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Modulatory Efficiency of Vitamin C (Ascorbic Acid) on Collagen-Induced Platelet Aggregation and Dysfunction

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Abstract

Platelet aggregation, coagulation, and activation are crucial for hemostasis. Collagen treatment can impair hemostatic processes leading to bleeding disorders like thrombosis; vitamin C may mitigate these effects. Hence, modulatory efficiency of vitamin C on collagen-induced platelet aggregation and dysfunction was investigated. Thirty (30) Wistar rats (135g-155g) were divided equally into; Group 1 (Control), Group 2 (Collagen-induced), and Group 3 (Collagen + Vitamin C treated). Platelet aggregation, prothrombin time, bleeding time, fibrinogen levels assessed coagulation and platelet function. Thromboxane B2 and P-selectin levels measured platelet and endothelial activation. Platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) evaluated platelet production and size variability. Statistical significance was set at $p < 0.05$. Group 2 exhibited higher platelet aggregation, prolonged prothrombin and bleeding times and elevated fibrinogen, thromboxane B2, and P-selectin levels, along with increased platelet count, MPV, PDW, and PCT, compared to Group 1. Group 3 showed significant reductions in all these parameters compared to Group 2 ($p < 0.05$). Vitamin C demonstrated significant modulatory effect on collagen-induced platelet aggregation and dysfunction which suggests that vitamin C may have therapeutic potential in mitigating platelet dysfunction and coagulation impairments associated with collagen-induced pathophysiological conditions.

Keywords: Platelets aggregation; Collagen-induced; Vitamin C.

INTRODUCTION

The preservation of vascular integrity and homeostasis depends heavily on platelets, which are tiny, non-nucleated cellular fragments generated from a group of precursor cells called megakaryocytes (Kroll and Kharghan 2012; Onwuka et al., 2022). Platelets bind to the site of injury in a blood artery to produce a hemostatic plug that stops excessive bleeding when the vessel is injured, exposing the sub-endothelial matrix (Broos et al., 2011; Periayah et al., 2017; Onwuka et al., 2023). Platelet aggregation is the final result of a series of events that occur during this process (Periayah et al., 2017). Platelet aggregation and dysfunction play a critical role in the pathogenesis of various cardiovascular diseases, including atherosclerosis, myocardial infarction, and stroke. These conditions are often associated with increased platelet reactivity and aggregation, leading to the formation of thrombi that can obstruct blood flow and cause severe health complications (Hosseini et al., 2012; Malik et al., 2021). Understanding the mechanisms underlying platelet aggregation and identifying potential modulators is essential for developing effective therapeutic strategies.

Collagen is a potent stimulator of platelet aggregation, initiating a cascade of events that lead to platelet activation and the subsequent formation of a hemostatic plug (Tomaiuolo et al., 2017). The interaction between collagen and platelets is a complex process involving various receptors and intracellular signaling pathways. Dysregulation of this process can result in either excessive bleeding or thrombotic events, underscoring the importance of maintaining a delicate balance in platelet function (Farndale 2004; Brass et al., 2013).

Vitamin C (ascorbic acid) is a vital nutrient known for its antioxidant properties and its role in various physiological processes (Pehlivan, 2017). Recent studies have suggested that vitamin C may have a modulatory effect on platelet function, potentially influencing platelet aggregation and overall cardiovascular health (Ashor et al., 2014). However, the precise mechanisms by which vitamin C impacts collagen-induced platelet aggregation

and dysfunction remain to be fully elucidated. This research therefore investigated the modulatory potential of vitamin C in collagen-induced platelet aggregation and dysfunction. By exploring the effects of vitamin C on platelet reactivity, aggregation, and related signaling pathways, this study seeks to provide a deeper understanding of its potential therapeutic benefits in preventing and managing diseases associated with abnormal platelet function. The findings from this study could have significant implications for developing novel therapeutic approaches that leverage the properties of vitamin C to modulate platelet function, thereby reducing the risk of thrombotic events and improving health outcomes.

