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The Effect of Administration Platycerium bifurcatum (Cav.) C.Chr Extract on Total Bilirubin Levels and Hepatosomatic Index Values of Ethanol-Induced Rats

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Abstract: The Liver is a vital metabolic organ. Liver injury is related to an inflammatory process that increases the hepatosomatic index (HSI). One indicator of liver damage is increased total bilirubin levels (hyperbilirubinemia). Currently, hyperbilirubinemia treatment is still limited to irradiation therapy and exchange transfusions, because there is no pharmacological specific treatment. This study aimed to evaluate the hepatoprotective effect of Platycerium bifurcatum (Cav.) C.Chr extract on total bilirubin levels and the hepatosomatic index (HSI) in male white rats. A laboratory-based experimental design was employed using a post-test only control group, involving 35 rats randomly assigned to seven groups: normal control, negative control, positive control (sacatonic active), solvent control (Na CMC), and treatment groups receiving ethanol extract at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW.The treatment was administered orally for 14 consecutive days. On the final day, all rats (except the normal group) were induced with absolute ethanol at 2 mL/200 gBW. After 12 hours, the animals were euthanized for liver and blood sample collection. Total bilirubin levels were measured, and the hepatosomatic index was calculated. Data were analyzed using one-way ANOVA followed by LSD post hoc test. The results demonstrated that administration of *Platycerium bifurcatum* (Cav.) C.Chr extract significantly reduced total bilirubin levels and HSI, particularly at doses of 50 mg/kgBW and 100 mg/kgBW, compared to the negative control group ($p<0.05$). These findings suggest that *Platycerium bifurcatum* (Cav.) C.Chr ethanol extract exhibits a hepatoprotective effect by reducing total bilirubin levels and HIS.

Keywords: Ethanol, Hepatosomatic index, Liver injury, *Platycerium bifurcatum* (Cav.) C.Chr, Total bilirubin
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1. INTRODUCTION

Paku tanduk rusa (*Platycerium bifurcatum* (Cav.) C.Chr.) belongs to the *Polypodiaceae* family, which contains secondary metabolite compounds, namely tannins, alkaloids, flavonoids, glycosides, phenolic compounds and steroids (Robert, Isaac and Joel, 2023). Previous studies have proven the activity of methanol extracts of *Platycerium bifurcatum* as hepatoprotector with parameters of liver weight ratio, SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvic Transaminase) and total bilirubin (Muoneke and Bruno, 2022). Platycerium bifurcatum exhibits antibacterial and in vitro cytotoxic activity against MCF-7 breast cancer cells (Chinaka, Okwudili and Nkiru, 2018; Jumaryatno *et al.*, 2023).

Platycerium bifurcatum has been tested for antioxidant activity using the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method at concentrations of 200 μg/mL and 400 μg/mL, which caused a decrease in free radicals by 41.17% and 75.82%, respectively (Chinaka, Okwudili and Nkiru, 2018). This antioxidant compound acts as a hepatoprotector, protecting the liver from toxic substances, compounds, and drugs (Suharyanto and Prima, 2020). One substance that is toxic to the hepatic organ is ethanol (Wardani *et al.*, 2024). Ethanol can induce cytochrome P450 enzymes, which can increase free radicals directly by forming superoxide radicals, which are toxic to the liver (Liu, Tsai and Hsu, 2021).

Total bilirubin examination is very important because it is one of the parameters to assess the secretory function of the liver. This examination consists of examining total serum bilirubin, direct serum bilirubin and indirect serum bilirubin. If liver function is impaired, total bilirubin levels will increase (Gondal and Aronsohn, 2016). In addition to bilirubin, hepatosomatic index (HSI) can also parameterise the presence of damage to the hepatic organ. HSI values that are greater than normal conditions can describe the swelling and inflammation in the liver due to necrosis of hepatic cells (Nessa, Martinus and Oktarina, 2022). Until now, the management of hyperbilirubinemia still uses irradiation therapy and exchange transfusions because no safe pharmacological drug therapy has been found for hyperbilirubinemia, especially for infants (Kemenkes RI, 2019). Therefore, by looking at the potential that exists in *Platycerium bifurcatum* (Cav.) C.Chr., the researcher, aims to determine the effect of giving ethanol extract of paku tanduk rusa on total bilirubin levels and hepatic weight of male white rats. The success of this study will be very helpful in the world of health, especially in the development of new traditional medicine therapies as hepatoprotectors, especially in cases of hyperbilirubinemia.

