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Research Article

In Vitro Evaluation of Ginger Extract and Ginger-Derived Nanolipids on Platelet Activation, Fibrinolysis, and Fibrin Formulation

Safaa S. Alfaris¹, Mohanad A. Albayati^{1*}

¹Dept. Physiology, Biochemistry and Pharmacology/ College of Veterinary Medicine/ University of Baghdad/ Iraq.

***Corresponding author:** Mohanad A. Albayati

*Email: aumnmumu@yahoo.com

Abstract

Platelet count show significant difference between plasma poor platelet and plasma rich platelet that increase in plasma rich platelet and show significant difference between the treatment groups, in alteplase show the highest value of platelet count 5.20 ± 0.66 , while in Ginger derived Nanoparticles show 2.40 ± 0.74 , and ginger extract show less with 3.00 ± 0.63 and the least positive control with 2.20 ± 0.37 in plasma poor platelet and in plasma rich platelet; Ginger derived Nanoparticles show 8.40 ± 0.67 show the highest value while in alteplase of platelet count 8.20 ± 0.86 , and ginger extract show less with 5.20 ± 0.37 and the least positive control with 2.00 ± 0.31 . fibrinogen quantification shows nonsignificant difference between plasma poor platelet and plasma rich platelet and show significant difference between the treatment groups, the alteplase treatment group decrease more than Ginger derived Nanoparticles and ginger extract groups in fibrinogen quantity in alteplase show the least value of fibrinogen quantification 18.25 ± 4.29 , while in Ginger derived Nanoparticles show 34.17 ± 3.39 , and ginger extract show more with 40.08 ± 3.58 and the highest positive control with 61.45 ± 0.35 in plasma poor platelet and in plasma rich platelet; in alteplase show the least value of fibrinogen quantification 25.77 ± 4.82 , while in Ginger derived Nanoparticles show 47.98 ± 4.32 , and ginger extract show more with 53.19 ± 2.92 and the highest positive control with 61.13 ± 0.31 . Fibrin quantification show nonsignificant difference between plasma poor platelet and plasma rich platelet, while in group of ginger extract show significant difference between plasma poor platelet and plasma rich platelet and show significant difference between the treatment groups, fibrin quantification shows nonsignificant increase in alteplase treatment group more than Ginger derived Nanoparticles and ginger extract groups in fibrin quantity, in alteplase show the highest value of Fibrin quantification 48.91 ± 10.56 , while in Ginger derived Nanoparticles show 41.54 ± 7.20 , and ginger extract show less with 40.08 ± 3.58 and the least positive control with 2.84 ± 0.26 in plasma poor platelet and in plasma rich platelet; alteplase of Fibrin quantification 41.71 ± 9.80 show the highest value while in Ginger derived Nanoparticles show 36.44 ± 7.73 and ginger extract show less with 8.59 ± 2.81 and the least positive control with 2.24 ± 0.53 . These findings suggest that ginger and its derivatives may have therapeutic potential in preventing and treating thrombotic disorders. Human clinical trials are necessary to assess the therapeutic potential of ginger-based interventions in patients with thrombotic disorders.

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Introduction

Platelets, essential components of the blood clotting process, play a crucial role in both physiological hemostasis and pathological thrombosis (Scridon, 2022). When activated, Medicinal plants have long been used for their therapeutic properties. Recent research has focused on their potential to modulate platelet activation and inhibit thrombus formation. Herbals plant had several beneficial effects such as antioxidants and anti-inflammatory effects (Hasan, 2019).

Ginger, a widely used spice, has been shown to possess anti-inflammatory and anti-platelet properties and fibrinolysis effect (AL-Bayaty, 2006; Al-Bayar, 2009; Nafia, 2012; Fakhri *et al.*, 2021; JAAFAR *et al.*, 2020).

Ginger-derived nanoparticles, engineered to enhance drug delivery, may offer additional benefits (Raheem and Hasan, 2021). The study investigated the fibrinolytic activity of acetone extracts of ginger and ginger-derived nanoparticles. Fibrinolytic Activity both ginger extract and nanoparticles demonstrated significant fibrinolytic activity, comparable to the standard drug alteplase (MustafaAl-Najjar *et al.*, 2016; Al-Nimer *et al.*, 2011). Anti-Platelet Aggregation the methanol extract of ginger and ginger-derived nanoparticles exhibited the highest anti-platelet aggregation activity (Fawzi, 2009; Al-Saigh, 2012), as measured by the percentage of fibrin and fibrinogen liberation.

Methodology protocol: the Experiment design to separation of platelet and to evaluation lysate production.