METHODOLOGY

Study Design

This study constitutes an experimental, controlled laboratory research conducted in compliance with the Guide for the Care and Use of Laboratory Animals, as outlined by the National Academy Press at 2101 Constitution Ave. NW, Washington, DC 20055, USA. The laboratory animals used were housed in adequately ventilated cages and acclimatized to laboratory environments for a period of 14 days. They were sustained on standard rat chow with unrestricted access to drinking water and subjected to natural alternating 12-hour day and 12-hour night cycles.

Subjects and Grouping

Thirty (30) animal subjects (Wistar rats weighing 135g-155g) were used and equally divided into three groups;

Group 1 : Control group (no treatment)
Group 2 : Collagen-induced platelet aggregation group
Group 3 : Collagen-induced platelet aggregation with vitamin C treatment group

Induction of Platelet Aggregation and Administration protocol

Collagen (bovine brand, Sigma-Aldrich, USA) was administered (i.p.) **at** a dose of 10 µg/kg body weight, once every three days for a period of 14 days (total of five injections). Vitamin C (ascorbic acid) **of** laboratory grade, obtained from the Biochemistry Department, Gregory University Uturu, was administered (p.o.) at a dose of 50 mg/kg body weight daily for 14 days.

Sample collection

At the end of the 14-day experimental period, blood samples were collected from all groups. The animals were euthanized under anesthesia using ketamine at a dose of 100 mg/kg. Blood samples were obtained via retro-orbital puncture using capillary tubes into 5 ml sample bottles. These samples were then used for assays.

Preparation of Platelet-rich plasma (PRP) and Platelet-poor plasma (PPP)

Blood samples for Platelet-rich plasma (PRP) and Platelet-poor plasma (PPP) was collected into sample bottles containing anticoagulant (3.2% sodium citrate solution) and gently inverted to mix the blood with the anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifuging the samples at 200 x g for 10 minutes at room temperature. The supernatants (PRP) were transferred into new tubes.

Platelet-poor plasma (PPP) was obtained after getting PRP, the plasma samples were subjected to a second centrifugation at an increased speed of 2,000 x g for 10 minutes at room temperature. This additional centrifugation step was performed to ensure the removal of any residual platelets, thereby yielding platelet-poor plasma (PPP). The supernatants (PPP) were also transferred into new tubes. The resultant PRP and PPP were used for assays.

Platelet Aggregation Assay

The supernatants (PRP) were used for platelet aggregation and were measured using platelet aggregometer (Chrono-log Model 700 Whole Blood/Optical Lumi-Aggregometer, USA). The degree of aggregation was recorded as a percentage of light transmission.

Prothrombin Time (PT) Measurement

Thromboplastin reagent and calcium chloride solution were pre-warmed to 37°C. Then, 100 µL of platelet-poor plasma (PPP) was pipetted into clean test tube. Following this, 100 µL of the pre-warmed thromboplastin reagent was added to the PPP, and the stopwatch was started. The mixture was incubated at 37°C for 2 minutes. Subsequently, 100 µL of the pre-warmed calcium chloride solution was added to the mixture, and the timer was started immediately. The mixture was observed, and the time taken for clot formation was recorded as the prothrombin time (PT).

Bleeding Time Measurement

Standardized incision (1-2 mm deep) was made on the rat's tail using scalpel blade. Stopwatch was started immediately after making the incision. Blood was gently blotted at regular intervals of 15 seconds using filter paper. This process continued until bleeding ceased. The duration from the initial incision to the cessation of bleeding was recorded as the bleeding time.

Fibrinogen, Thromboxane B2 and P-selectin (CD62P) Assay

Platelet-poor plasma (PPP) was used to measure fibrinogen, thromboxane B2, and P-selectin (CD62P) using enzyme-linked immunosorbent assays (ELISAs) specific for each of these markers, following the manufacturer's protocol.

Measurement of Platelet Counts, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT)

Platelet counts, MPV, PDW and PCT ware measured in blood sample using hematological analyzer (Swelab Alfa, Boule, USA).

Statistics

Statistical analysis was performed using GraphPad Prism software (v8). Comparisons between groups were made using one-way ANOVA followed by Bonferroni's post-hoc tests. A p-value < 0.05 was considered statistically significant.

