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THE EFFECT OF COMBINING ASTAXANTHIN AND VITAMIN E ON NEUTROPHILS, LEUKOCYTES, AND PLATELET COUNTS

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ABSTRACT
Inflammation is a defensive response that eliminates lesions caused by injury and can be induced by 1% carrageenan, leading to increased blood neutrophil, leukocyte, and platelet counts. Astaxanthin and vitamin E are antioxidants with potential anti-inflammatory properties; however, the effects of their combination remain underexplored. This study evaluates the impact of combined astaxanthin and vitamin E administration on neutrophil, leukocyte, and platelet counts in white male Wistar rats using a carrageenan-induced inflammation model with a Post Test Only Control Group Design. Thirty white male Wistar rats were selected using a simple random sampling method and randomly assigned into three groups: KN (normal), KS (disease, induced with 1% carrageenan), and KP (treatment, receiving astaxanthin 0.72 mg/kgBW and vitamin E 24.1 mg/kgBW with 1% carrageenan). Following treatment, neutrophil, leukocyte, and platelet counts were measured using a hematology analyzer and subjected to normality and homogeneity testing, followed by parametric and nonparametric analyses with post hoc testing. The results show that combining astaxanthin and vitamin E has an anti-inflammatory effect by lowering neutrophil, leukocyte, and platelet counts in carrageenan-induced male Wistar rats.

Keywords: astaxanthin; leukocyte; neutrophil; platelet; vitamin

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INTRODUCTION

Inflammation is a defensive reaction involving cells, mediators, and blood vessels, including platelets, to eliminate foreign substances (Kumar et al., 2023). The polysaccharide lambda-carrageenan is a known pathogenic factor that binds to Toll-like receptor 4 (TLR-4) and activates the NF-κB pathway, resulting in cytokine production and inflammation in macrophages, which subsequently release inflammatory mediators. This process promotes the migration of neutrophils and leukocytes to areas with damaged vascular endothelium (Bekkouch et al., 2023; Komisarska et al., 2024). Endothelial damage activates enzymes such as phospholipase, which convert phospholipids into arachidonic acid. The cyclooxygenase (COX) enzyme metabolizes arachidonic acid to produce thromboxane A2 (TXA2), while lipoxygenase (LOX) generates reactive oxygen species (ROS) (Hannoodee & Nasuruddin, 2024; J. Liu et al., 2023; Suhana et al., 2025; Tao et al., 2024). TXA2 activates platelets, increasing platelet count (Johny et al., 2021; Kumar et al., 2023). Elevated neutrophil and leukocyte counts indicate ongoing inflammation, which, if unresolved, progresses to chronic inflammation characterized by increased platelet levels. Chronic inflammation is a key factor in diseases such as asthma, diabetes, and heart disease. The World Health Organization reported that cardiovascular disease accounted for 31% of all deaths in 2016, with over 85% resulting from strokes and heart attacks, predominantly in low- and middle-income countries (Pereira et al., 2021; WHO, 2021).

Astaxanthin is a red carotenoid pigment derived from xanthophyll, which imparts coloration to certain plants and algae. It is found in microalgae, shrimp, crab, shellfish, and salmon (Maharani et al., 2023). Astaxanthin acts as an antioxidant; its chemical structure allows it to donate electrons or bind free radicals, thereby neutralizing reactive oxygen species (ROS) and preventing oxidative damage (Pereira et al., 2021). Additionally, astaxanthin exhibits anti-inflammatory activity by inhibiting the NF-κB signaling pathway and suppressing COX-2 expression, both of which are involved in inflammatory responses. This leads to reductions in neutrophil, leukocyte, and platelet counts (Hamed & Generali, 2022; Yin et al., 2021). Vitamin E also demonstrates potent antioxidant and anti-inflammatory properties. As a fat-soluble compound, it converts peroxyl radicals into lipid hydroperoxides within cell membranes, thereby preventing cellular damage (Putri et al., 2024). Previous research showed that the combination of astaxanthin and vitamin E confers anti-inflammatory benefits, as indicated by decreased neutrophil levels and reduced neutrophil migration to sites of injury (Aripin et al., 2022).