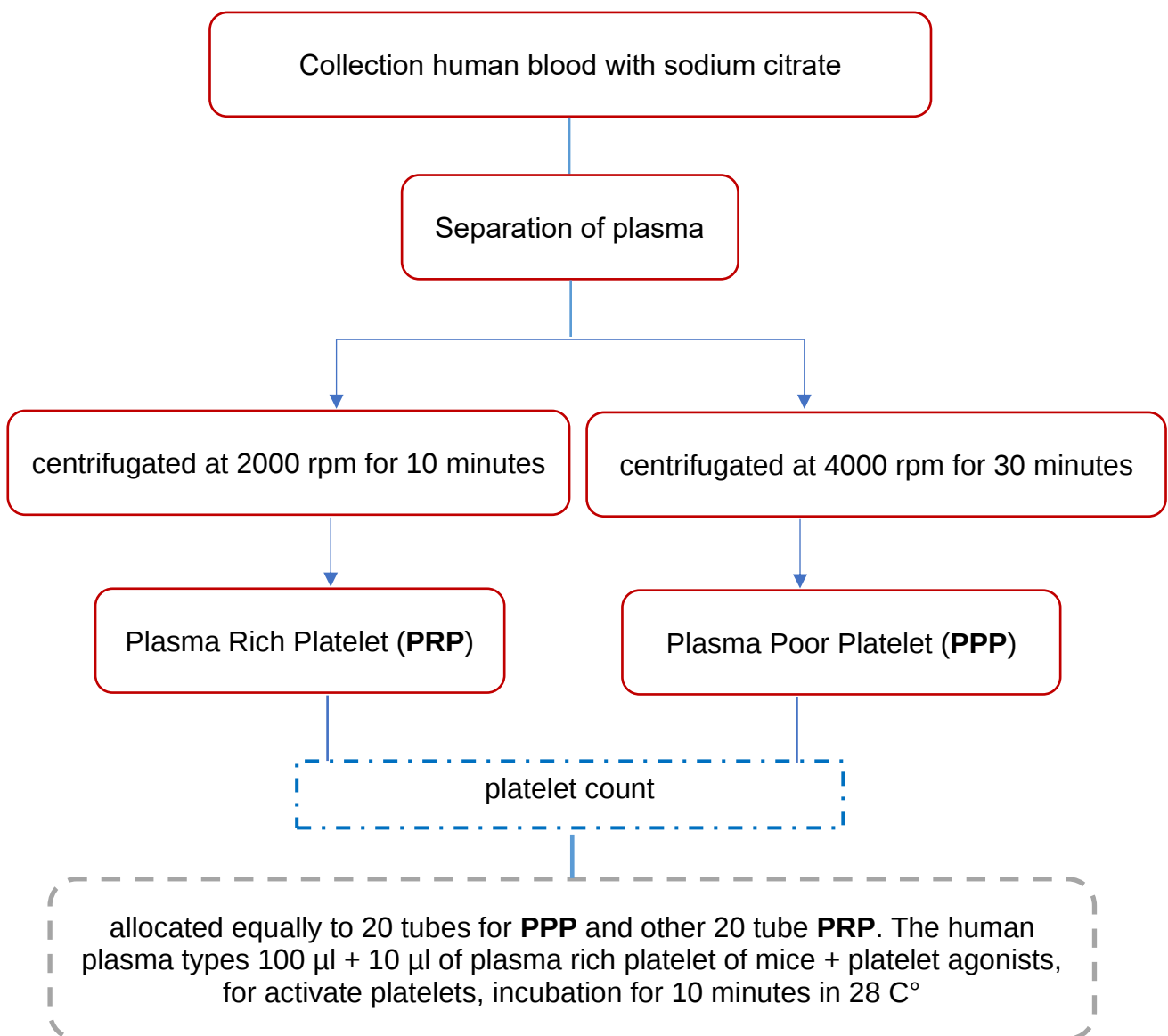


Figure 1: isolation of platelet Plasma Rich Platelet (PRP) and Plasma Poor Platelet (PPP)

Experiment design:

I. Blood collected: the blood was aspirated from two model from human and mice achieved activation fibrin production. Blood aspired 10 ml from human and 1.3 ml from mice and the human blood was set

in two coated tube of Sodium citrate (5 ml for each) and the mice in one tube with rolling for one minute at 25 C°.

II. Isolation platelets:

- a. Plasma Poor Platelet (**PPP**): The human blood was centrifugated at 4000 rpm for 30 minutes obtained plasma poor platelet (**PPP**).
- b. Plasma Rich Platelet (**PRP**): centrifugated human blood test tube 2000 rpm for 10 minutes to obtain plasma rich platelet (**PRP**), the mice Plasma Rich platelet was done as same procedure. The platelets in isolated plasma were estimated by using autoanalyzer (hemolyzer) for ensure platelet count in both **PPP** and **PRP**; pragmatically gently handle to avoidance platelets activation (Burzynski *et al.*, 2019). The **PPP** number was $14 \times 10^9/l$ and **PRP** platelet number was $23 \times 10^9/l$.
- c. **Washing platelets maneuvers**
- d. The human plasma both types were allocated equally to the test tube, 20 tubes for **PPP** and other 20 tube **PRP**. The human plasma types 100 μl were mix with 10 μl of plasma rich platelet of mice and add platelet agonists, list to at table (1), for activate platelets, the contain was incubation for 10 minutes in 28 C° (Burzynski *et al.*, 2019).

Table 1: the platelet agonist with concentrations(Burzynski *et al.*, 2019):

Platelet agonist	Concentration
Collagen	5 $\mu g/ml$
Collagen-related	50 $\mu g/ml$
Peptide (CRP)-XL Thrombin	1 U/ml
ADP	10 μM
Platelet Factor Activating	0.3 μM

Group design in Vitro

- 1. Control group (placebo)
- 2. Ginger extract treated group (0.02%, 0.03%, 0.04%, 0.05%, and 0.06%).
- 3. GDNPs ginger derived Nano-lipids treated group (0.02%, 0.03%,0.04%, 0.05%, and 0.06%).
- 4. Alteplase treated group was grouped in to with five concentrations (0.02%, 0.03%, 0.04%, 0.05%, 0.06%).