RESULTS

Vitamin C Modulation of Degree of Platelet Aggregation in Collagen-Induced Platelet Aggregation and Dysfunction

The degree of platelet aggregation is expressed as percentage of light transmission (%LT). Group 2: Collagen-induced group showed a significant increase in platelet aggregation compared to the Group1: Control group ($p < 0.05$). Group 3: Collagen + Vitamin C treated group showed a significant reduction in platelet aggregation compared to the Group 2: Collagen-induced group ($p < 0.05$), indicating the modulating effect of vitamin C on collagen-induced platelet aggregation (Figure 1).

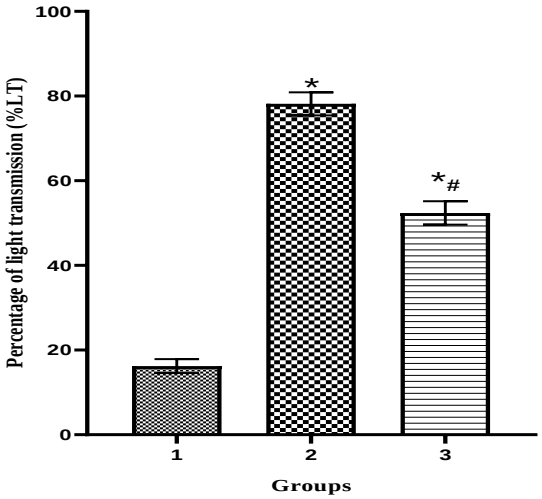

Figure 1. Degree of Platelet Aggregation expressed as percentage of light transmission (%LT): Values are expressed as mean ± SEM, * $p < 0.05$ compared to Group 1: Control group, # $p < 0.05$ comparison between Group 2: Collagen-induced group and Group 3: Collagen + Vitamin C treated group.

Vitamin C Modulation of Prothrombin Time in Collagen-Induced Platelet Aggregation and Dysfunction

Group 2: Collagen-induced group showed significantly prolonged prothrombin time compared to the Group 1: Control group ($p < 0.05$), suggesting impaired coagulation function due to collagen treatment. Group3: Collagen + Vitamin C group showed significantly shorter prothrombin time compared to Collagen-induced group ($p < 0.05$), indicating that vitamin C may mitigate the coagulation impairment caused by collagen treatment (Figure 2).

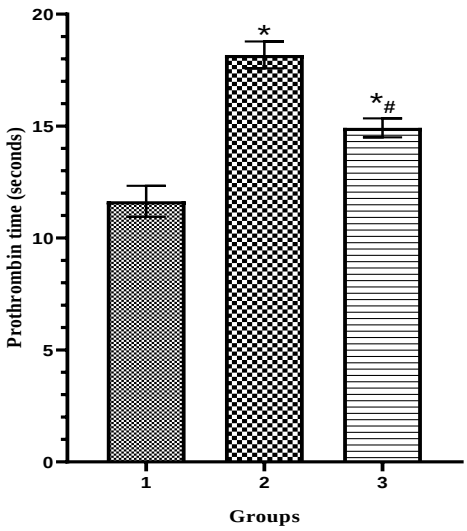

Figure 2. Prothrombin Time (seconds): Values are expressed as mean ± SEM, * $p < 0.05$ compared to Group 1: Control group, # $p < 0.05$ comparison between Group 2: Collagen-induced group and Group 3: Collagen + Vitamin C treated group.

Vitamin C Modulation of Bleeding Time in Collagen-Induced Platelet Aggregation and Dysfunction

Group 2: Collagen-induced group showed significantly prolonged bleeding time compared to the Group 1: Control group ($p < 0.05$), suggesting impaired platelet function and primary hemostasis due to collagen treatment. Group 3: Collagen + Vitamin C group showed significantly shorter bleeding time compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help mitigate the impairment in platelet function caused by collagen treatment (Figure 3).