2. METHODS

2.1 Tools and Materials

The tools used are a set of glassware, rotary evaporator, hotplate, analytical scales, mortar and stampher, Non-EDTA tube, animal surgery board, syringe, coolbox, Mindray BA-88A photometer (clay adams), maceration bottles, Bunsen burner, measuring pipettes, sterile surgical instruments, droppers, 100 mesh sieves, stirring rods, and oral sonde. The materials used were paku tanduk rusa leaves, 96% ethanol, Na CMC, absolute ethanol, phytochemical test reagents (Wagner, Mayer, Dragendrof and Liberman Burchard reagents), H_2SO_4, $FeCl_3$, HCL, gelatine, chloroform, plastic aluminium foil, male white rats (aged 2-3 months, minimum body weight 120 g), distilled water, and BR 551 rat feed.

2.2 Methods

This research has obtained ethical approval from The Ethical Committe of Medical Research Faculty of Dentistry, University of Jember, Indonesia, with certificate No.2568UN25.8/KEPK/DL/2024. This research was an experimental study using a Post-Test Control Group Only design. The treatment consisted of seven groups with five animals each. The treatment groups included a normal group, solvent control, negative control, positive control, and groups receiving Platycerium bifurcatum extract at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW for 14 days. On the last day, all rats (except the normal group) were induced with 100% ethanol (absolute) at a single dose of 2 mL/200 gBW and underwent surgery after 12 hours.

a. Plant verification

Plant verification of paku tanduk rusa was conducted at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University.

b. Preparation of the sample

The process of making dried plant material for paku tanduk rusa begins with collecting raw materials from the forest of Kuti Agung Village, Seluma, Bengkulu Province. The plant material underwent wet sorting, followed by washing, chopping, and drying in an oven at 45 °C for 10 days (10 × 24 hours), after which it was ground into a crude powder.

Extraction of paku tanduk rusa simplisia using the maceration method with the ratio of extract and solvent is 1:5. Two hundred grams of simplisia were soaked in 1 litre of 96% ethanol for 2 x 24 hours with occasional stirring. Then remaceration was carried out until a clear mixture was obtained (Malino *et al.*, 2024). The percentage of yield can be calculated with the following formula (Fawwaz *et al.*, 2024).

$$\%yield = \frac{Total\ weight\ extract(g)}{Total\ weight\ of\ sample\ (g)} \times 100\%$$

c. Phytochemical screening

1. Alkaloid**:** 1-2 mL extract solution was put into a test tube, add 1 mL of HCl 2 N and 9 mL of distilled water then the solution was heated for 2 minutes on a water bath after which it was filtered then the filtrate was divided into three tubes and added 1-2 drops of Wagner reagent for tube 1, Mayer for tube 2, and Dragendrof for tube 3 (Harbone, 1998).
2. Flavonoids: 1 mL of extract solution was put into a test tube, 2-5 drops of 10% NaOH were added. Flavonoids are positive if there is a red to brown color change (Harbone, 1998).
3. Saponins: A sample of 0.5 grams was mixed with 10 mL of hot water and then shaken vigorously for 10 seconds until foam appeared. Then, 1 drop of HCl 2 N is added. The presence of stable foam after adding HCL 2N indicates positive for saponins (Harbone, 1998).
4. Tannins**:** Test with FeCl3: 1-2 mL of extract solution is added with 2-3 drops of 1% $FeCl_3$ solution. If the solution changes color to blackish green or dark blue, it is positive for tannins. Test with gelatin: 1-2 mL of extract solution is added with the gelatin solution. Positive results are indicated by the presence of white sediment (Harbone, 1998).
5. Steroids and Terpenoids**:** 0.5 grams of extract was dissolved with ethanol, put into a cup, then 1 mL of ether, evaporated until dry, then 3-5 drops of Liberman Bouchard reagent. The formation of a green color marks positive results for steroids, while triterpenoids are marked by a red-purple color (Harbone, 1998).

