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Protective effect of vitamin C against ethanol induced oxidative stress in Wistar rats

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Abstract
Inflammation is a natural immune response to harmful agents. This study aimed to explore the protective effect of vitamin C against alcohol-induced toxicity on hematological inflammatory markers. Forty healthy adult male Wistar rats were acclimatized and divided into eight groups. Group A served as the control, while Group B received alcohol. Groups C, D, and E were given varying doses of vitamin C, and Groups F, G, and H received alcohol followed by vitamin C treatments. After twenty-one days, blood samples were collected and analyzed for various markers. Rats receiving alcohol only showed increased white blood cell count (WBC), platelet count (PLT), neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR), along with decreased antioxidant enzyme activity. However, rats receiving vitamin C alone or in combination with alcohol exhibited reduced inflammatory markers and increased antioxidant activity compared to the alcohol-only group. The study demonstrated that commercial-grade vitamin C at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg effectively reduced chronic low-grade inflammation. Overall, the findings suggest that vitamin C supplementation may mitigate the inflammatory effects of alcohol consumption.

Keywords: Alcohol; Anti- inflammatory; Antioxidant; Hematological indices; Hematological ratios

INTRODUCTION

The immune system's response to harmful stimuli, such as pathogens, damaged cells and their content, damaged cells, and toxic compounds, results in inflammation, which is essential for health as it removes injurious stimuli and initiates the healing process [1]. During acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection, contributing to the restoration of tissue homeostasis and resolution of the acute inflammation [2]. However, chronic inflammation, which can result from uncontrolled acute inflammation, may contribute to various chronic inflammatory diseases [3]. Clinically, inflammatory markers are used to assess the presence or absence of an active inflammatory disease process and the activity of a known disease [4]. Although traditionally, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were the key inflammatory markers [5], hematological ratios, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and

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lymphocyte-to-monocyte ratio (LMR), have recently come into use as markers of systemic inflammation. They are highly sensitive measures of inflammation in various fields, including oncology, cardiology, nephrology, diabetes, infectious diseases, and autoimmune rheumatic diseases. [6-8]. Elevated inflammatory markers may be caused by infections, inflammatory disease, malignancy, major depressive disorder, or myocardial infarction [9].

Chronic low-grade inflammation is a condition characterized by a state of persistent, low-level inflammation that occurs over an extended period. It is associated with several chronic diseases, including obesity, type 2 diabetes, atherosclerosis, cancer, and neurodegenerative disorders. The pathophysiology of chronic low-grade inflammation involves complex interactions between various components of the immune system, including pro-inflammatory cytokines, chemokines, and immune cells [10]. The primary trigger for chronic low-grade inflammation is thought to be metabolic dysfunction, which can result from a range of factors, including a high-fat diet, physical inactivity, alcohol consumption, and chronic stress. These factors can induce changes in the gut microbiota and increase the permeability of the intestinal barrier, allowing gut-derived microbial products to leak into the systemic circulation. These microbial products, such as lipopolysaccharides (LPS), can activate immune cells, such as macrophages and dendritic cells, to produce pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-), interleukin-6 (IL-6), and interleukin-1 beta (IL-1). These cytokines can further activate immune cells and promote the recruitment of additional immune cells to the site of inflammation, perpetuating the inflammatory response [11]. In addition to gut-derived microbial products, other factors can also contribute to chronic low-grade inflammation, including oxidative stress, mitochondrial dysfunction, and cellular senescence. These factors can induce the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which can activate the immune system and promote the release of pro-inflammatory cytokines. The chronic activation of the immune system in response to these various stimuli can lead to a state of systemic inflammation, which can cause tissue damage and contribute to the development of chronic diseases [12]. The mechanisms underlying the pathophysiology of chronic low-grade inflammation are complex and involve the interplay between various biological systems.