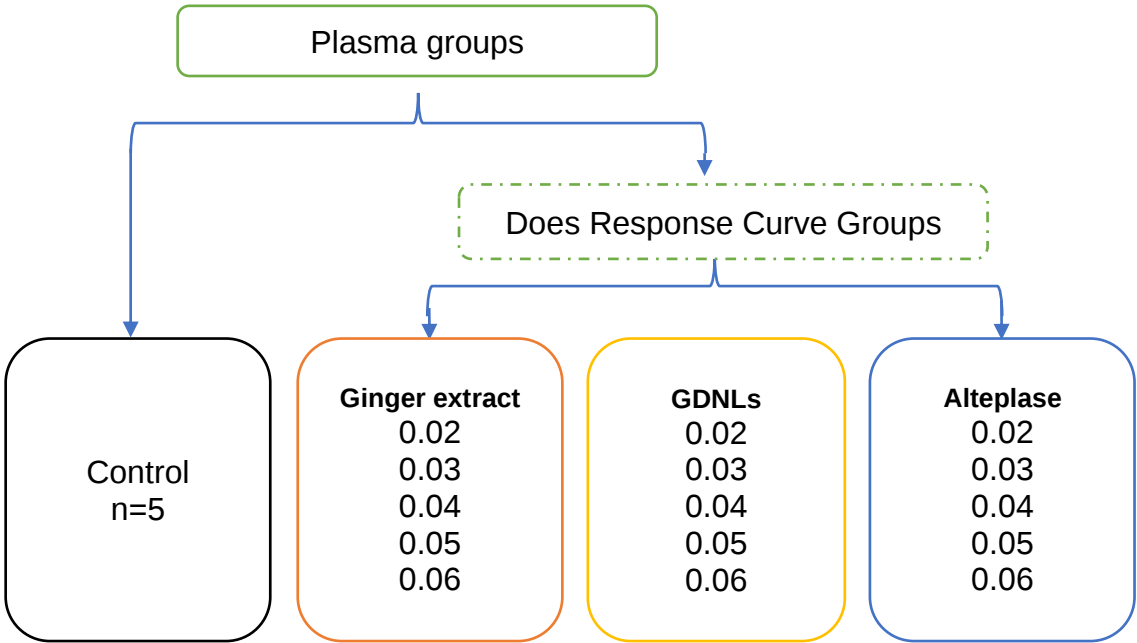


Figure 2: Group design in Vitro platelet for Plasma Poor Platelet (PPP) and plasma poor platelet (PPP) with grouping of treatment with Ginger extract, Ginger derived nanoparticles, Alteplase and positive Control group

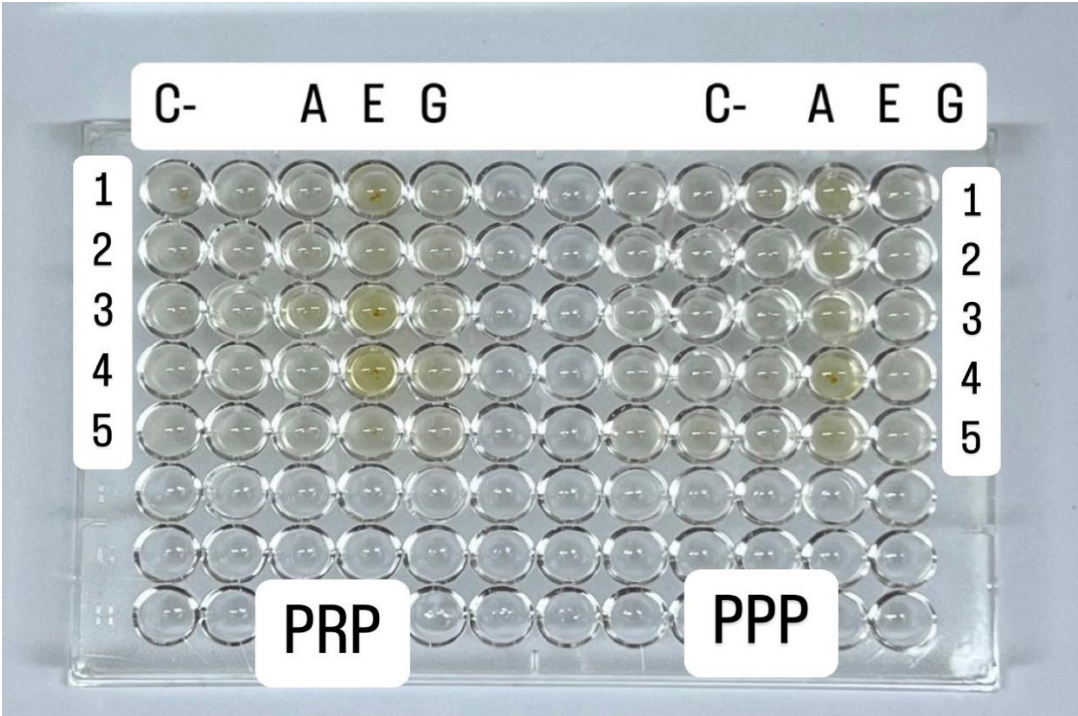


Figure 3: Plasma Poor Platelet (PPP) and plasma poor platelet (PPP) with grouping of treatment with Ginger extract, Ginger derived nanoparticles, Alteplase and positive Control group

Preparation of sample for experiment parameters:
To conduct the experiments for platelet count and fibrin/fibrinogen determination, the sample is divided into two parts:

- **First part for Platelet Count** A specific volume of the sample is allocated for platelet count analysis. This portion is prepared for analysis in autoanalyzer apparatus, a specialized device designed to count platelets accurately.