d. Group division of animals

35 male white rats (Wistar strain, aged 2-3 months, minimum body weight 120 g), were divided into seven groups, each with five animals. The treatment was carried out for 14 days and on the last day, after 2 hours of treatment the rats were induced with absolute ethanol 2 mL/200 gBW, and after 12 hours surgery was performed to take hepatic organs and blood serum samples. The following is the division of groups in this study:

1. The normal group was only given food and drink, and on the last day, was not induced by absolute ethanol.
2. The solvent control group was given 0.5% Na CMC solution equivalent to 50 mg/kgBW, and on the last day, induced by absolute ethanol 2 mL/200 gBW.
3. The negative control group was given drinking water and purified food for 14 days and on the last day was induced by absolute ethanol 2 mL/200 gBW.
4. The positive control group was given Sakatonik Activ Syrup at a dose of 334.7 mg/kgBW for 14 days and on the last day, was induced with absolute ethanol at 2 mL/200 gBW.
5. Treatment group 1st dose was given ethanol extract of *Platycerium bifurcatum* (Cav.) C.Chr. orally at a dose of 50 mg/kgBW for 14 days and induced absolute ethanol 2 mL/200 gBW on the last day.
6. The 2nd dose treatment group was given ethanol extract of *Platycerium bifurcatum* (Cav.) C.Chr. orally at a dose of 100 mg/kgBW for 14 days and induced absolute ethanol 2 mL/200 gBW on the last day.
7. The 3rd dose treatment group was given ethanol extract of paku tanduk rusa. Orally at a dose of 200 mg/kgBW for 14 days and on the last day induced absolute ethanol 2 mL/200 gBW.

e. Surgery, hepatic organ collection and examination of total bilirubin levels

Chloroform was used for inhalation to induce anaesthesia and facilitate cervical dislocation, not as a euthanasia agent. Afterwards, the rats were dissected using sterile scissors from the abdomen to the thorax, and the hepatic organs were removed and weighed. The following is a formula to calculate the percentage of hepatosomatic index value (Syahidah, Pertiwi and Adfa, 2022).

$$\%HSI = \frac{Weight\ of\ hepatic\ organ}{Rat\ body\ weight} \times 100\%$$

Blood samples were taken from the aortic vessel near the heart using a 3 mL syringe and then inserted into a non-EDTA tube in a cool box to ensure that the blood samples were not lysed, then centrifuged at 3000 rpm for 15 minutes using a Clay Adams® centrifuge. Total bilirubin levels were measured at the Biomedical Laboratory of Bhayangkara Hospital, Bengkulu City. Measurement of total bilirubin level using Mindray BA-88A® photometer.

f. Data analysis

Data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 27.0.1. A one-way ANOVA test was performed to determine whether there were significant differences between the groups. Prior to the ANOVA, the data were tested for normality and homogeneity with the condition that (p>0.05). If the ANOVA showed a significant result (p<0.05), it was followed by a Least Significant Difference (LSD) post hoc test to identify which specific groups differed significantly (p<0.05).

3. RESULTS AND DISCUSSION

This study has obtained ethical feasibility permission from the Health Research Ethics Commission, Faculty of Dentistry, University of Jember, with No.2568UN25.8/KEPK/DL/2024. Based on the plant verification that has been carried out, a plant verification letter with No. 336/UN30.28.LAB.BIOLOGY/PP/2024.The verification results show that the plant species is *Platycerium bifurcatum* (Cav.) C.Chr with the order Polypodiales and the family Polypodiaceae.