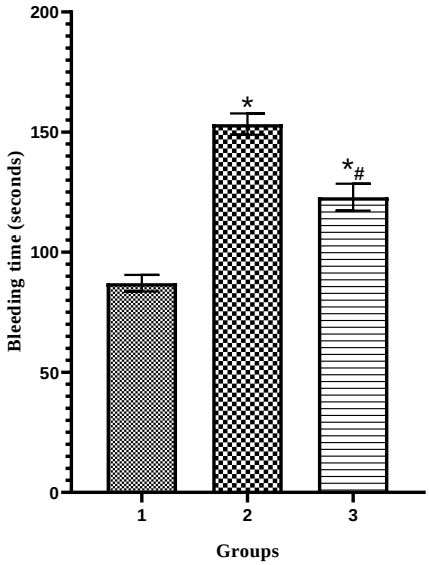

Figure 3. Bleeding Time (seconds): Values are expressed as mean ± SEM, * $p < 0.05$ compared to Group 1: Control group, # $p < 0.05$ comparison between Group 2: Collagen-induced group and Group 3: Collagen + Vitamin C treated group.

Vitamin C Modulation of Fibrinogen, Thromboxane B2 and P-selectin (CD62P) in Collagen-Induced Platelet Aggregation and dysfunction

Group 2: Collagen-induced group shows significantly higher fibrinogen levels compared to the Group 1: Control group ($p < 0.05$), suggesting an increase in coagulation activity or inflammatory response due to collagen treatment. Group 3: Collagen + Vitamin C group shows significantly lower fibrinogen levels compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help normalize the fibrinogen levels affected by collagen treatment (Figure 4A). The Collagen-induced group shows significantly higher thromboxane B2 levels compared to the Control group ($p < 0.05$), suggesting increased platelet activation due to collagen treatment, while the Collagen + Vitamin C group shows significantly lower thromboxane B2 levels compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may reduce the increased platelet activation caused by collagen treatment (Figure 4B). The Collagen-induced group also showed significantly higher P-selectin (CD62P) levels compared to the Control group ($p < 0.05$), suggesting increased platelet and endothelial cell activation due to collagen treatment. The Collagen + Vitamin C group showed significantly lower P-selectin levels compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may reduce the increased activation of platelets and endothelial cells caused by collagen treatment (Figure 4C).

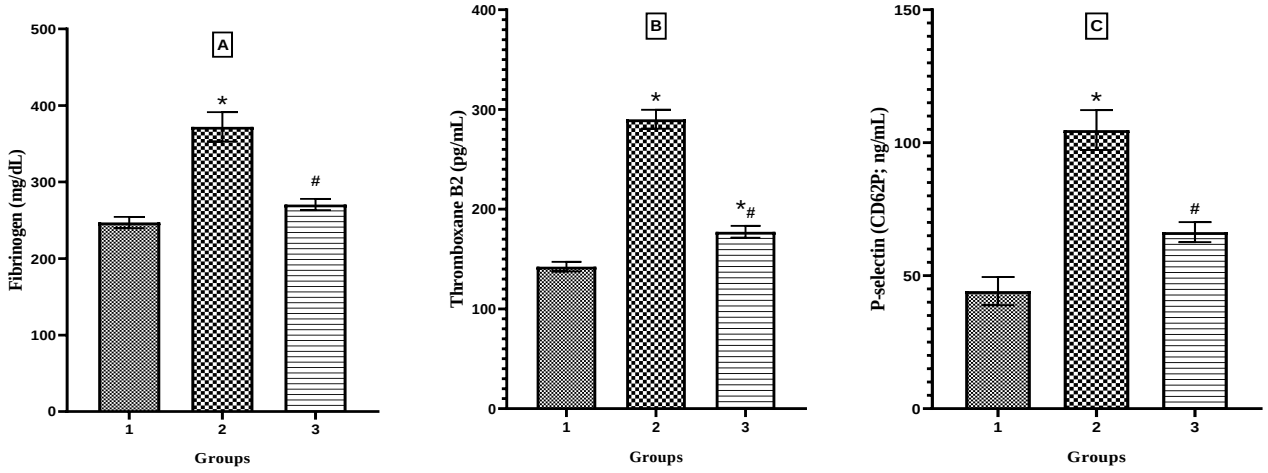

Figure 4. [A] Fibrinogen (mg/dl); [B] Thromboxane B2 (pg/ml); [C] P-selectin (CD62P: ng/mL): Values are expressed as mean ± SEM, * $p < 0.05$ compared to Group 1: Control group, # $p < 0.05$ comparison between Group 2: Collagen-induced group and Group 3: Collagen + Vitamin C treated group.

