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Penderita Diabetes Mellitus Buatan dengan  
Penggunaan Simplisia Kayu Manis (*Cinnamon  
burmanii*) dan Kembang Bulan (*Tithonia diversifolia*)

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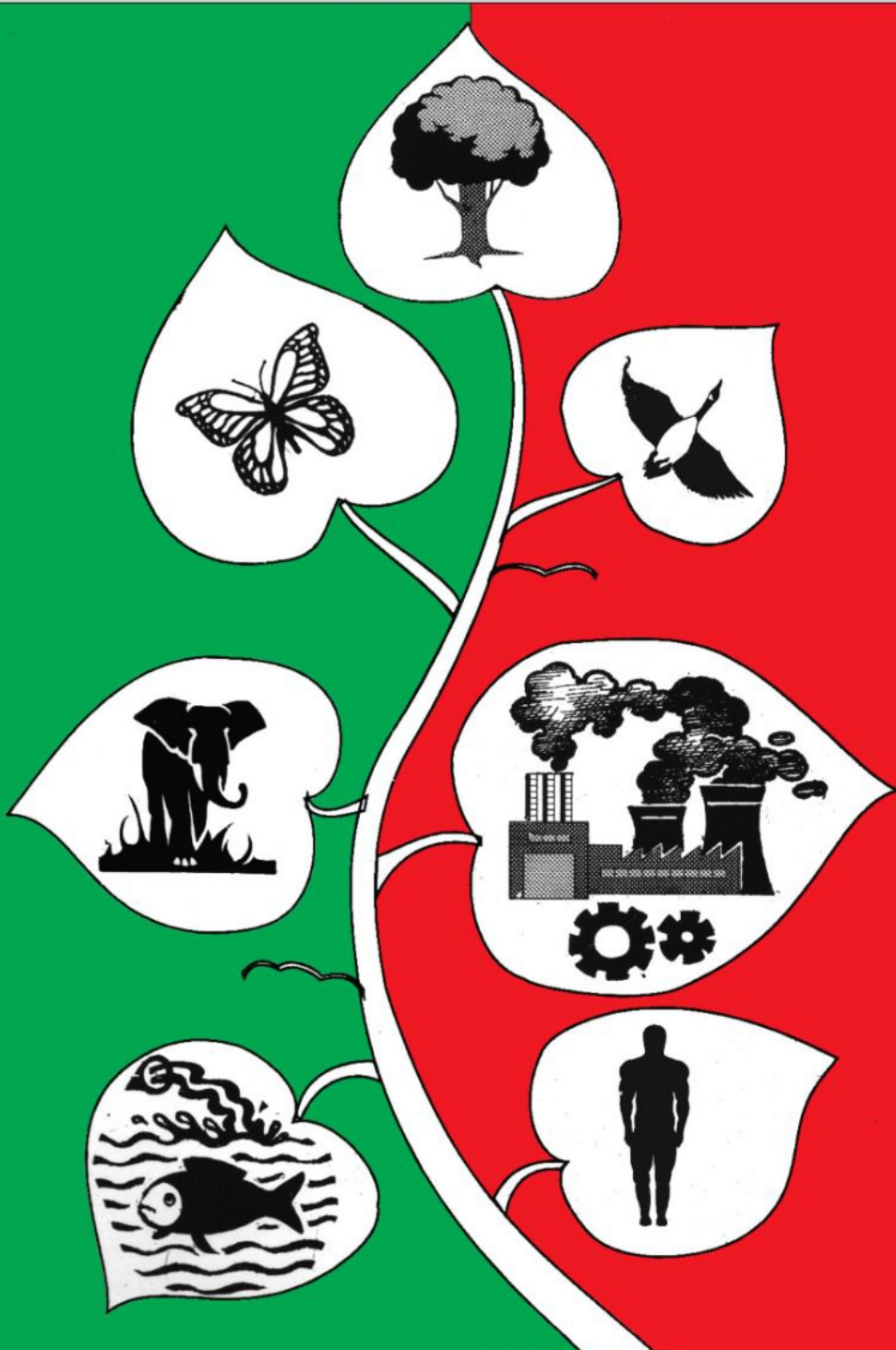
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# The effectiveness of *Cinnamomum (Cinnamomum burmannii)* Essential Oil on the Reduction of Inflammation Levels in White Rat Livers (*Rattus norvegicus*) Induced by Streptozotocin