In this study, a wet sample of paku tanduk rusa as much as 1.5 kg was used and a simplisia powder weight of 252 g was obtained. The results of the maceration extraction process of paku tanduk rusa samples can be seen in Table 1.

Table 1: Results of simplisia weight, paku tanduk rusa extract and % Yield

Wet sample	Dry powder	Extracted Powder	Thick Extract	% Yield
1,58 kg	252 g	200 g	24, 85 g	12, 42%

The extraction process was carried out for 2 x 24 hours with a ratio of sample and solvent (96% ethanol) 1: 5, then filtered and the remaining filtrate was remacerated until clear in color. After 13 remaserations, a clear mixture was obtained, indicating that the active substance had been completely dissolved (Sulistyarini *et al.*, 2016).This clear solution also indicates the process has reached the saturation poin (Syahidah, Pertiwi and Adfa, 2022). The use of polar solvents in maceration aims to dissolve polar compounds such as alkaloids, flavonoids, tannins, and saponins, following the principle of "like dissolve like" (Sinaga *et al.*, 2024). From 200 g of macerated paku tanduk rusa leaf simplisia, 24.85 g of thick extract was obtained after evaporation. The yield of this extract is 12.48%, which is considered good because it exceeds 10% (Asendy, Widarta and Nocianitri, 2018).

Table 2: Result of phytochemical screening

Screening	Reagent	Observation Result	Final result
Alkaloid	Wagner	Brown precipitate	+
	Mayer	White precipitate	+
	Dragendorf	Orange to light brown precipitate	+
Flavonoid	NaOH 10%	discoloration to brown	+
Saponin	Aquadest, HCL 2N	Stable nozzle 2.3 cm	+
Tanin	$FeCl_3$ 1%	Deep blackish green discoloration	+
	Gelatin	White precipitate	+
Steroid	Liberman Burchard	color change to green	+
Triterpenoid	Liberman Burchard	No color change	-

Description: + (positive metabolite secunder), -(negative metabolite secunder)

Based on the phytochemical screening carried out, the results obtained as can be seen in Table 2 where the ethanol extract of paku tanduk rusa positively contains alkaloid compounds, flavonoids, tannins, saponins, and steroids and negative results on terpenoid compounds.

Phytochemical screening showed positive results for alkaloids with Wagner, Mayer, and Dragendorf reagents. In the Mayer reagent, a white precipitate is formed due to the reaction of alkaloids with mercury-iodide ions, in Wagner, a brown precipitate is formed from the reaction of I_3 ions with alkaloid nitrogen and in Dragendorf, an orange to brown precipitate is formed from the reaction of alkaloids with bismuth iodide ions (Marliana SD, Suryanti V and Suyono, 2015).

Flavonoid testing shows a brown color change due to the reaction with NaOH, which decomposes flavone into acetophenone (Suharyanto and Prima, 2020). Saponin testing produces stable foam as high as 2,3 cm after adding 2N HCl, meeting positive standards, indicating glycoside compounds forming foam in water (Pratiwi *et al.*, 2024). Tannin testing with $FeCl_3$ 1% showed a concentrated green to almost black color change and precipitate formation with gelatin, indicating the interaction of tannin with gelatin to form an insoluble complex (Samaria and Megawati, 2024; Suhaila *et al.*, 2024; Widowati, Parnanto and Muthoharoh, 2020).