Vitamin C Modulation of Platelet Counts, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) in Collagen-Induced Platelet Aggregation and dysfunction

Group 2: Collagen-induced group showed significantly higher platelet count compared to the Group 1: Control group ($p < 0.05$), suggesting an increase in platelet production or reduced platelet clearance due to collagen treatment. Group 3: Collagen + Vitamin C group showed significantly lower platelet count compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help normalize the platelet count affected by collagen treatment. Collagen-induced group showed significantly higher MPV compared to the Control group ($p < 0.05$), suggesting an increase in platelet size due to collagen treatment. Collagen + Vitamin C group showed significantly lower MPV compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help modulate the increase in platelet size caused by collagen treatment. The Collagen-induced group showed significantly higher PDW compared to the Control group ($p < 0.05$), suggesting increased variability in platelet size due to collagen treatment. The Collagen + Vitamin C group showed significantly lower PDW compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help reduce the variability in platelet size caused by collagen treatment. The Collagen-induced group also showed significantly higher PCT compared to the Control group ($p < 0.05$), suggesting an increase in platelet mass due to collagen treatment.; while Collagen + Vitamin C group showed significantly lower PCT compared to the Collagen-induced group ($p < 0.05$), indicating that vitamin C may help modulate the increase in platelet mass caused by collagen treatment (Table 1).

Table 1. Platelet Counts, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) in experimental groups.

Groups	Platelet Count ($x10^9$/L)	MPV (fL)	PDW (%)	PCT (%)
1. Control	722.0 ± 36.8	4.84 ± 0.92	8.36 ± 0.82	0.13 ± 0.02
2. Collagen-induced	957.0 ± 23.5*	10.34 ± 0.65*	15.00 ± 0.99*	0.27 ± 0.02*
3. Collagen + Vitamin C	826.0 ± 31.6#	7.60 ± 0.27*#	11.08 ± 0.46#	0.18 ± 0.01#

Values are expressed as mean ± SEM, * $p < 0.05$ compared to Group 1: Control group, # $p < 0.05$ comparison between Group 2: Collagen-induced group and Group 3: Collagen + Vitamin C treated group.

DISCUSSION

Platelet aggregation (the clumping of platelets in the blood, vital for blood clotting) has been associated with thrombosis due to excessive aggregation leading to excessive clotting. It has also been linked to platelet dysfunction, resulting in bleeding disorders (Ghoshal and Bhattacharyya, 2014; Krishnegowda and Rajashekaraiah, 2015). This study evaluated the potential of vitamin C to modulate platelet aggregation and dysfunction by assessing its effects on collagen-induced platelet aggregation and dysfunction. The results demonstrated that vitamin C significantly reduced the degree of platelet aggregation in collagen-induced platelet aggregation and dysfunction in Wistar rats. This suggests that vitamin C has a potential therapeutic effect in modulating platelet function and could be beneficial in preventing or treating conditions associated with excessive platelet aggregation. This is in line with studies that suggests potential beneficial role of vitamin c on platelets function (Wilkinson et al., 1999; Ashor et al., 2014).

A prothrombin time measures the time it takes for blood to clot and assesses the extrinsic pathway of coagulation. Prolonged prothrombin time indicates impairment of clotting factors and may be associated with abnormal platelet aggregation (Kamal et al., 2007). The prothrombin time in this study suggest that collagen treatment impairs the extrinsic pathway of blood coagulation in Wistar rats, as evidenced by the prolonged prothrombin time. However, co-administration of vitamin C appears to counteract this effect, reducing the prothrombin time. These findings indicate a potential protective role of vitamin C in maintaining normal coagulation function in the presence of collagen-induced dysfunction.