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## ABSTRACT

The purpose of this study was to determine the effectiveness of administration of cinnamon (*Cinnamomum burmannii*) essential oil on hemorrhage, congestion, and inflammatory cell infiltration in the liver of white rats (*Rattus norvegicus*) induced by streptozotocin to make artificial Diabetes Mellitus (DM). Thirty adult male rats, aged 2-4 months, weighing 130-140 g were divided into six groups. K (-) control group was given drug solvent, K (+) DM rats were given drug solvent, P1 group DM rats were given glibenclamide 0.45 mg / Kg BW + drug solvent, P2, P3, P4 were group of DM rats given oil therapy Cinnamon volatile with doses of 100mg/kg BW, 200mg/kg BW, and 400mg/kg BW, respectively. Therapy was given orally for 14 days. At the end of the study, all experimental animals were euthanized and their livers were taken. The liver was made histopathological preparations with Hematoxylin eosin staining and calculated bleeding, congestion, and inflammatory cell infiltration. The results showed that the lowest number of hemorrhage, congestion, and inflammatory cell infiltration was in the negative control group (K-), the highest number in the positive control group (K+), and the lowest in the treatment group was P3. The results showed a decrease, but no significant difference in hemorrhage. There are significant differences in inflammatory cell congestion and infiltration. The results of this study concluded that cinnamon essential oil can reduce the level of inflammation.

**Key words :** Streptozotocin, *Cinnamomum* essential oil, Liver, White rats, Diabetes mellitus

## Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by an increase in blood sugar levels. Broadly speaking, DM is divided into two types,

namely: DM type 1 and DM type 2. DM type 1 is caused because the pancreas is not able to produce insulin or the pancreas produces little insulin. Type 2 diabetes is caused because the body is unable to use insulin or the occurrence of insulin resistance

(World Health Organization, 2019). Inhibited production of the insulin hormone or the body cannot use the insulin hormone produced effectively, causing the body to experience excess levels of sugar in blood plasma or hyperglycemia (Ministry of Health, 2014).

High blood glucose levels due to diabetes in the long term can cause serious damage to organs (World Health Organization, 2019). One of the organs that is often damaged is the liver because of its role in the process of metabolism and detoxification of materials and chemicals that enter the body (Aisyah, 2015; Merdana *et al.*, 2019).

The number of side effects of DM treatment, the increasing number of DM patients every year, and the frequent occurrence of clinical complications in DM patients encourage people to switch to trying alternative medicine by utilizing herbal plants. Herbal treatment is a form of medical therapy that is more affordable, has mild side effects, and is easy to obtain (Budiastuti *et al.*, 2020). For this reason, the authors chose the Cinnamon (*Cinnamomum burmannii*) plant for herbal medicine (Budiastuti *et al.*, 2020).

Cinnamon (*Cinnamomum burmannii*) has various bioactive components, including essential oils, cinnamaldehyde, flavonoids, coumarins, cinnamic acid, and other aromatic compounds (Al-Dhubiab, 2012). The efficacy of cinnamon essential oil can function as an antibacterial against *Staphylococcus aureus* (Hakim *et al.*, 2020; Effendi *et al.*, 2019; Tyasningsih *et al.*, 2019; Yunita *et al.*, 2020), and also as an antibacterial against Methicillin resistant *Staphylococcus aureus* (MRSA) (Fadlilah *et al.*, 2021; Rahmaniar *et al.*, 2020; Ramandinianto *et al.*, 2020). Cinnamon which has active ingredients such as cinnamaldehyde compounds are able to inhibit oxidative stress by increasing antioxidants in the liver, and reducing serum TNF- kadar levels. Polyphenols are able to suppress the expression of Nuclear Factor Kappa B (NF- $\kappa$ B). Flavonoid compounds are able to reduce the expression of various different pro-inflammatory cytokines/chemokines such as Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-1, (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Monocyte Chemoattractant Protein-1 (MCP-1). All the activities of these compounds can inhibit the expression of cytokines thereby reducing the inflammatory reaction (Sentangelo *et al.*, 2007; Liao *et al.*, 2012). According to Ekaprasada (2009) cinnamaldehyde is able to reduce levels of Nitric oxide (NO). NO is a

vascular vasodilator agent. When NO is suppressed, blood vessels will not experience vasodilation, so that it can reduce hemodynamic disorders such as congestion and hemorrhage Budiastuti *et al.*, 2020).