Testing steroids using the Liberman-Burchard reagent produces a green color change, indicating the formation of a complex with H_2SO_4 in anhydrous acetate solvent (Fransiska *et al.*, 2021). Triterpenoid testing is negative due to the nonpolar nature of the compound (Mierza *et al.*, 2023). These screening results follow previous research stating that ethanol extracts of paku tanduk rusa contain alkaloids, flavonoids, tannins, saponins, and steroids (Muoneke and Somtochukwu, 2022). However, unlike the earlier findings, triterpenoids were not detected in this study, although they had previously tested positive. This inconsistency might have occurred because triterpenoids are polar compounds and were less efficiently extracted using the polar solvent ethanol used in this research. Moreover, differences in growing locations could have influenced the secondary metabolite content, as environmental factors such as temperature, humidity, and altitude are known to affect plant chemical composition (BPOM RI, 2023).

This study will look at the effect of giving ethanol extract of paku tanduk rusa (*Platycerium bifurcatum* (Cav.) C.Chr) on the ratio of hepatic weight (hepatosomatic index). The results of the hepatic weight ratio can be seen in Table 3.

Table 3: Average result of weight, liver and hepatosomatic index (HSI)

Treatment Groups	$\bar{x}$ Weight Body ± SD (g)	$\bar{x}$ Liver Weight ± SD (g)	$\bar{x}$ HSI ± SD (%)
Normal	206.0 ± 13.52	5.8 ± 0.43	2.8 ± 0.10^{abc}
Negative	193.3 ± 6.65	6.9 ± 0.45	3.5 ± 0.14^{abcd}
Solvent (Na CMC 0,5%)	164.3 ± 50.81	4.8 ± 1.13	3.0 ± 0.81^{b}
Positive (Sakatonik activ)	175.6 ± 8.50	4.8 ± 1.00	2.7 ± 0.51^{e}
D1 (50 mg/kgBW)	167.0 ± 21.63	4.4 ± 1.15	2.6 ± 0.69^{ef}
D2 (100 mg/kgBW)	181.6 ± 12.05	4.1 ± 1.25	2.2 ± 0.61^{efg}
D3 (200 mg/kgBW)	185.6 ± 5.85	5.4 ± 0.62	2.9 ± 0.30^{ef}

Description: (D): Dose of *Platycerium bifurcatum* (Cav.) C.Chr extract, ($\bar{x}$): mean, (SD): Standard deviation, (a): significantly different from D1, (b): significantly different from D2 (c): significantly different from D3, (d): significantly different from control +, (e): significantly different from negative control, (f): significantly different with normal group, (g): significantly different with solvent control

Hepatosomatic index (HSI) measures the ratio of hepatic weight to body weight, which reflects the physiological condition of the hepatic (Sasmita, Kuswanti and Khaleyla, 2024; Pangestuningsih and Rukminingsih, 2022). The results showed that the normal group had an HSI of 2.8 ± 0.10%, which was lower than that of the negative control (3.5 ± 0.14%) and solvent control (3.0 ± 0.80%). The extract-treated groups (doses I, II, and III) showed significantly different HSI values compared to the normal group (p<0.05). Among these, the extract at dose II (100 mg/kgBW) was the most effective in reducing HSI to 2.2 ± 0.61% (p< 0,05). The negative control group had the highest HSI, indicating liver damage, but its HSI was significantly reduced in the positive control group (2.7 ± 0.51%) (p<0.05). Importantly, the significant reduction in HSI observed in extract-treated groups, particularly dose II, compared to the negative control (p<0.05) indicates a hepatoprotective effect of the ethanol extract of Platycerium bifurcatum. Furthermore, the solvent control (Na CMC) did not produce a significant reduction in HSI (p>0.05), confirming that the observed effects were due to the extract itself. The significant difference between dose II and the solvent control (p<0.05) further supports the efficacy of the extract.

Ethanol extract of paku tanduk rusa reduced HSI at doses of 50 mg/kgBW (2.6 ± 0.69%), 100 mg/kgBW (2.2 ± 0.61%), and 200 mg/kgBW (2.9 ± 0.30%). The dose of 100 mg/kgBW showed the best effect (p<0.05), although the difference between doses was not significant (p>0.05). The decrease in HSI is thought to be due to the antioxidant and anti-inflammatory activities of the flavonoids in the extract, which help prevent oxidative stress and inflammation (Agbo *et al.*, 2014; Chinaka, Okwudili and Nkiru, 2018; Muoneke and Somtochukwu, 2022).