Excessive alcohol consumption is a persistent medical and social issue in many countries worldwide, leading to a fatty liver and adversely impacting physical, mental, social, and psychological functions [13]. Chronic alcoholism can also cause neurological lesions and even cerebral atrophy [14]. The liver extensively metabolizes alcohol, generating acetaldehyde through enzymatic activity in the cytosol, microsomes, and peroxisomes [15]. Ethanol is also metabolized selectively in brain microsomes by cytochrome P-450 II E1, resulting in the generation of reactive oxygen species (ROS) [16]. Chronic ethanol treatment leads to increased production of ROS, causing cellular damage until removed by the antioxidant system, which comprises antioxidant enzymes and substances [17]. Vitamin C is a potent water-soluble antioxidant that is able to donate a hydrogen atom to form a stable ascorbyl free radical and has been shown to neutralize ROS and reduce oxidative stress [18–20]. This study aims to evaluate the protective effect of commercial-grade vitamin C against alcohol-induced inflammation in adult male Wistar rats.

EXPERIMENTAL METHODS

Animal care and grouping: Forty adult healthy male Wistar rats weighing between

150 and 250g were utilized in this study. The rats were raised under laboratory conditions in plastic and wire mesh cages located in the animal house of the Obafemi Awolowo College of Health Science at the Sagamu campus of Olabisi Onabanjo University in Ago-Iwoye, Ogun State, Nigeria. A two-week acclimatization period was observed during which the rats were fed a standardized pellet diet and provided with unlimited access to water. The care and management of the animals were in compliance with the internationally recognized standard guidelines for the use of animals established by the National Research Council [21]. The rats were randomly divided into eight groups of five rats each, and treatments were done for twenty-one days. Animals in each group received:

Group A: 1 ml of Distilled water only
Group B: 6000 mg/kg body weight of alcohol (30% v/v)
Group C: 100 mg/kg body weight of vitamin C
Group D: 200 mg/kg body weight of vitamin C
Group E: 300 mg/kg body weight of vitamin C
Group F: 6000 mg/kg body weight of alcohol (30% v/v) and 100 mg/kg body weight of vitamin C after 2 hours
Group G: 6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of vitamin C after 2 hours
Group H: 6000 mg/kg body weight of alcohol (30% v/v) and 300 mg/kg body weight of vitamin C after 2 hours

All treatments were done through the oral route of administration

Procedure for blood collection: blood was collected retrobulbarly. A capillary tube was inserted dorsally into the eye, and blood was allowed to flow by capillary action through the capillary tube into an EDTA sample bottle and a plane sample bottle.

Procedure for determination of serum antioxidant enzymes activity: The serum to be assessed for oxidative studies was homogenized in phosphate buffer in a ratio of four to one, and glutathione reductase (GSH) activities in the serum were determined using the method described by Sedlak and Lindsay [22], catalase (CAT) activities were determined using the method described by Sinha [23], and superoxide dismutase (SOD) activities were determined using the method described by Sun and Zigman [24].

Haematological analysis: White blood cell count (WBC), neutrophil, lymphocyte, and platelet counts (PLT) were estimated using an automated Beckman-Coulter hematological analyzer. Hematological ratio calculation Neutrophils to lymphocyte ratio (NLR) and platelets to lymphocyte ratio (PLR) were calculated by the method described by Aly et al. [25].

Statistical analysis: All analysis was done using SPSS (version 16) and Microsoft Excel (2019) using the student's T-test. Data were expressed as mean ± SEM, with $P < 0.05$ considered statistically significant. In the results section, ^{A}-values were significant when compared to group A; ^{B}-values were significant when compared to group B; ^{C}-values were significant when compared to group C; ^{D}-values were significant when compared to group D; E-values were significant when compared to group E, ^{F}-values were significant when compared to group F; and ^{G}-values were significant when compared to group G.

RESULTS

Effect of vitamin C and alcohol on platelet count, white blood cell, neutrophils and leukocyte count. Table 1 presents the effect of vitamin C and alcohol on white blood cell (WBC) and platelet counts in rats. Group B, which received only alcohol, had a significantly higher platelet count compared to Group A, which received only distilled water. Groups C, D, and E, which received different doses of vitamin C, had lower WBC counts compared to group B. Group F, which received both alcohol and vitamin C, had a higher platelet count compared to groups D and E but a lower count compared to group B. Group G, which received a higher dose of vitamin C, had a lower platelet count compared to group F, while group H, which received a higher dose

of vitamin C, had a similar platelet count to group F. Overall, the results suggest that vitamin C may have a protective effect on WBC count, but the effect on platelet count may depend on the dose and timing of vitamin C administration.