Based on the content of compounds in cinnamon that can reduce proinflammatory cytokines and suppress NO production, the authors believe that compounds in cinnamon essential oil can repair liver cell damage due to the inflammatory response. Therefore, the authors wanted to conduct a study on the effectiveness of giving cinnamon essential oil to reduce the level of inflammation (in terms of hemorrhage, congestion, and inflammatory cell infiltration) in the liver of white rats (*Rattus norvegicus*) induced by Streptozotocin.

## Materials and Methods

30 male white rats (*Rattus norvegicus*) of Wistar strain were divided into six groups, each groups of five each. Negative control (K-) is a group of rats that are not induced by STZ and given solvent drug therapy, positive control (K+) is a group of white mice that are induced by STZ with a dose of 45 mg / Kg BW intraperitoneally and given solvent drug therapy, treatment 1 (P1) is a group of white rats induced by STZ at a dose of 45 mg / Kg BW intraperitoneally and treated with glibenclamide at a dose of 0.45 mg / kg BW orally, treatment 2 (P2), treatment 3 (P3), treatment 4 (P4), namely group of white rats induced by STZ at a dose of 45 mg / Kg BW intraperitoneally and treated with essential oils, each dose: 100 mg / kg BW, 200 mg / kg BW, 400 mg / kg BW orally.

The preparation of therapeutic preparations is by adding 1% tween 80 to a mortar, adding distilled water and stirring. Add cinnamon essential oil to form thick mucilage, stir for 5 minutes. CMC-Na 1% was developed by adding hot water to the mucilage and adding distilled water again.

White mice were adapted for seven days before treatment. 45 mg/kg BW STZ injected intraperitoneally. The rats were fasted during 12 hours before STZ induction. Streptozotocin was dissolved in 0.01M citrate buffer, pH 4.5 and always freshly prepared for use within 10-15 minutes. STZ induction with a single dose in all groups except negative controls. After induction, rats were given food and drink ad libitum (Saputra *et al.*, 2018).

White rats were given a 10% sucrose or dextrose solution for 12-24 hours to avoid sudden hypoglyce-

mic occurrence (Frode *et al.*, 2008). Measurement of blood glucose levels in white rats was carried out on day 3 (72 hours) after STZ induction (Saputra *et al.*, 2018). The test animal is said to experience hyperglycemia condition if the blood glucose level is more than 140 mg/dL (Wang, 2010). If it has been declared free from sudden hypoglycemic and it is confirmed that the blood sugar test results are as expected, then cinnamon essential oil is given. Cinnamon essential oil is given orally once a day using a gastric probe for fourteen days.

At the end of the study, white rats were euthanized using ketamine 100 mg/Kg BW and xylazine 10 mg/Kg BW, for liver extraction. The liver was made histopathological preparations using Hematoxylin eosin staining. Histopathological preparations were observed using a microscope, magnification 400x with five different fields of view for each variable. Variables consist of hemorrhage, congestion, and inflammatory cell infiltration.

## Results and Discussion

In the Kruskal Wall is statistical test, there was a significant difference ( $p < 0.05$ ), the test was continued with the Mann Whitney test and the following data were obtained:

In table 1, the results of the Mann Whitney follow-up statistical test for hemorrhage show that there is a significant difference ( $p < 0.05$ ) between the K(-) group and the K(+), P1, P2, and P4 group, but there is no significant difference ( $p > 0.05$ ) with P3. There was no significant difference ( $p > 0.05$ ) between treatment groups P1, P2, P3, and P4. For congestion there was a significant difference ( $p < 0.05$ ) between group K(-) and group K(+), P1, P2, P4, but there was no significant difference ( $p > 0.05$ ) with P3.