- **Second part for Fibrin and Fibrinogen Determination** the remaining volume of the sample is used for fibrin and fibrinogen quantification.

Results
Platelet count, fibrin and fibrinogen quantification show in figures 1,2,3,4,5,6 respectively. Platelet count show significant difference between plasma poor platelet and plasma rich platelet that increase in plasma rich platelet and show significant difference between the treatment groups as show in figures 1,2:

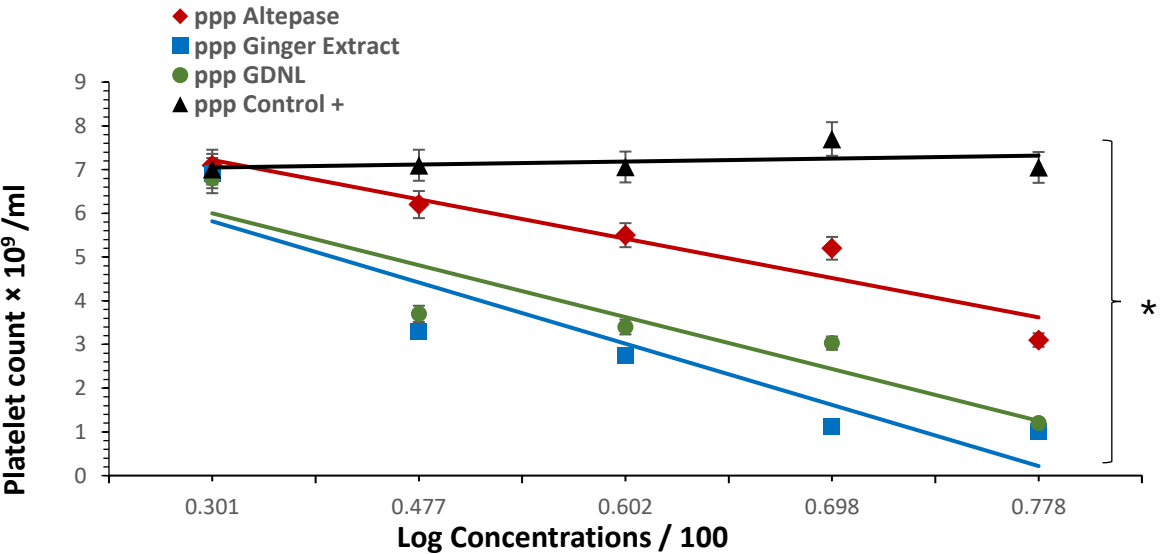


Figure 4: Effect of treatment and type in Platelet count PPP

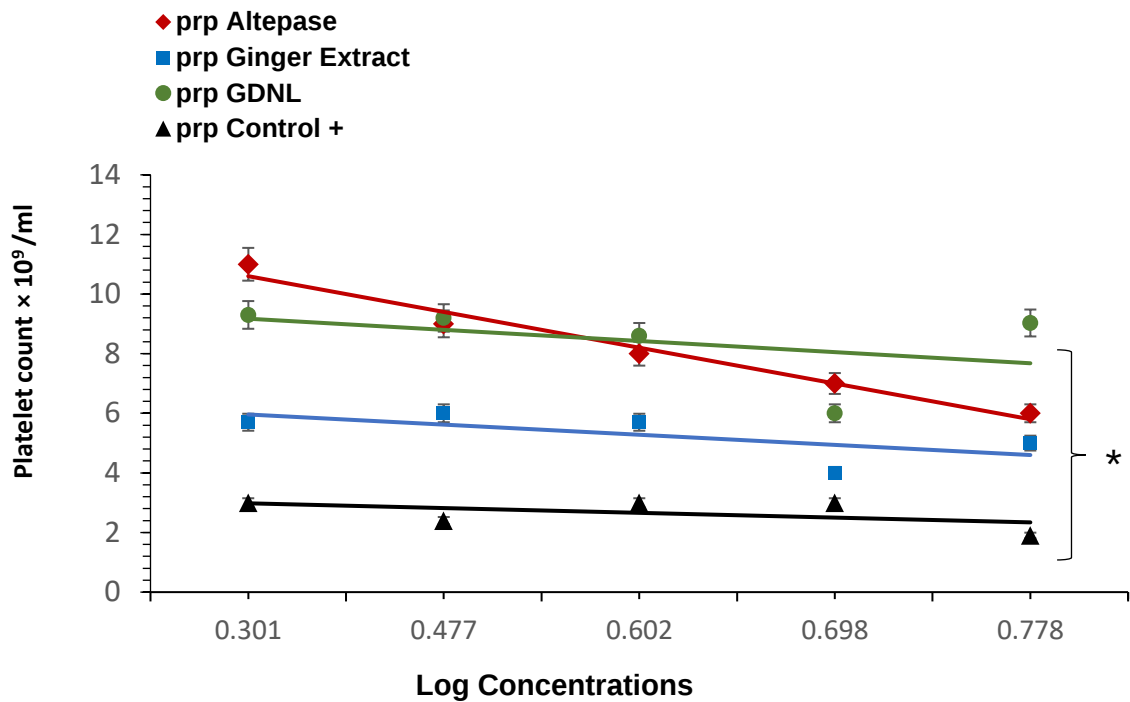


Figure 5: Effect of treatment and type in Platelet count PRP

fibrinogen quantification shows nonsignificant difference between plasma poor platelet and plasma rich platelet and show significant difference between the treatment groups, the alteplase treatment group decrease

more than Ginger derived Nanoparticales and ginger extract groups in fibrinogen quantity as show in figures 3,4:

