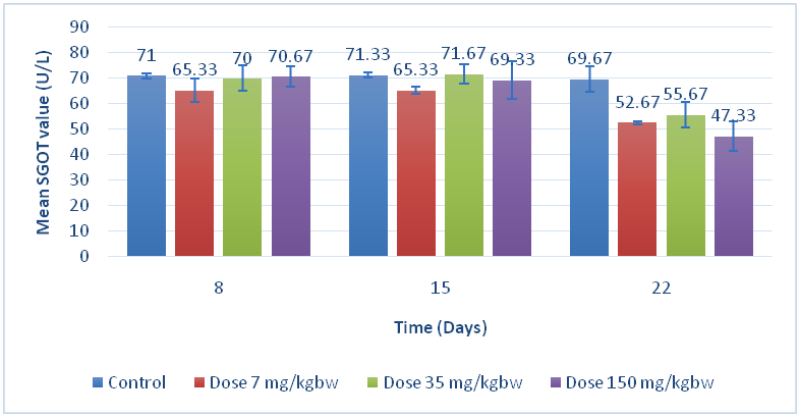

Fig. 3: Mean SGOT value (U/l) of treatment group and duration of administration of pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract graph

The same as the previous result, the mean SGOT values were substantially impacted by the dosages, length of administration of pegagan embun extract, and interaction between doses and duration of administration ($p<0.05$) based on the results assessed by two-way

ANOVA. Duncan's multiple range test on doses shows that extract treated group with a dose of 7, 35, and 150 mg/kgBW showed a significant decrease in mean SGOT value compared to the control group. Duncan's multiple range test on the duration of administration shows that the mean SGOT value on the 22nd day differed significantly from the mean SGOT value on the 8th and 15th days. The SGOT value decreases considerably on the 22nd day. It can also be concluded that the SGOT level of the treatment group was within the expected value and relatively nontoxic (table 2).

DISCUSSION

Medicinal plants are widely accepted worldwide as effective treatments for various ailments because their pharmacologically significant phytoconstituents are considered to be natural and hence associated with little to no toxicity. Therefore, regardless of the length of use, toxicity studies are crucial in determining therapeutic plants' safety or adverse effects [18]. Subacute toxicity (also known as repeat dose toxicity) is the study of adverse reactions that follow the administration of a test sample in a single dosage or several doses each day for a duration of between 14 and 28 d [19].

When determining the safety or toxicity of medicinal plants, it's crucial to consider the liver, which serves as the body's main organ for metabolism, detoxification, and dispersion of foreign substances [20]. The aminotransferases, which comprise SGOT and SGPT, are reliable indicators of liver health and significant biomarkers for predicting toxicity. These enzymes are ordinarily contained within liver cells; however, when the liver is damaged, the liver cells release these enzymes into the blood, raising the blood levels of the SGOT and SGPT enzymes. Because it is produced within the liver cells as opposed to other tissues, SGPT is a more sensitive serum marker enzyme for liver disease than SGOT. SGOT is also produced by the heart muscle, skeletal muscle, brain, lungs, leucocytes, red blood cells, kidney, and pancreas [21]. The considerable change in enzyme levels in the rats in this study who received sub-acute oral doses may indicate that the liver's function was impacted [22].

This study shows that pegagan embun extract sub-acute administration at doses of 7, 35, and 150 mg/kgBW is generally nontoxic based on the level of SGPT and SGOT. The extract-treated group with a dose of 7 mg/kgBW significantly reduced the mean SGPT value compared to the control, 35, and 150 mg/kgBW groups. There was no significant difference between the treatment 35 and 150 mg/kgBW extract group compared to the control group. Meanwhile, for SGOT level, the all-treatment extract group reduced the SGOT level of rats significantly compared to the control group. This matches the previous Hazarika (2019) study that reported the LD50 of *Hydrocotyle sibthorpioides* Lam. extract in white female Wistar rats is>2000 mg/kgBW and relatively very safe on acute oral administration. Based on the Hazarika study, there are no significant changes in the rat's body weight, behavior, renal function, liver function, and lipid profile on acute administration [23].

According to this study, in comparison to other groups, the animals given the lowest dosage (7 mg/kg BW) showed the highest decrease in SGOT and SGPT levels. This could be brought on by pegagan embun extract, which research suggests may have hepatoprotective properties. The level of SGPT and SGOT can be decreased because the flavonoid compound, rutin, in the pegagan embun extract has antioxidant properties and acts as a potential hepatoprotector. Khan *et al.* (2012) reported that rutin significantly protects against CCl_4-induced liver injury in Sprague–Dawley white male rats by suppressing SGPT, SGOT, ALP, and γ-GT in the serum [24]. Yesmin *et al.* (2019) research on the antioxidant activity and hepatoprotective potential of *Piper chaba* roots on paracetamol-induced liver injury reported that administration of *Piper chaba* roots ethanol extract on a low dose (200 mg/kgBW) is more effective in lowering the SGPT, SGOT, ALP, and bilirubin level than the higher dose (400 mg/kgBW) [25]. When antioxidant treatment was used, this event was frequent to happened [26, 27].

Another study from Hazarika found that Rats' cognitive behavior was greatly improved by chloroform and methanolic extracts of *H. sibthorpioides* compared to the control. According to biochemical research, the cortex and hippocampus had higher amounts of antioxidants and lower levels of glutamate and proinflammatory cytokines. The researcher came to the conclusion that *H. sibthorpioides* extracts in chloroform and methanol increased the amount of antioxidants in the brain, lowered pro-inflammatory cytokines and glutamate, and so avoided the monosodium-glutamate-induced-excite-neurotoxicity [28]. Quanfang *et al.* also examined the effect of *Hydrocotyle sibthorpioides*-derived genistein on Chronic Alcohol-Induced Hepatic Fibrosis. In rats, persistent alcohol administration causes liver fibrosis and liver damage, both of which are ameliorated by genistein [29].

Due to its many qualities, such as its anti-inflammatory, antioxidant, antidiabetic, cardiovascular, neuroprotective, and anticancer effects, rutin has been demonstrated to have a wide range of therapeutic uses. Numerous pathways have been identified over time as being in charge of its antioxidant activity in both *in vitro* and *in vivo* studies. It was first noted that its chemical composition might directly scavenge Reactive Oxygen Species (ROS). The expression of various antioxidant enzymes, including Catalase (CAT) and Superoxide Dismutases (SOD), is thought to be elevated, which in turn enhances the synthesis of Glutathione (GSH) and is thought to upregulate cellular oxidative defense mechanisms. Thirdly, rutin prevents the production of ROS by inhibiting the enzyme xanthine oxidase. Given those mentioned above, it is apparent why rutin can treat various medical disorders where oxidative stress is a contributing factor [30].

CONCLUSION

This study concluded that pegagan embun (*Hydrocotyle sibthorpioides* Lam.) extract sub-acute administration at doses of 7, 35 and 150 mg/kgBW and duration of administration at 7, 14 and 21 d are relatively non-toxic to the liver and safe to be used as an immunostimulant. There were no sign of damage showed in the liver of treated rats based on the levels of SGOT and SGPT.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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