There was a significant difference ( $p < 0.05$ ) between treatment groups (P1, P2, P3, and P4). For inflammatory cell infiltration, there was a significant difference ( $p < 0.05$ ) between group K(-) and group K(+), P1, P2, P4, but there was no significant difference ( $p > 0.05$ ) with P3. There was a significant difference ( $p < 0.05$ ) between treatment groups (P1, P2, P3, and P4).

The positive control group (K+) which has the highest mean value in the calculation of hemorrhage, congestion, and inflammatory cell infiltration are 0.96, 2.64, and 2.52, respectively. While the negative control group (K-) has a mean the lowest in the calculation of hemorrhage, congestion, inflammatory cell infiltration, respectively 0.16, 0.24, and 0.20.

The lowest average was obtained in treatment group three (P3) with a therapeutic dose of essential oil of 200 mg/ kg BB. The average levels of hemorrhage, congestion, and inflammatory cell infiltration in the P3 group were as follows: 0.44, 0.40, and 0.36, respectively. The second lowest average in treatment group four (P4) with cinnamon essential oil therapy 400 mg/ kg BB. The mean levels of hemorrhage, congestion, and inflammatory cell infiltration in the P4 group were as follows: 0.64, 0.68, and 0.64, respectively. The third lowest average was in treatment one (P1) with glibenclamide therapy, the average levels of hemorrhage, congestion, and inflammatory cell infiltration were as follows: 0.72, 1.16, and 1.16, respectively. The highest average in the treatment group was in group two (P2) with cinnamon essential oil therapy of 200 mg/Kg BW. P2 showed the highest mean level of hemorrhage, congestion, and inflammatory cell infiltration, which were :0.84, 1.60, and 1.52, respectively.

Treatment (P2) with a dose of 100 mg/Kg BW and treatment (P4) with a dose of 400 mg/Kg BW,

**Table 1.** The average score of hemorrhage, congestion, and inflammatory cell infiltration in the liver histopathology of white rats (*Rattus norvegicus*) in groups K(-), K(+), P1, P2, P3, and P4.

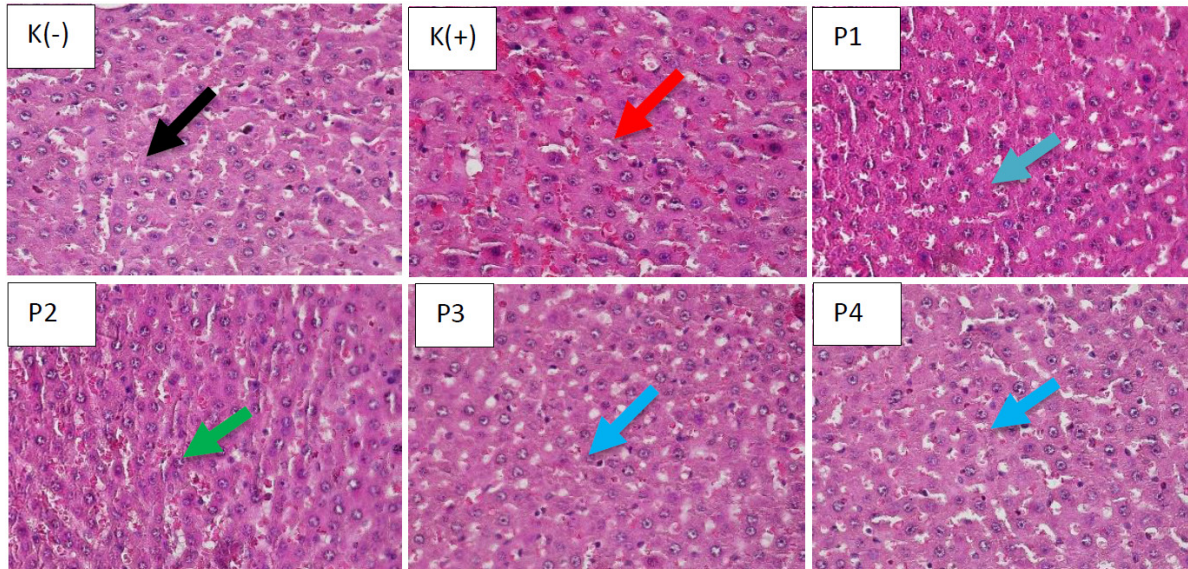
Groups	Score of hemorrhage (Mean $\pm$ SD)	Score of congestion (Mean $\pm$ SD)	Score of inflammatory cell infiltration (Mean $\pm$ SD)
K-	0.16a $\pm$ 0.17	0.24a $\pm$ 0.09	0.20a $\pm$ 0.14
K+	0.96cd $\pm$ 0.09	2.64e $\pm$ 0.17	2.52e $\pm$ 0.18
P1	0.72bc $\pm$ 0.11	1.16c $\pm$ 0.22	1.16c $\pm$ 0.09
P2	0.84c $\pm$ 0.09	1.60d $\pm$ 0.35	1.52d $\pm$ 0.23
P3	0.44ab $\pm$ 0.26	0.40a $\pm$ 0.14	0.36a $\pm$ 0.09
P4	0.64b $\pm$ 0.17	0.68b $\pm$ 0.11	0.64b $\pm$ 0.09