Increased activation of pro-inflammatory mediators and blood components, particularly neutrophils, leukocytes, and platelets, is anticipated to decrease following administration of a combination of astaxanthin and vitamin E. This combination is proposed to exert anti-inflammatory effects by suppressing NF-κB and COX-2 activity and maintaining the balance between ROS and TXA2 (Laja, 2021; Mar et al., 2024). This rationale underpins the present investigation into the anti-inflammatory effects of combined astaxanthin and vitamin E on neutrophil, leukocyte, and platelet counts in male Wistar rats induced with carrageenan.

METHOD

This study utilized a Post-Test Only Control Group design, with 30 samples collected concurrently. The research subjects were male Wistar strain white rats (Rattus norvegicus), aged two months and weighing 175–200 grams, maintained at the Nutrition Study Center, Food and Nutrition Research (PSPG), Gadjah Mada University. Sample size was determined using the Federer (1956) formula, resulting in nine rats per group. To account for potential mortality or dropout, the formula 1/(1–f) was applied, with f representing a 10% dropout rate. Consecutive sampling was performed based on predefined inclusion and exclusion criteria. All 30 rats underwent a seven-day adaptation period with standardized food and water. Following adaptation, rats were randomly assigned to three groups (normal, disease, and treatment), each comprising ten animals, using a simple random sampling (lottery) method. The treatment group received a combination of astaxanthin (0.72 mg/kg BW) and vitamin E (24.1 mg/kg BW). One hour post-treatment, both the disease and treatment groups were induced with an injection of 0.1 mL of 1% carrageenan and observed for 24 hours. The normal group received only standard feed without further intervention.

The combination solution of astaxanthin and vitamin E was prepared by dissolving 0.72 mg/kgBW of Natural MPL brand astaxanthin powder (sourced from the microalgae Haematococcus pluvialis) and 24.1 mg/kgBW of IPI brand vitamin E in distilled water at a 1:1 ratio. The 1% carrageenan solution was prepared by dissolving 100 mg of carrageenan (an inflammatory agent) in 10 mL of 0.9% sterile physiological sodium chloride (NaCl) solution. Blood samples were collected from the orbital sinus (a blood vessel behind the eyes) of the rats, and the numbers of neutrophils, leukocytes, and platelets were measured using a hematology analyzer, a device that automatically counts different blood cells.

Data analysis in this study used IBM SPSS Statistics version 30 software. The analysis of leukocyte and platelet count data began with the Shapiro-Wilk normality test and Levene's homogeneity-of-variance test, which yielded p-values < 0.05, indicating non-normality and heteroscedasticity. Therefore, the analysis was continued with the non-parametric Kruskal-Wallis test and the Mann-Whitney test. Meanwhile, the Shapiro-Wilk normality test and Levene's homogeneity test for neutrophil count showed p-values > 0.05, indicating that the data were normal and homogeneous.

Analysis of neutrophil count data continued with the parametric One-Way ANOVA and Post-Hoc LSD tests.

RESULT

The study was conducted at the Faculty of Medicine, Sultan Agung Islamic University, encompassing proposal preparation, material procurement, research implementation, and data analysis. Subsequent experimental procedures were carried out at the Nutrition Study Center, Food and Nutrition Research (PSPG), Gadjah Mada University, utilizing 30 white male Wistar rats that were acclimated for seven days and randomly assigned to three groups.

Neutrophils

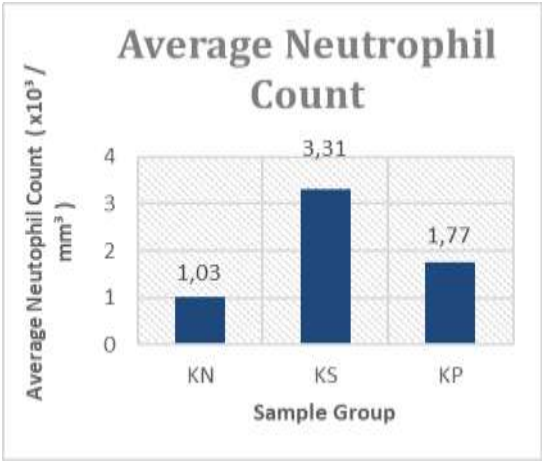

Figure 1. Average Neutrophil Count Between Groups

Based on Figure 1, the highest average neutrophil count was observed in the diseased group (KS), at 3.31 × 10³/mm³. The Treatment Group (KP) had a lower average neutrophil count of 1.77 × 10³/mm³ compared to the Diseased Group (KS). Data for each group will be tested for normality with *the Shapiro-Wilk* test and homogeneity with *the Levene Test.*