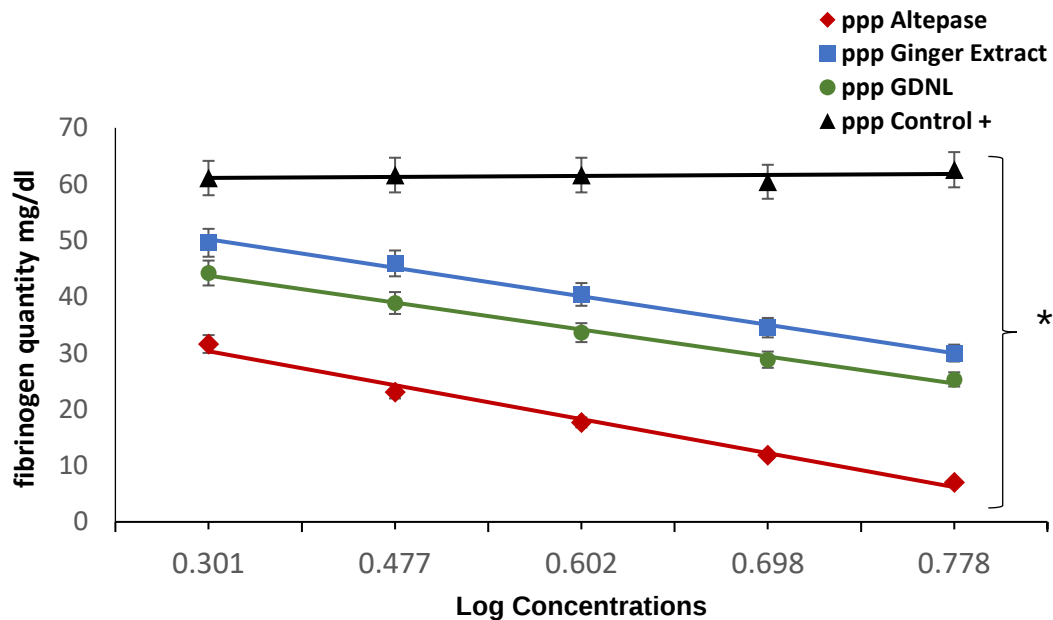


Figure 6: Effect of treatment and type in fibrinogen PPP

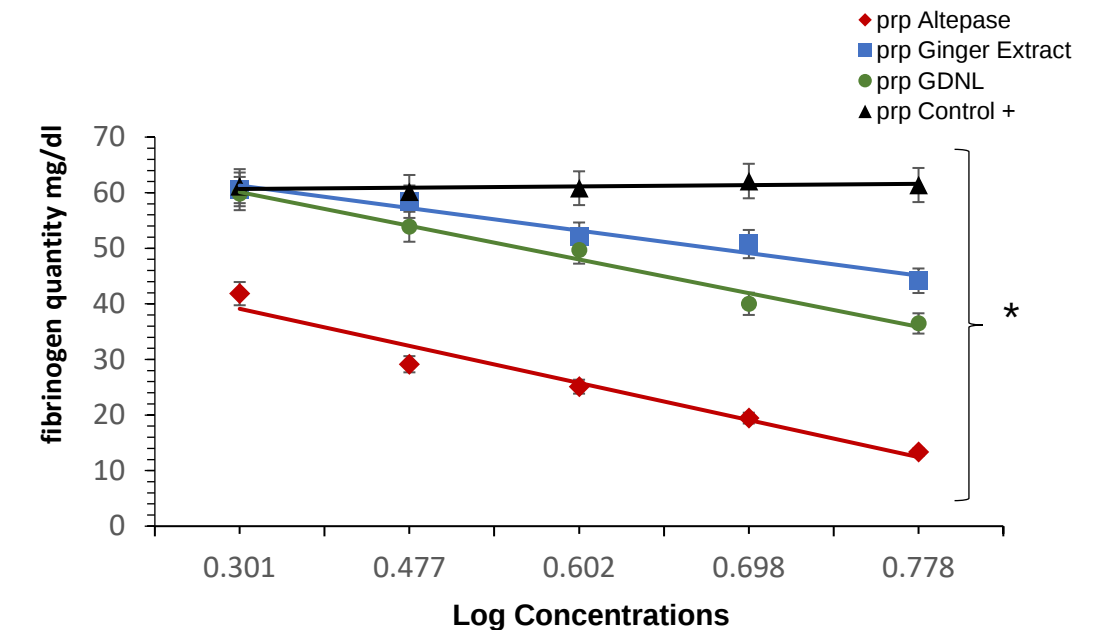


Figure 7: Effect of treatment and type in fibrinogen PRP

Fibrin quantification show nonsignificant difference between plasma poor platelet and plasma rich platelet, while in group of ginger extract show significant difference between plasma poor platelet and plasma rich platelet and show significant difference between the

treatment groups, fibrin quantification shows nonsignificant increase in alteplase treatment group more than Ginger derived Nano-lipids and ginger extract groups in fibrin quantity as show in figures 5,6:

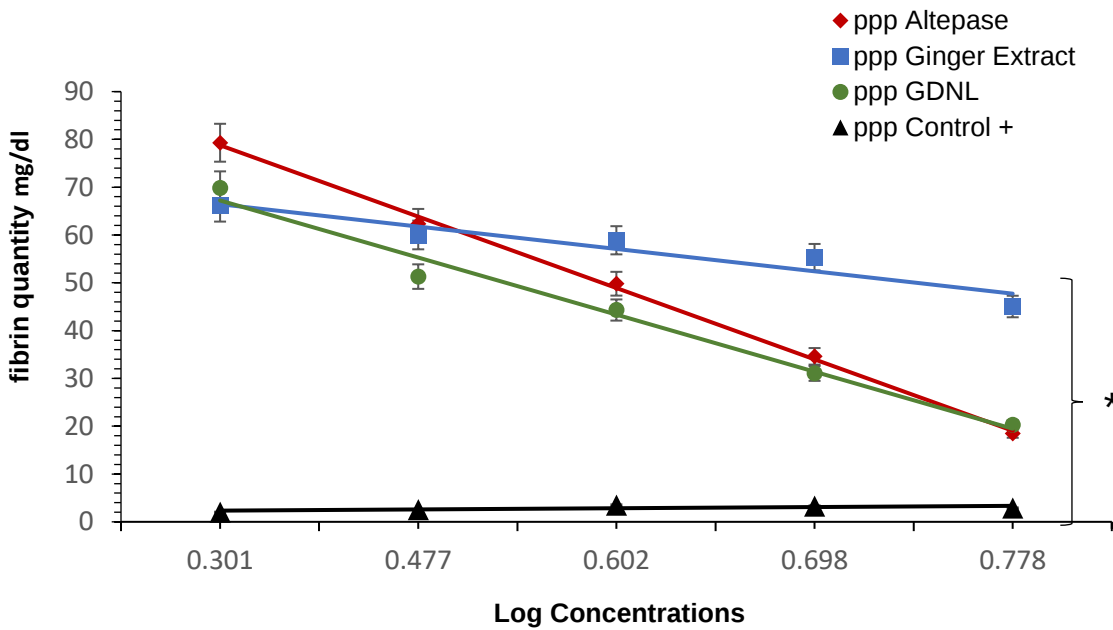


Figure 8: Effect of treatment and type in fibrin PPP

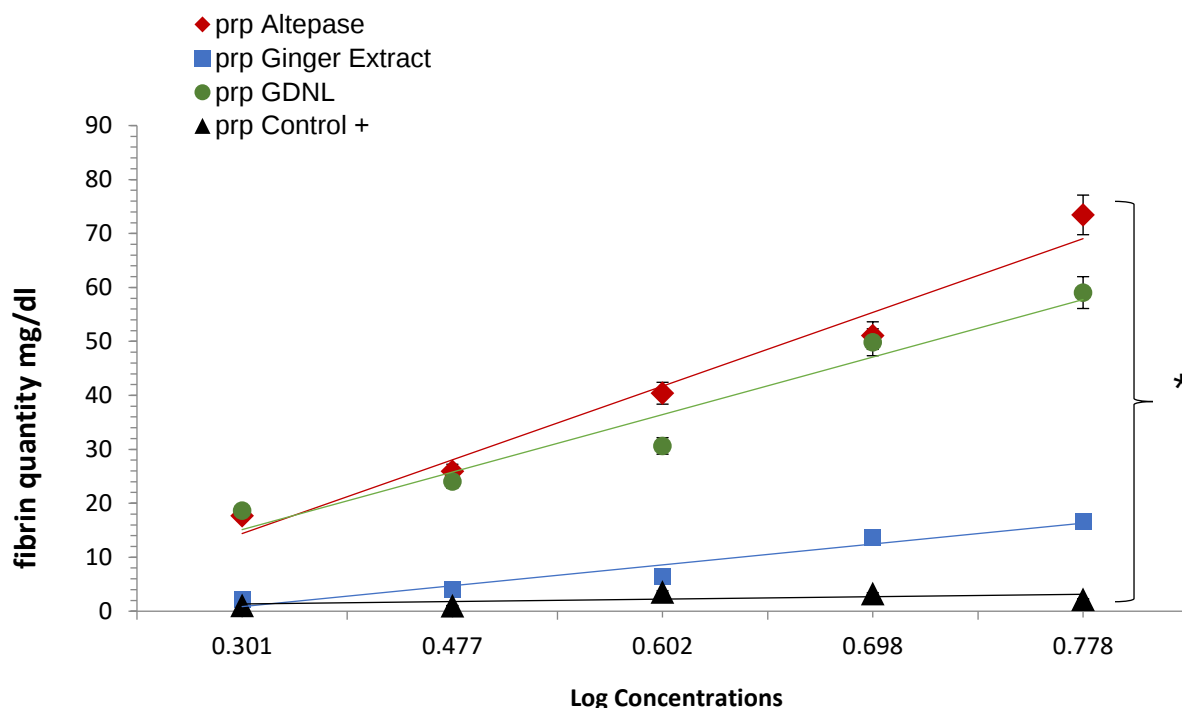


Figure 9: Effect of treatment and type in fibrin PRP

Effect of treatment and type in Platelet count

The data presented in the figures 1,2 evaluates the effect of different ginger extract, Nano-lipids ginger treatments compared with reference Alteplase on platelet count, considering two types of platelet preparations: plasma poor platelet (PPP) and plasma rich platelet (PRP).