Effect of the concurrent administration of alcohol and vitamin C on antioxidant enzyme activity in serum of male Wistar rats. The table presents the effects of concurrent administration of alcohol and vitamin C on antioxidant enzyme activity in the serum of male Wistar rats. The results show that the group treated with only alcohol had significantly reduced levels of all three antioxidant enzymes compared to the group treated with only distilled water. However, the groups treated with different doses of vitamin C showed significantly increased levels of antioxidant enzymes compared to the group treated with only alcohol. The groups treated with a combination of alcohol and vitamin C also showed increased antioxidant enzyme activity compared to the group treated with only alcohol, but the extent of the increase varied depending on the dose of vitamin C.

Effect of the concurrent administration of alcohol and vitamin C on selected hematological indices (NLR and PLR) in adult male Wistar rats. The graph in Figure 1, shows the effect of the interaction between alcohol and vitamin C on PL-RATIO. Group A (distilled water only) had the lowest PL-RATIO while Group B (alcohol only) had the highest. Groups C, D, and E (varying doses of vitamin C) showed a decrease in PL-RATIO compared to Group B. Group F (alcohol and low dose of vitamin C) showed a slight decrease in PL-RATIO compared to Group B, while Groups G and H (alcohol and higher doses of vitamin C) showed a more significant decrease in PL-RATIO compared to Group B. The letters "a" through "f" indicate which groups were significantly different from each other. The graph in figure 2, shows the effect of the interaction between alcohol and vitamin C on NL-RATIO in rats. The groups were treated with different doses of alcohol and vitamin C, and the results are compared to the control group (A). The graph indicates that the NL-RATIO increased significantly in groups treated with alcohol (B) compared to the control group. However, treatment with vitamin C (C, D, E) resulted in a decrease in the NL-RATIO. The combination of alcohol and vitamin C (F, G, H) also resulted in a significant decrease in the NL-RATIO compared to alcohol alone (B). The results suggest that vitamin C can counteract the negative effects of alcohol on the NL-RATIO in rats.

DISCUSSION

The study evaluated the anti-inflammatory activity of vitamin C by examining the impact of alcohol toxicity on selected hematological indices and ratios. The results revealed that rats administered with alcohol only showed an increase in white blood cell count, platelet count, and PL/NL ratio compared to the control group. However, rats administered with vitamin C and those given both alcohol and vitamin C showed a decrease in these indices compared to the alcohol group. Chronic alcohol use can disrupt various physiological, biochemical, and metabolic processes in the blood cells, leading to inflammatory and other metabolic disorders. Alcohol-related medical conditions are commonly associated with chronic inflammation, and studies suggest that it contributes to the initiation and progression of many diseases. Reactive oxygen species, produced during alcohol metabolism, can activate a key inflammation transcription factor (NF-B), leading to inflammation [26]. Wang et al. [27] reported a high level of circulating pro-inflammatory cytokines in individuals with alcoholic liver diseases.

Table 1: Protective effect of vitamin c against alcohol induced toxicity on the white blood cells and platelets count male Wistar rats

Group	Treatment	WBC (10^3)	PLT (10^3/uL)
A	Distilled water only	7.28±2.22	669.8±72.29
B	6000 mg/kg of alcohol (30% v/v)	7.94±0.09	791±64.47^{b}
C	Vitamin C, 100 mg/kg	4.62±0.71^{A,,B}	591.4±163.77
D	Vitamin C, 200 mg/kg	5.8±0.37^{B,C}	521.4±199.20^{B,C}
E	Vitamin C, 300 mg/kg	6.08±1.44^{C,D}	702.4±11.08^{B,D}
F	6000 mg/kg of alcohol (30% v/v) and Vitamin C, 100 mg/kg after 2 h	6.52±1.25^{B,C,E}	786.4±98.88^{A,D,E}
G	6000 mg/kg of alcohol (30% v/v) and Vitamin C, 200 mg/kg after 2 h	6.2±0.37$^{B,C\ E}$	508.6±75.67^{A,B,C,E,F}
H	6000 mg/kg of alcohol (30% v/v) and Vitamin C, 300 mg/kg after 2 h	6.14±0.43^{B,C,E}	668.4±40.30^{B,F,G}

values are mean ± SEM. (P <0.05)