Different superscripts (abcde) in the same column indicate a significant difference ( $p < 0.05$ )

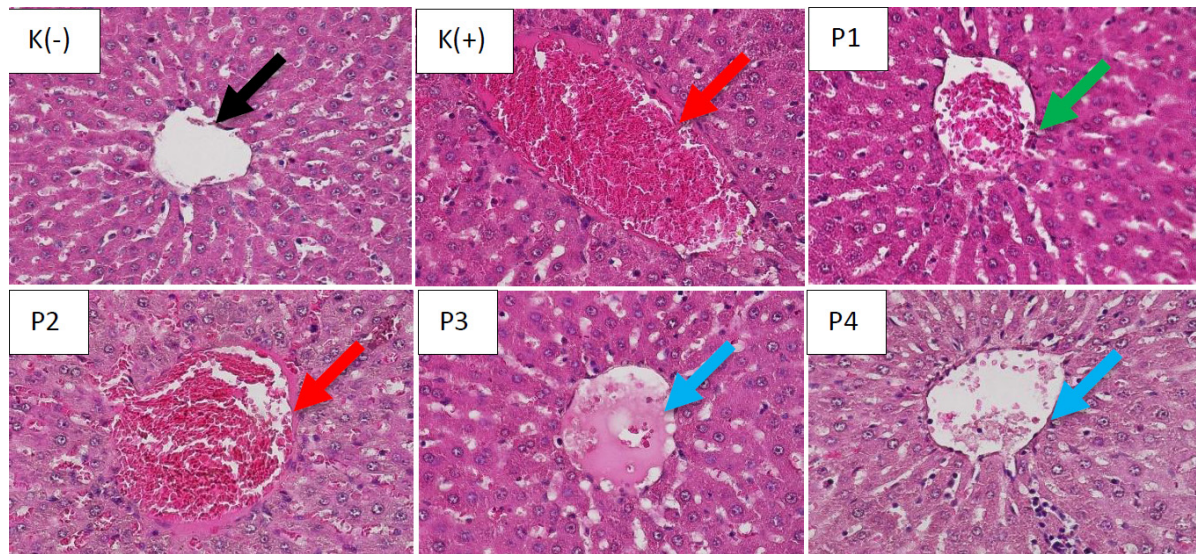


gave no better results than treatment (P3). This condition is thought to be related to the dosing regimen. P2 is suspected to have a dose regimen that is lower than the therapeutic dose, while P4 has a dose regimen that is higher than the therapeutic dose. Ac-

cording to Husnasya and Ihsan (2018), the dosage regimen is said to be irrational if the drug level is excessive or insufficient. An excess drug level is said if the maximum steady-state concentration is equal to or exceeds the minimum toxic concentration and



**Fig. 1.** Hemorrhage in the liver histopathology of white rats (*Rattus norvegicus*) in 400x magnification with HE staining in groups K(-), K(+), P1, P2, P3, and P4. The black arrows in Figure 2 show the histopathology of the liver of white rats without bleeding (normal). The red arrows indicate the histopathology of the white rat liver with a lot of hemorrhage. The green arrow indicates the histopathology of the liver of white rats with moderate hemorrhage. The blue arrows indicate the histopathology of the white rat liver with slight hemorrhage.