Platelet Count in Plasma Poor Platelet (PPP): The control group had a platelet count of 2.20 ± 0.37 , which represents the baseline level of platelet count in the absence of any treatment. **Alteplase:** This treatment significantly increased ($p \leq 0.05$) the platelet count to 5.20 ± 0.66 , with the difference being statistically significant compared to the control.

Ginger Extract: The platelet count for the ginger extract treatment was 3.00 ± 0.63 , which was significantly ($p \leq 0.05$) higher than both the control but lower than Alteplase. **Ginger-derived Nano-lipids:** This treatment showed a platelet count of 2.40 ± 0.74 , which was similar to the control and did not show a statistically significant ($p > 0.05$) difference from them.

Platelet Count in Plasma Rich Platelet (PRP): The platelet count in the PRP control group was 2.00 ± 0.31 , similar to the PPP control group, indicating a baseline platelet level. **Alteplase:** In PRP, Alteplase significantly ($p \leq 0.05$) increased the platelet count to 8.20 ± 0.86 . This result demonstrates that Alteplase has a potent effect on platelet enhancement, especially in PRP preparations.

Ginger Extract: The ginger extract treatment in PRP resulted in a platelet count of 5.20 ± 0.37 , which is significantly higher than the control but lower than Alteplase. **Ginger-derived Nano-lipids:** The treatment with ginger-derived nano-lipids had the highest ($p \leq 0.05$) platelet count in the PRP group, with a mean of 8.40 ± 0.67 . This value was significantly ($p \leq 0.05$) higher compared to all other treatments, making it the most

effective in enhancing platelet count in PRP preparations.

Groups that share the (Alteplase and ginger-derived Nano-lipids and ginger extract in PRP) are significantly ($p \leq 0.05$) different from PPP.

Discussion

The treatments showed varying effects on platelet count, with Alteplase and Ginger-derived Nano-lipids proving to be the most effective in increasing platelet counts, especially in the PRP group. This suggests that these treatments may be more beneficial when using PRP preparations compared to PPP. On the other hand, the Ginger Extract also showed an enhancement in platelet count but was not as potent as Alteplase and ginger-derived Nano-lipids, particularly in PRP preparations.

The analysis indicates that the treatments significantly alter platelet counts depending on the preparation type, with PRP generally exhibiting higher counts than PPP across the board. This is an important finding, as it suggests that the type of platelet preparation (PPP vs. PRP) interacts with the treatment to influence the outcome.

Surprisingly, ginger-derived nano-lipids resulted in the highest platelet count among all treatments in the PRP group, significantly surpassing both alteplase and ginger extract. This suggests that nano-lipid formulation may enhance the platelet-boosting properties of ginger compounds. **Alteplase:** Remains a potent agent for increasing platelet counts in both PPP and PRP preparations. **Ginger Extract:** Shows promise as a natural platelet enhancer, although its effects may be less pronounced than alteplase. **Ginger-Derived Nano-lipids:** Exhibit a remarkable potential for significantly enhancing platelet counts in PRP preparations,

warranting further investigation and potential clinical applications.

Effect of Treatment and Type on Fibrinogen Levels

The following data presents the effect of various treatments on fibrinogen levels in two types of platelet preparations: plasma poor platelet (PPP) and plasma rich platelet (PRP).

Fibrinogen Levels in Plasma Poor Platelet (PPP)

Control: The fibrinogen level in the control group (no treatment) was 61.45 ± 0.35 , which is considered the reference point value for fibrinogen in the PPP preparation. **Alteplase:** as reference treatment with Alteplase significantly ($p \leq 0.05$) lowered the fibrinogen level to 18.25 ± 4.29 , which was the lowest among other treatments and control. The PPP and PRP indicate no significant difference within the Alteplase group.

Ginger Extract: The ginger extract treatment resulted in a fibrinogen level of 40.08 ± 3.58 , which was higher ($p \leq 0.05$) than that of Alteplase but significantly ($p \leq 0.05$) lower than the control. This indicates a moderate reduction in fibrinogen following treatment. **Ginger-Derived Nano-lipids:** The fibrinogen level for the ginger-derived nano-lipids group was 34.17 ± 3.39 , which is higher ($p \leq 0.05$) than both Alteplase and ginger extract but still significantly ($p \leq 0.05$) lower than the control.

Fibrinogen Levels in Plasma Rich Platelet (PRP)

Control: The negative control in PRP showed a fibrinogen level of 61.13 ± 0.31 , which was nearly identical to the PPP control and reflects the baseline fibrinogen value in PRP. **Alteplase:** In the PRP group, Alteplase resulted in a fibrinogen level of 25.77 ± 4.82 , which was significantly ($p \leq 0.05$) lower than the control and indicates a strong inhibitory effect on fibrinogen levels, consistent with its action in PPP.