Table 1.
Results of Normality Test Analysis, Homogeneity, and One-Way ANOVA

	p-value		
Group	Normalitas	Homogenitas	*One Way ANOVA*
KN	0,254*	0,195**	0,000
KS	0,610*		
KP	0,570*		

Description: * = normal data distribution; ** = homogeneous data variance.

Based on Table 1., the results of the normality and homogeneity tests indicate p > 0.05; thus, the data variances within each group are normal and homogeneous, and the three groups meet the requirements for the parametric *One-Way ANOVA*. The one-w*ay ANOVA test* showed a significant difference (*p < 0.05) in neutrophil counts among the three research groups. Further analysis to determine the difference in the average number of neutrophils between groups was performed using the Post Hoc LSD* test.

Table 2.
Results of Statistical Analysis of the Number of Neutrophils between Test Group

Group		***Post Hoc LSD***
KN	KS	0,000*
	KP	0,000*
KS	KN	0,000*
	KP	0,000*

KP	KN	0,000*
	KS	0,000*

Description : * = Available difference average amount significant neutrophils *(p* <0.05 *)* between group study

The Post Hoc LSD test in Table 2, for each group study, shows there is a significant difference from other groups ($p < 0.05$).

Leukocytes

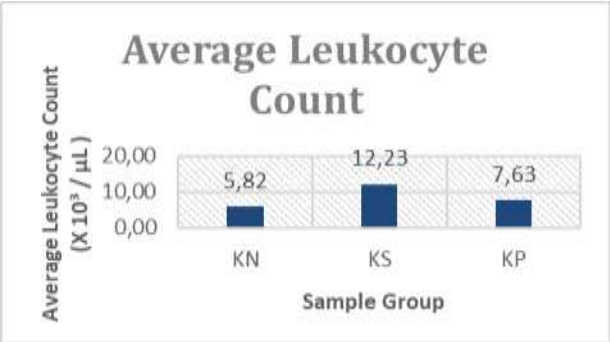

Figure 2. Average Leukocyte Count in Each Group

The average leukocyte count was highest in the KS group at 12.23 × 10^3/µL and lowest in the KN group at 5.82 × 10^3/µL. The KP group exhibited a lower leukocyte count than KS (7.63 × 10^3/µL), but higher than KN. These results suggest that administration of a combination of astaxanthin and vitamin E exerts anti-inflammatory effects by reducing leukocyte counts. Overall, carrageenan induction increases leukocyte counts, whereas the combination treatment reduces them toward normal levels.

Leukocyte count data for each group were analyzed using the *Shapiro-Wilk test for normality*. Analysis results showed that KN and KS have normal distributions ($p \geq 0.05$), with $p = 0.792$ and 0.523, respectively. In KP, a non-normal distribution was observed ($p < 0.05$; $p = 0.007$). The normality test results are shown in Table 3. Homogeneity was analyzed using *Levene's* Test, which yielded $p = 0.001$ ($p < 0.05$), indicating that the homogeneity of leukocyte counts across all three groups was not met. Analysis of the differences in the third group using the Kruskal-Wallis Test, because there is a non-normal data distribution. *Kruskal-Wallis test results:* $p = 0.001$ ($p < 0.05$), indicating a significant difference across the groups. Based on the test results, the *Mann-Whitney Post Hoc* Test was conducted to determine whether there was a significant difference in leukocyte counts between the partner groups.