Table 2: Effect of the concurrent administration of alcohol and vitamin C on serum antioxidant enzyme activity of male Wistar rats

Group	Treatment	GSH (µmol/ml)	SOD (µmol/ml/min/mg/pro)	CAT (µmol/ml/min/mg/pro)
A	Distilled water only	189.88±10.02	1.49±0.07	4.73±0.75
B	6000 mg/kg of alcohol (30% v/v)	45.23±6.77^{A}	0.68±0.20^{A}	1.61±0.31^{A}
C	Vitamin C, 100 mg/kg	180.55±33.92^{B}	2.27±0.21^{A}	78.67±0.33$^{A, B}$
D	Vitamin C, 200 mg/kg	180.95±18.61$^{A, B}$	2.37±0.13$^{A, B}$	7.12±1.04$^{A, B}$
E	Vitamin C, 300 mg/kg	200.56±20.52$_{A, B}$	2.59±0.17$^{A,C\ D}$	9.74±0.39$^{A, C, D}$
F	6000 mg/kg of alcohol (30% v/v) & Vitamin C, 100 mg/kg after 2 h	140.64±26.63 $^{A, C\ D, E}$	1.06 ± 0.18$^{A, B, E}$	5.08 ±1.15$^{B, D,E}$
G	6000 mg/kg of alcohol (30% v/v) & Vitamin C, 200 mg/kg after 2 h	142.73 ±48.79^{B,CDEF}	1.15 ±0.51^{A,B,E}	5.80 ±1.56^{B,C,D}
H	6000 mg/kg of alcohol (30% v/v) & Vitamin C, 300 mg/kg after 2 h	187.10 ±15.29$^{A, C, D, E, G}$	1.50 ±7.55	6.08 ±1.62^{C,D,F}

values are mean ± SEM. (P <0.05)

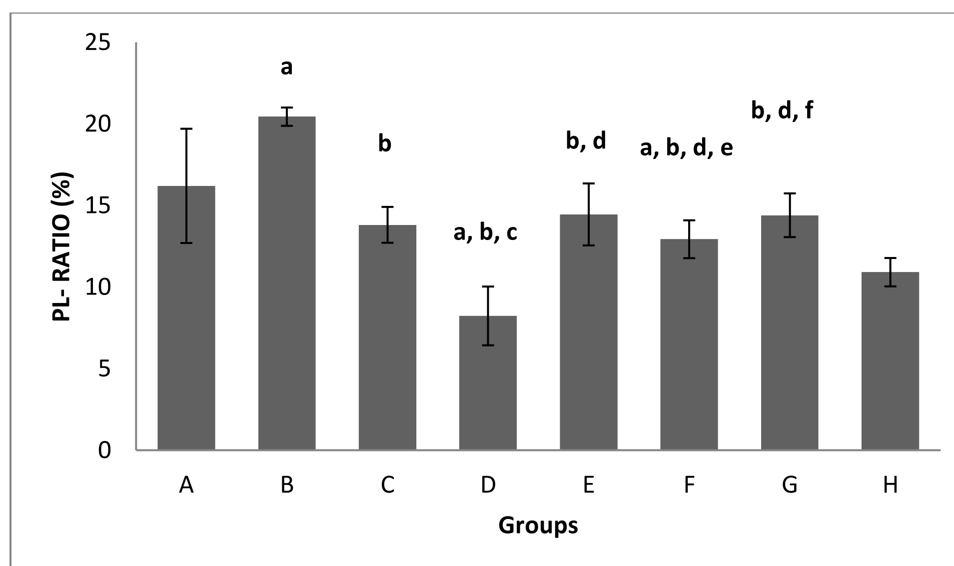

Figure 1: Effect of the alcohol vitamin C interaction on PL- RATIO. A; Distilled water only, B; 6000mg/kg body weight of alcohol (30% v/v), C; 100mg/kg body weight of vitamin C, D; 200mg/kg body weight of vitamin C, E; 300mg/kg body weight of vitamin C, F; 6000mg/kg body weight of alcohol (30% v/v) and 100mg/Kg body weight of vitamin C after two hours, G; 6000mg/kg body weight of alcohol (30% v/v) and 200mg/kg body weight of vitamin C after two hours, H; 6000mg/kg body weight of alcohol (30% v/v) and 300mg/kg body weight of vitamin C after two hours