**Fig. 2.** Overview of congestion in the liver histopathology of white rats (*Rattus norvegicus*) 400x magnification with HE staining in groups K(-), K(+), P1, P2, P3, and P4. The black arrow indicates the histopathological picture of the liver of normal white rats without any congestion. The red arrows indicate the histopathological features of the liver of white rats with severe congestion. The green arrow indicates the histopathological picture of the rat liver with moderate congestion. Blue arrows show histopathological features of rat liver with mild congestion.



the drug level is said to be insufficient if the minimum steady-state concentration is less than the minimum effective concentration.

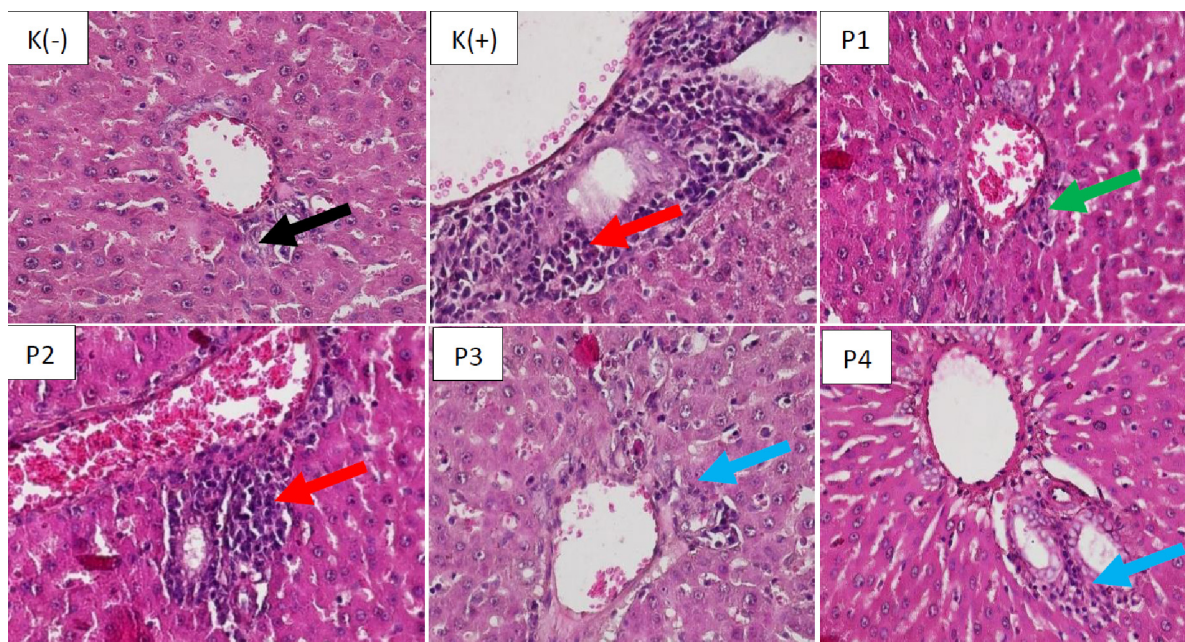
Decrease in the level of hemorrhage, congestion, inflammatory cell infiltration on P2 with a dose of 200 mg/Kg BW until it was close to the negative group. This can be explained that the dose contains sufficient antioxidants to neutralize free radicals so that provide maximum therapeutic effect. The following discusses the mechanism of cinnamon (*Cinamomum burmannii*) essential oil in reducing the amount of hemorrhage, congestion, and inflammatory cell infiltration.

Hemorrhage showed no significant difference between groups. This is because cinnamon contains coumarin (13.39%) and transcinnamaldehyde (60.17%) (Wang *et al.* 2010). Coumarin and cinnamaldehyde have pharmacological activity opposite to cinnamaldehyde. Coumarin has pharmacological activity as an anticoagulant, coumarin inhibits the synthesis of prothrombin and prevents the formation of blood clotting factor preparations (factors II, VII, IX, X) (Pengelly, 2005). Coumarin has been shown to prolong bleeding time (Hidayah, 2016). Cinnamaldehyde has anti-inflammatory

pharmacological activity by suppressing NO production (Hong *et al.*, 2012). NO is a vasodilator agent on blood vessels. When blood vessels are maintained vasodilation, hemodynamic disturbances will be avoided. The content of cinnamaldehyde is more than coumarin so that blood vessels are maintained vasodilation and cause erythrocytes not to easily come out into the tissue, but the different therapeutic effects between coumarin and cinnamaldehyde may cause a decrease in the number of inflammatory hemorrhages not significantly different.