Bleeding time assesses the overall ability of the body to form a platelet plug and stop bleeding. Prolonged bleeding time has been associated with impaired hemostasis, platelet dysfunction, or coagulopathy, which can also be linked to abnormal platelet aggregation (Jandrey, 2012; Wohlauer et al., 2012). In this study, the results of bleeding time suggest that collagen treatment impairs primary hemostasis in Wistar rats, as evidenced by the prolonged bleeding time. However, co-administration of vitamin C appears to mitigate this effect by reducing the bleeding time. These findings indicate a potential protective role of vitamin C in maintaining normal platelet function and primary hemostasis in the presence of collagen-induced disorders, consistent with studies reporting the beneficial impacts of vitamin C on platelet function and hemostasis (Violi, 2010).

Fibrinogen (clotting factor I) is crucial in platelet aggregation; elevated fibrinogen levels can enhance platelet aggregation and thrombus formation (Kattula et al., 2017). Thromboxane B2 is a potent platelet agonist that, upon activation, enhances platelet activation and serves as a marker of platelet activation (Smyth, 2010).

P-selectin (CD62P) is expressed on the surface of activated platelets and endothelial cells; its elevation indicates platelet activation and aggregation, as P-selectin activated by thromboxane mediates platelet-platelet interactions leading to aggregation (Chandler et al., 2010). In this study, collagen treatment enhanced coagulation response, platelet activation, and endothelial cell activation in Wistar rats, as evidenced by increased fibrinogen, Thromboxane B2, and P-selectin levels. Co-administration of vitamin C appears to reduce this effect, suggesting a potential protective role of vitamin C in modulating coagulation, platelet activation, and endothelial cell activation, thereby modulating platelet aggregation in the presence of collagen-induced dysfunction.

During platelet aggregation, there is an increase in platelet count, contributing to the aggregation and clotting process (Stissing et al., 2011). Mean Platelet Volume (MPV) rises, indicating the presence of larger platelets, which are more active in aggregation and release more granules, thereby promoting clot formation (Vizioli et al., 2009). Elevated Platelet Distribution Width (PDW) and Plateletcrit (PCT) indicate greater variation in platelet size and a higher percentage of platelets in the blood, respectively, which can enhance platelet aggregation (Cetin et al., 2017). This study demonstrated that collagen treatment significantly increases platelet concentration, MPV, PDW, and PCT in Wistar rats, suggesting substantial impacts on platelet size, variability, and mass. However, the co-administration of vitamin C appears to mitigate these effects, normalizing the values. These findings suggest a potential protective role of vitamin C in maintaining normal platelet homeostasis, function, and morphology in the context of collagen-induced bleeding disorders. This corresponds to studies that suggested that vitamin C can be beneficial for platelet homeostasis, function, and morphology in pathological conditions (Ferroni et al., 2012, May and Harrison, 2013).

CONCLUSION

Vitamin C significantly mitigated platelet aggregation and dysfunction in Wistar rats by reversing the effects of collagen on degree of platelet aggregation, prothrombin time, bleeding time, fibrinogen, thromboxane B2, P-selectin, platelet concentration, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). This suggests a potential therapeutic role for vitamin C in preventing or treating excessive platelet aggregation, abnormal coagulation, and abnormal platelet activation, thus maintaining normal platelet homeostasis, function, and morphology which could be beneficial for bleeding disorders, especially thrombosis.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

REFERENCES

Ashor AW, Lara J, Mathers JC, Siervo M. Effect of vitamin C on endothelial function in health and disease: a systematic review and meta-analysis of randomised controlled trials. Atherosclerosis. 2014; 235(1):9-20.

Brass LF, Newman DK, Wannemacher KM, Zhu L, Stalker TJ. Signal transduction during platelet plug formation. Platelets. 2013; 2:319-46.

Broos K, Feys HB, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Platelets at work in primary hemostasis. Blood reviews. 2011; 25(4):155-67.

Cetin MS, Cetin EH, Akdi A, Aras D, Topaloglu S, Temizhan A, Aydogdu S. Platelet distribution width and plateletcrit: novel biomarkers of ST elevation myocardial infarction in young patients. Polish Heart Journal (Kardiologia Polska). 2017; 75(10):1005-12.

Chandler AB, Earhart AD, Speich HE, Kueter TJ, Hansen J, White MM, Jennings LK. Regulation of CD40L (CD154) and CD62P (p-selectin) surface expression upon GPIIb-IIIa blockade of platelets from stable coronary artery disease patients. Thrombosis research. 2010; 125(1):44-52.