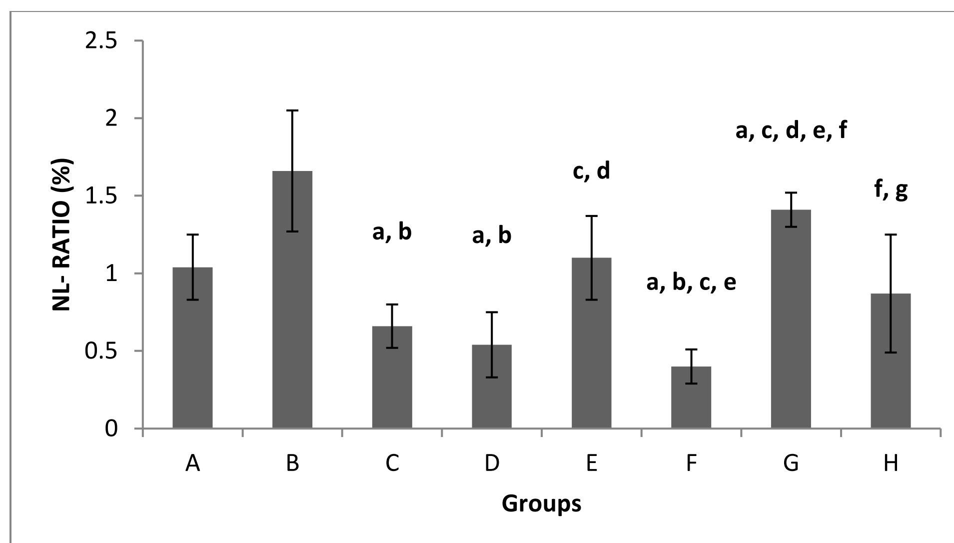

Figure 2: Effect of the alcohol vitamin C interaction on NL- RATIO. A; Distilled water only, B; 6000mg/kg body weight of alcohol (30% v/v), C; 100mg/kg body weight of vitamin C, D; 200mg/kg body weight of vitamin C, E; 300mg/kg body weight of vitamin C, F; 6000mg/kg body weight of alcohol (30% v/v) and 100mg/Kg body weight of vitamin C after two hours, G; 6000mg/kg body weight of alcohol (30% v/v) and 200mg/kg body weight of vitamin C after two hours, H; 6000mg/kg body weight of alcohol (30% v/v) and 300mg/kg body weight of vitamin C after two hours

Low-grade inflammation is linked to several chronic diseases in the elderly, such as cardiovascular disease, cancer, and diabetes. For example, immune mechanisms play a role in the formation and activation of atherosclerotic plaques leading to cardiovascular disease, and over-expression of TNF- is associated with insulin resistance and type 2 diabetes. Chronic inflammation is also considered a basic pathogenic process in cancer development [28–29]. Chronic alcohol consumption can also increase oxidative stress in the body, which is a state of imbalance between the production of reactive oxygen species (ROS) and the ability of the body to detoxify or repair the resulting damage. The accumulation of ROS can damage cells and tissues, leading to the activation of the immune system and the production of inflammatory cytokines [30]. Alcohol can activate immune cells, such as macrophages and dendritic cells, which can produce pro-inflammatory cytokines. These cytokines, including tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6), can cause chronic inflammation in the body. Studies suggest that a higher WBC contributes to the development of metabolic disorders. Pro-inflammatory cytokines, such as IL-6 and IL-8, induce WBC production, and elevated levels of these cytokines may cause higher WBC counts. Also, the reduction in antioxidant enzyme activity showed an increase in the production of free radicals, which also contribute to the development of inflammation [31–33]. Farhangi et al. [34] suggest that increased levels of CRP are responsible for the increased WBC count. In this study, the group administered alcohol alone showed an increase in WBC levels and a decrease in antioxidant enzyme activity, indicating inflammation. Vitamin C, a strong reducing agent, can regulate the resolution of the inflammatory process, stimulate tissue repair, and modulate the release of catabolic inflammatory cytokines, chemotaxis of immune cells, and activation of phagocytosis [35]. The anti-inflammatory properties of vitamin C are evident in the table, as it caused a decrease in WBC counts and an increase in antioxidant enzyme activity across groups administered with alcohol and vitamin C and the vitamin C only group when compared to the alcohol only group. The group that received 6000 mg/kg of