Table 3.
Results of Normality Test Analysis, Homogeneity, and Kruskal-Wallis

	p-value		
Group	*Shapiro Wilk*	*Levene*	*Kruskal Wallis*
KN	0,792*	0,001	0,001
KS	0,523*		
KP	0,007		

Description : * = normal data distribution

The Post Hoc Test in Table 4. shows a significant difference ($p < 0.05$) across all partner groups. The third group holds the same mark ($p = 0.001$), indicating a significant difference between the partner groups.

Table 4.
Results of Leukocyte Count Analysis Between Two Groups

Group		*p Value* Uji *Mann-Whitney*
Normal (KN)	Disease (KS)	0,001*
	Treatment (KP)	0,001*
Disease (KS)	Treatment (KP)	0,001*

Description : * = Available difference average amount significant leukocyte between group study

Platelet

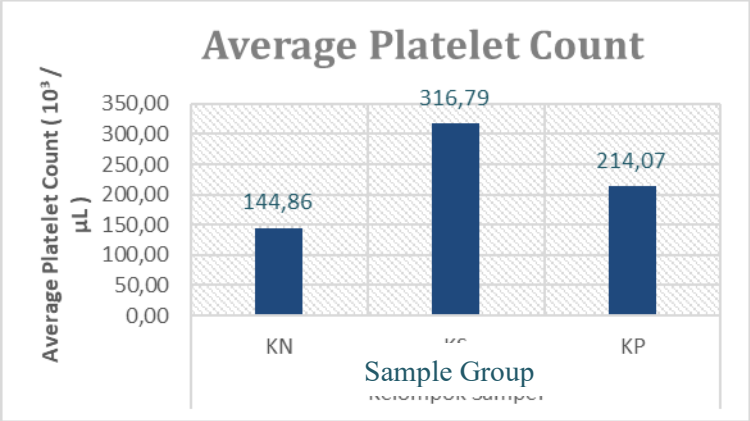

Figure 3. Average Platelet Count in Each Group

The average platelet count for each group is presented in Figure 3. The KS group exhibited the highest average platelet count (316.79 × 10^3/µL), while the KN group had the lowest (144.86 × 10^3/µL). The KP group showed an intermediate value (214.07 × 10^3/µL), higher than KN but lower than KS. These findings demonstrate that the combination of astaxanthin and vitamin E reduces platelet counts. Overall, carrageenan induction increases platelet counts, whereas the combination treatment lowers platelet counts toward normal levels.

Quantitative data on platelets in each group were tested for normality using the *Shapiro-Wilk* test and for homogeneity using the Levene test. *The results of the* normality test indicated normal data distribution in the KN and KS groups (p = 0.843 and p = 0.234, respectively), whereas in the KP group, the test showed non-normal data distribution (p = 0.000 or p < 0.05), as shown in Table 5. The results of the homogeneity test indicate a p-value of 0.044 (p<0.05), indicating that the variation in platelet quantity between the third group is not homogeneous. Analysis of differences in total platelet data in accordance with Table 6., using the *Kruskal-Wallis test, yielded p=0.000 (p<0.05), indicating* at least two groups with significantly different average platelet counts. Analysis to be continued with *the Mann-Whitney Post Hoc* Test for known difference in platelets between groups.

Table 5.
Results of Normality Test Analysis, Homogeneity, and Kruskal-Wallis

	p-value		
Group	*Shapiro Wilk*	*Levene*	*Kruskal Wallis*
KN	0,843*	0,044	0,000
KS	0,234*		
KP	0,000		

Description : * = normal data distribution

Table 6.
Results of Leukocyte Count Analysis Between Two Groups

Group	*p-value*

		Man Whitney Test
Normal (KN)	Disease (KS)	0,000
	Treatment (KP)	0,000
Disease (KS)	Treatment (KP)	0,000

The Mann-Whitney test in Table 6 illustrates that there is a difference in the amount of platelets between the KN group and the KS and KP groups, as well as a difference in the amount of platelets between the KS and KP groups. *p-value* from the third group is the same as the value, that is, *p* 0.000 ($p < 0.05$), which shows a significant difference between the groups.