The congestion showed a significant difference because cinnamaldehyde in cinnamon is an antioxidant agent that can fight the formation of ROS by activating Nuclear factor-erythroid-2 related factor 2 (Nrf2). In addition, Nrf2 can also maintain the level of Nitric Oxide (NO), which is a vasodilator agent in blood vessels (Ekaprasada, 2009). Choi *et al.* 2010 stated that eugenol was able to reduce intracellular oxidative stress, increase the activity of superoxide dismutase and catalase, as well as increase the activity of superoxide dismutase inhibit NO production. When the blood vessels are maintained vasodilation, congestion can be avoided.

Infiltration of inflammatory cells showed a sig-



**Fig. 3.** The histopathology of inflammatory cell infiltration in the liver of white rats (*Rattus norvegicus*) at 400x magnification with HE staining in groups K(-), K(+), P1, P2, P3, and P4. Black arrows indicate normal white mouse liver histopathology (without inflammatory cell infiltration). The red arrow indicates the histopathology of the rat liver white with severe inflammatory cell infiltration. The green arrow indicates the histopathology of the white rat liver with moderate (not too severe) inflammatory cell infiltration. Blue arrows indicate the histopathology of white rat liver with mild inflammatory cell infiltration.

nificant difference because cinnamaldehyde was able to inhibit the TLR4 pathway (Youn *et al.*, 2008; Liao *et al.*, 2011). Cinnamaldehyde is able to release crosstalk between oxidative stress and TLR4 signaling, because cinnamaldehyde is effective in suppressing the expression of TLR4 and protein-associated adapters. Its activity reduces inflammatory cytokine transcription factors that are responsible for the inflammatory response that occurs during acute liver injury. Inhibition of the TLR4 signaling pathway has been associated with inhibition of receptor oligomerization (Youn *et al.*, 2008). The decrease in inflammatory cell infiltration is also associated with the ability of cinnamaldehyde as an agent capable of lowering the level of TNF- $\alpha$  involved in NF- $\kappa$ B activation (Schmid, 2008).

To stop inflammatory reactions and hemodynamic disturbances due to free radicals, antioxidants are needed. According to Budiastuti (2020) the bioactive components of cinnamon essential oil are proven to have antioxidant activity. Flavonoids in cinnamon can reduce the expression of pro-inflammatory cytokines / chemokines such as TNF- $\alpha$ , IL-1  $\beta$ , IL-6, IL-8, MCP-1 (Liao *et al.*, 2011). Cinnamaldehyde has a protective effect by inhibiting the inflammatory response through inhibition of the TLR4 / NF- $\kappa$ B pathway (Schmid, 2008). The polyphenols in cinnamon have been shown to exert anti-inflammatory activity by suppressing the activation of NF- $\kappa$ B (Santangelo *et al.*, 2007). Cinnamic acid in cinnamon plays an important role in the inhibition of oxidative stress. There is a significant increase in Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) and significant reduction of Malondialdehyde (MDA) with cinnamic acid treatment (Liao *et al.*, 2012). Choi *et al.* 2010 states that eugenol is capable of inhibiting Nitric Oxide (NO) production. NO is a vasodilator agent in blood vessels. When the blood vessels are maintained, their vasodilation will avoid hemodynamic disorders Budiastuti *et al.*, 2020).

## Conclusion

Cinnamon essential oil (*Cinnamomum burmannii*) can reduce the level of inflammation (in terms of hemorrhage, congestion, and inflammatory cell infiltration) in the liver of white rats (*Rattus norvegicus*) induced by streptozotocin. The most effective therapeutic dose of cinnamon essential oil in reducing the level of inflammation is 200 mg/Kg BW

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