Bilirubin is a yellow pigment resulting from the destruction or breakdown of erythrocytes (red blood cells) which are secreted into bile by hepatic cells (Aji, Arania and Maharyunu, 2021). An increase in total bilirubin levels in the

blood or commonly called hyperbilirubinemia indicates impaired liver and bile function (Andrini Djojosugito, 2021).The results of the examination of total bilirubin levels can be seen in Table 4.

Table 4: Average result of Bilirubin Total Levels

Treatment groups	$\bar{x}$ Bilirubin Total Levels ± SD (mg/dL)
Normal	0.083 ± 0.00^{a}
Negative	0.103 ± 0.01^{ab}
Solvent (Na CMC)	0.090 ± 0.03^{a}
Positive (sakatonic activ)	0.086 ± 0.01^{a}
Dose 1 (50 mg/kgBW)	0.050 ± 0.01^{bcde}
Dose 2 (100 mg/kgBW)	0.080 ± 0.01^{d}
Dose 3 (200 mg/kgBW)	0.086 ± 0.01

Description: (KgBW): Kilogram body weight, ($\bar{x}$): average, (SD): Standard deviation, (a): significantly different from dose 1, (b): significantly different from dose 2, (c): significantly different from control +, (d): significantly different from control -, (e): significantly different from Normal group

The results of the examination of total bilirubin levels showed that the normal group had a value of 0.083 ± 0.00 mg/dL, lower than the negative control group (0.103 ± 0.01 mg/dL) and solvent control (0.090 ± 0.03 mg/dL), which had increased total bilirubin levels. Post Hoc LSD statistical test showed that the normal group was significantly different from the first dose of ethanol extract of paku tanduk rusa (50 mg/kgBW) with a value of $p<0.05$, indicating a decrease in total bilirubin levels at that dose. However, the significant difference at dose I was due to one lysed sample, which affected the results.

The negative control, which was given 2 mL/200gBW ethanol, showed the highest total bilirubin level, which was 0.103 ± 0.01 mg/dL, and significantly different from the normal control group ($p<0.05$), indicating that ethanol increased total bilirubin levels, although it did not significantly affect the increase when compared to the normal control. The negative control group was significantly different from dose I and dose II with the value ($p<0.05$), indicating that the ethanol extract of paku tanduk rusa can significantly reduce total bilirubin levels.

The solvent control group (Na CMC 0.5%) had a total bilirubin level of 0.090 ± 0.03 mg/dL, which was higher than the normal control group, but not significantly different from the other groups ($p>0.05$). However, the solvent control group was significantly different from the first dose (50 mg/kgBW) ($p<0.05$), indicating that the first dose of ethanol extract of paku tanduk rusa had a significant effect in reducing total bilirubin levels.

The positive control group (sakatonik activ) showed an average total bilirubin level of 0.086 ± 0.01 mg/dL, which was lower than the negative control and significantly different from dose I (50 mg/kgBW) ($p<0.05$). However, there was no significant difference between the positive control and dose II (100 mg/kgBW) and dose III (200 mg/kgBW) ($p>0.05$), indicating that the ethanol extract of paku tanduk rusa at doses of 100 mg/kgBW and 200 mg/kgBW had an effect equivalent to the positive control. The average total bilirubin level at dose I (50 mg/kgBW) was 0.050 ± 0.01 mg/dL, which was lower than dose II (100 mg/kgBW) and dose III (200 mg/kgBW). Post Hoc LSD test showed significant differences between dose I with normal control, dose I, dose II, and negative control ($p<0.05$). Meanwhile, dose II (100 mg/kgBW) had a total bilirubin level of 0.080 ± 0.01 mg/dL and was not significantly different from the positive control ($p<0.05$), indicating that dose II had almost the same effect as the positive control in reducing total bilirubin levels.