Farndale RW, Sixma JJ, Barnes MJ, De Groot PG. The role of collagen in thrombosis and hemostasis. Journal of Thrombosis and Haemostasis. 2004; 2(4):561-73.

Ferroni P, Vazzana N, Riondino S, Cuccurullo C, Guadagni F, Davi G. Platelet function in health and disease: from molecular mechanisms, redox considerations to novel therapeutic opportunities. Antioxidants & Redox Signaling. 2012; 17(10):1447-85.

Ghoshal K, Bhattacharyya M. Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. The Scientific World Journal. 2014; 2014(1):781857.

Hosseini E, Ghasemzadeh M. Different stages of platelet adhesion to the site of vascular injury. Iranian Journal of Blood and Cancer. 2012; 4(3):133-42.

Jandrey KE. Assessment of platelet function. Journal of Veterinary Emergency and Critical Care. 2012; 22(1):81-98.

Kamal AH, Tefferi A, Pruthi RK. How to Interpret and Pursue an Abnormal Prothrombin Time, Activated Partial Thromboplastin Time, and Bleeding Time in Adults. InMayo Clinic Proceedings 2007; 7(82):864-873.

Kattula S, Byrnes JR, Wolberg AS. Fibrinogen and fibrin in hemostasis and thrombosis. Arteriosclerosis, thrombosis, and vascular biology. 2017; 37(3):e13-21.

Krishnegowda M, Rajashekaraiah V. Platelet disorders: an overview. Blood Coagulation & Fibrinolysis. 2015; 26(5):479-91.

Kroll MH, Kharghan VA. Platelets in pulmonary vascular physiology and pathology. Pulmonary circulation. 2012; 2(3):291-308.

Malik A, Rehman FU, Shah KU, Naz SS, Qaisar S. Hemostatic strategies for uncontrolled bleeding: A comprehensive update. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2021; 109(10):1465-77.

May JM, Harrison FE. Role of vitamin C in the function of the vascular endothelium. Antioxidants & redox signaling. 2013; 19(17):2068-83.

Onwuka OM, Nkpurukwe CI, Osuji AC. Comparative analysis of the impact of artificially and naturally ripened fruit on obesity-induced biochemical and hematological alterations. Gastroenterol Hepatol Open Access. 2023; 14(6):175-9.

Onwuka OM, Okerulu AL, Nwosu NC. Administration of chemically ripened banana (Musa acuminate) juice to male Wistar rats depletes blood cells via impaired hematopoiesis. Bio-Research. 2022; 20(2):1552-9.

Pehlivan FE. Vitamin C: An antioxidant agent. Vitamin C. 2017; 2:23-35.

Periayah MH, Halim AS, Saad AZ. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. International journal of hematology-oncology and stem cell research. 2017; 11(4):319.

Smyth EM. Thromboxane and the thromboxane receptor in cardiovascular disease. Clinical lipidology. 2010; 5(2):209-19.

Stissing T, Dridi NP, Ostrowski SR, Bochsen L, Johansson PI. The influence of low platelet count on whole blood aggregometry assessed by Multiplate. Clinical and Applied Thrombosis/Hemostasis. 2011; 17(6):E211-7.

Tomaiuolo M, Brass LF, Stalker TJ. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. Interventional cardiology clinics. 2017; 6(1):1.

Violi F, Pignatelli P, Basili S. Nutrition, supplements, and vitamins in platelet function and bleeding. Circulation. 2010; 121(8):1033-44.

Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. International journal of clinical practice. 2009; 63(10):1509-15.

Wilkinson IB, Megson IL, MacCallum H, Sogo N, Cockcroft JR, Webb DJ. Oral vitamin C reduces arterial stiffness and platelet aggregation in humans. Journal of cardiovascular pharmacology. 1999 Nov 1;34(5):690-3.

Wohlauer MV, Moore EE, Thomas S, Sauaia A, Evans E, Harr J, Silliman CC, Ploplis V, Castellino FJ, Walsh M. Early platelet dysfunction: an unrecognized role in the acute coagulopathy of trauma. Journal of the American College of Surgeons. 2012; 214(5):739-46.

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