DISCUSSION

Average amount of neutrophils (3.31 x10^3/mm^3), leukocytes (12.23 x 10^3/μL), and platelets (316.79 x 10^3/μL) in KS- induced carrageenan 1% shows improvement compared to with KN and has a significant difference ($p < 0.05$). This is in line with the theory that induction 1% carrageenan will activate the NF through signaling TLR-4, which produces various proinflammatory cytokines (TNF-α, IL-6, and IL-1β) and chemokines (Guo et al., 2024; Komisarska et al., 2024; Kumar et al., 2023; Suhana et al., 2025; Widyarini et al., 2023) . Furthermore, it promotes the activation and differentiation of hematopoietic progenitors, enhances proliferation, and inhibits leukocyte apoptosis (Guo et al., 2024; Komisarska et al., 2024).

Cytokines produced from signaling NF-kB stimulate G-CSF, which stimulates proliferation, differentiation, and activation of neutrophils to cause the occurrence of inflammation, so that the number of neutrophils in the blood increases (Mescher, 2023; Nurkhasanah et al., 2023). Temporary that, IL-1β and enzymes phospholipase change phospholipids become sour arachidonic acid arachidonate will metabolized become two track main enzymes, namely track cyclooxygenase with help COX enzymes become prostaglandins (PGD 2 and PGE 2), prostacyclin, and TXA2, as well as track lipoxygenase stimulated by the LOX enzyme produces lipoxins, leukotrienes (LTB 4, LTD 4, LTE 4), and products side job in the form of ROS (Guo et al., 2024; Hannoodee & Nasuruddin, 2024; Komisarska et al., 2024; Kumar et al., 2023; Widyarini et al., 2023).

PGD2 and PGE2 contribute to vasodilation, facilitating leukocyte migration to the site of inflammation (Kumar et al., 2023). In addition, PGE2 increases IL-1β and TNF-α levels, which, in turn, influence neutrophil mobilization and granulopoiesis via GM-CSF (Khullar et al., 2024). In addition, LTB4 acts as a chemotactic agent for neutrophils, while LTD4 and LTE4 enhance blood vessel permeability, facilitating the migration of leukocytes (Kumar et al., 2023). Products derived from arachidonate damage the endothelium and trigger platelet aggregation, increasing platelet numbers (Tao et al., 2024).The effect of giving a combination of astaxanthin with vitamin E can be seen from the differences in average amounts of neutrophils (1.77 x10^3 / mm^3), leukocytes (7.63 x 10^3 / μL), and KP platelets (214.07 x 10^3 / μL), with results showing a lower value compared to KS, with a marked $p < 0.05$, which means the difference is significant. Research results. This is in line with Pratiwi (2015) about the combination of astaxanthin with vitamin E, which provides an effective anti-inflammatory effect by hindering the NF-kB pathway, thereby suppressing proinflammatory gene expression and inhibiting cyclooxygenase, which affects neutrophil mobilization, resulting in a decline in neutrophil levels in the blood. Besides that, the findings this is also in line with theory that astaxanthin blocks NF- k B signaling via emphasis degradation IκB -α which results in inhibition Monocyte Chemoattractant Protein-1 (MCP-1) and other molecules inflammation others (IL-6, VEGF, ICAM-1) and inhibit ROS production through improvement enzyme antioxidants, heme oxygenase-1 (HO-1), Nrf2 target protein, and glutathione peroxidase-1 so that will happen balance between ROS and TXA2 (Laja, 2021; Nair et al., 2023; Patil et al., 2022). Vitamin E inhibits the NF-κB pathway and suppresses

the secretion of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and PGE2 (Mar et al., 2024; Ungurianu et al., 2021; Wang et al., 2022). Analysis of average neutrophil, leukocyte, and platelet counts between the KN and KP groups revealed a significant difference ($p < 0.05$). However, administration of a combination of astaxanthin 0.72 mg/kgBW and vitamin E 24.1 mg/kgBW did not fully restore these counts to normal levels in a clinical context.

CONCLUSION

A combination of astaxanthin (0.72 mg/kgBW) and vitamin E (24.1 mg/kgBW) demonstrates an anti-inflammatory effect by reducing neutrophil, leukocyte, and platelet counts in white male rats induced with 1% carrageenan.

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