Overall, the ethanol extract of Platycerium bifurcatum (paku tanduk rusa) at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW significantly reduced total bilirubin levels compared to the negative control group ($p<0.05$). This reduction in total bilirubin, along with the decrease in hepatosomatic index (HSI), indicates a hepatoprotective effect of the extract against ethanol-induced liver damage. Among the tested doses, 100 mg/kgBW was the most effective, as it resulted in the greatest reduction in total bilirubin levels, which was also statistically significant ($p<0.05$). Although no significant difference was observed between the extract-treated groups and the positive control ($p>0.05$), the findings suggest that the 100 mg/kgBW dose of Platycerium bifurcatum ethanol extract provides optimal hepatoprotective activity in mitigating liver injury caused by ethanol exposure.

Sakatonik activ as a positive control, a product with high iron content, also functions as an antioxidant. The iron in sakatonik activ plays a role in increasing the activity of the enzyme superoxide dismutase (SOD), which is

important in cell defence against oxidative stress, by converting superoxide radicals into oxygen and hydrogen peroxide that are less harmful to the body's cells (Utami and Farida, 2022).

The mechanism of reducing total bilirubin levels is also thought to be related to the antioxidant activity of secondary metabolites in paku tanduk rusa extract. Active compounds such as flavonoids (quercetin), alkaloids, tannins, and saponins in the extract have the ability to reduce oxidative stress and inflammation in the hepar. For example, quercetin, which is a flavonoid compound, can increase the levels of GSH (glutathione) and catalase enzymes, which play a role in protecting hepatic cells from free radical damage (Muoneke and Somtochukwu, 2022). In addition, alkaloids have hepatoprotective activity through reducing oxidative stress and increasing SOD (Superoxide dismutase) activity, which can protect hepatic cells from further damage (Sirin, Nigdelioglu Dolanbay and Aslim, 2023). Saponins also function as hepatoprotectors by inhibiting lipid peroxide formation and reducing inflammation in the liver (Timilsena, Phosanam and Stockmann, 2023). Tannins, with OH^- groups, function as free radical scavengers and reduce damage to the hepar through the free radical reduction process (Damayanti, 2023).

The ethanol extract of paku tanduk rusa at 100 mg/kgBW dose significantly reduced total bilirubin levels, comparable to the effects observed with other known hepatoprotective plants. For instance, in a CCl_4-induced toxicity model, Curcuma longa restored serum bilirubin levels close to normal through antioxidant and anti-inflammatory mechanisms (Ezzat *et al.*, 2020). In addition, Phyllanthus niruri was also reported to significantly reduce bilirubin levels and improve liver enzyme function (SGOT, SGPT) in CCl_4-induced rats ($p < 0.05$) (Lee *et al.*, 2017). In the present study, the effect of paku tanduk rusa extract showed a comparable magnitude of bilirubin reduction, with the 100 mg/kgBW dose being the most optimal, as evidenced by statistically significant results ($p < 0.05$). These findings suggested that paku tanduk rusa possesses hepatoprotective potential similar to that of *Curcuma Longan* L. and *Phylantus niruri*. However, to confirm the relative effectiveness among these hepatoprotective plants, further studies are needed to directly compare various doses and biochemical parameters in a quantitative manner.

4. CONCLUSION

Administration of ethanol extract of paku tanduk rusa (*Platycerium bifurcatum* (Cav.) C.Chr) for 14 days had activity in reducing total bilirubin levels and hepatosomatic index (HSI) values of male white rats (*Rattus norvegicus*) of the Wistar strain induced by absolute ethanol of 2 mL/200 gBB. Based on the results obtained, 100 mg/kgBW is the most effective dose. For the next research, it is better to conduct tests at different lengths of time and test them against other liver function parameters such as SGOT, SGPT, bile acids, and so on.

5. ACKNOWLEDGMENT

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