alcohol and 300 mg/kg of vitamin C had the lowest inflammation level across the concurrent groups.

According to the study by Page [36], platelets are cells involved in inflammation that serve multiple functions beyond hemostasis and thrombosis. Platelets contain numerous inflammatory peptides and proteins that are released during inflammatory conditions, such as cytokines, chemokines, and eicosanoids, enabling platelets to recruit leukocytes to the site of injury or inflammation. Platelets are present in the circulation of patients with various inflammatory diseases, including sepsis, cerebrovascular ischemia, and diabetes. In addition to posing a risk for excessive thrombosis, activated platelets in the circulation also transmit a pro-inflammatory signal. Platelet-endothelial interactions mediate the accumulation of monocytes and the deposition of pro-inflammatory cytokines [37–38]. In this study, groups administered alcohol only showed an increase in platelet count, indicating the presence of inflammation, as stated above. Ellulu et al. [39] explained that vitamin C's anti-inflammatory properties and antioxidant capacity can be attributed to its ability to modulate the DNA binding activity of NF-B, which is primarily promoted by oxidative stress and leads to cytokine-induced expression of cell adhesion molecules in the vascular endothelium and to the TNF- and IL-6-induced production of CRP by the liver. In this study, there was a decrease in the platelet count in the groups administered with only vitamin C and the concurrent group when compared to the alcohol-only group. This corresponds with other studies that have shown a decrease in platelet count after vitamin C administration [40–41].

Elevated levels of systemic inflammatory markers are associated with an increased risk of developing cardiovascular diseases [42]. Chronic low-grade inflammation has also been linked to several other health issues such as diabetes mellitus, hypertension, metabolic syndrome, and certain lifestyle habits [43–46]. The neutrophil-to-lymphocyte ratio (NLR) is a useful biomarker that reflects both lymphocyte and neutrophil counts and can predict the risk of developing health issues. A reduction in the lymphocyte count and an increase in the neutrophil count contribute to the power of NLR as an inflammatory factor. Several studies have shown that NLR is effective in identifying individuals who are at risk of developing sensorineural hearing loss or adverse cardiac events [47–48]. The platelet-to-lymphocyte ratio (PLR) is another biomarker that can reveal shifts in platelet and lymphocyte counts due to acute inflammatory and prothrombotic states [49]. PLR has been extensively studied in neoplastic diseases that are accompanied by immune suppression and thrombosis, and it can be predicted by combined blood cell counts and their ratios [50].

The platelet-to-lymphocyte ratio (PLR) is a useful inflammatory marker, particularly when considered alongside other hematologic indices such as the neutrophil-to-lymphocyte ratio (NLR). The NLR provides additional information about disease activity, the presence of neutrophilic inflammation, infectious complications, and severe organ damage in systemic lupus erythematous. PLR and NLR are highly predictive in rheumatic diseases that predominantly involve neutrophil inflammation, such as Behçet disease and familial Mediterranean fever. Additionally, high PLR, along with an elevated platelet count, can be potentially useful in diagnosing some systemic vasculitides, particularly giant-cell arteritis [49, 51]. In this study, we observed an increase in the PLR and NLR in rats administered with alcohol only. However, in the concurrent groups, there was a decrease in the NL and PL ratios, which was also observed in the groups administered with only vitamin C.

Conclusion. The findings of this study revealed that male Wistar rats administered commercial grade vitamin C at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg reduced chronic low-grade inflammation, as evidenced by a reduction in WBC count, platelet count, and NLR and PLR. However, further molecular investigations are necessary to better understand the anti-inflammatory mechanisms of commercial grade vitamin C.

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