Ginger Extract: The fibrinogen level with ginger extract was 53.19 ± 2.92 , higher ($p \leq 0.05$) than the level observed with Alteplase, but still significantly ($p \leq 0.05$) lower than the control, suggesting a moderate effect on fibrinogen reduction. **Ginger-Derived Nanoparticles :** This treatment led to a fibrinogen level of 47.98 ± 4.32 , which was the second-highest level after the control, and significantly ($p \leq 0.05$) higher than Alteplase in the PRP group, though lower than the control.

The comparing treatments between PPP and PRP, different in Ginger extract and Ginger-Derived Nano-lipids denote significant Ginger-Derived Nanoparticles differences between the fibrinogen levels in the two platelet preparations for a given treatment as indicated to PPP was lower PRP.

Control: In both PPP and PRP, the positive Control exhibited relatively high fibrinogen levels, as expected, since no fibrinolytic agents were applied. The fibrin levels in the control group were slightly higher in PPP than in PRP, which is consistent with the generally lower protein concentrations found in PPP preparations.

Discussion

Alteplase consistently lowered fibrinogen levels in both PPP and PRP, with the greatest reduction occurring in PPP. Alteplase is a known fibrinolytic agent, and its

ability to decrease fibrinogen aligns with its mechanism of action, which involves the breakdown of fibrin and the promotion of clot dissolution. This suggests that Alteplase may be particularly useful in clinical settings requiring rapid fibrinogen reduction, such as in patients with thrombotic disorders, Alteplase is a recombinant tissue plasminogen activator (rt-PA) used primarily to treat acute ischemic stroke and myocardial infarction (Gurman *et al.*, 2015). It functions by activating the fibrinolytic system, a natural process that dissolves blood clots. Its mechanism of action: Plasminogen Activation: Alteplase binds to fibrin, a protein component of blood clots, This binding enhances the enzyme's ability to convert plasminogen, an inactive precursor, into plasmin. Fibrinolysis: Plasmin is a potent protease that breaks down fibrin, leading to the dissolution of the blood clot. This process, known as fibrinolysis, restores blood flow to the affected tissue (Singh *et al.*, 2023).

Ginger Extract and Ginger-Derived Nano-lipids also resulted in a reduction of fibrinogen levels, though to a lesser extent than Alteplase. Ginger extract, in particular, had a more moderate effect in PRP compared to PPP, suggesting that the type of platelet preparation may influence the treatment's effectiveness. Ginger has been shown in other studies to possess anti-inflammatory and fibrinolytic properties, which may explain its ability to reduce fibrinogen levels (Harapan, 2024). Ginger-derived Nanoparticles, while showing similar effects, appear to be slightly more potent than ginger extract alone, possibly due to the enhanced bioavailability and targeted delivery of the nano-lipid formulation. Ginger, while a potent medicinal herb, does not directly impact the fibrinolytic system in the same way as specific medications like tissue plasminogen activator (t-PA). However, ginger's anti-inflammatory and antioxidant properties may indirectly influence fibrinolysis by:

Reducing Inflammation: Inflammation can impair fibrinolysis by inhibiting the activity of plasminogen activators and promoting the formation of fibrin clots. By reducing inflammation, ginger may indirectly contribute to improved fibrinolytic activity (Tang *et al.*, 2021). **Improving Blood Flow:** Ginger's vasodilatory properties can help improve blood flow, which may enhance the delivery of fibrinolytic factors to blood clots (Fakhri *et al.*, 2021).

Effect of Treatment and Type on Fibrin Levels

The figure presents the results of the effect of various treatments on fibrin levels in two types of platelet preparations: plasma poor platelet (PPP) and plasma rich platelet (PRP).

Fibrin Levels in Plasma Poor Platelet (PPP)

Control: The fibrin level in the PPP positive control group was 2.84 ± 0.26 , which represents the baseline fibrin content in the absence of treatment. This value served as the reference point for comparing the effects of other treatments. **Alteplase:** The treated with Alteplase significantly ($p \leq 0.05$) increased the fibrin level to 48.91 ± 10.56 , which was the highest value among all treatments in PPP. This substantial increase in fibrin levels is consistent with the known mechanism of

action of Alteplase, which promotes the conversion of fibrinogen to fibrin during thrombolysis.

Ginger Extract: Ginger extract resulted in a fibrin level of 40.08 ± 3.58 , which was higher than the control but lower than the Alteplase treatment. This suggests that ginger extract has a moderate effect on fibrin formation in PPP, likely due to its known bioactive compounds with mild fibrinolytic properties.

Ginger-Derived Nanoparticles: The fibrin level for the ginger-derived nano-lipids treatment in PPP was 41.54 ± 7.20 , slightly higher ($p \leq 0.05$) than the ginger extract but still lower than Alteplase.

Fibrin Levels in Plasma Rich Platelet (PRP)

Control: The fibrin level in the PRP negative control group was 2.24 ± 0.53 , which is slightly lower than the PPP control but still within the expected range for baseline fibrin levels in the absence of treatment.

Alteplase: In PRP, Alteplase treatment led to a fibrin level of 41.71 ± 9.80 , which is significantly ($p \leq 0.05$) higher than the control and shows a similar pattern to that observed in PPP.

Ginger Extract: The fibrin level with ginger extract in PRP was 8.59 ± 2.81 , much lower ($p \leq 0.05$) than in both the control and the Alteplase groups. This suggests that ginger extract has a pronounced fibrinolytic effect in PRP, which may be due to its bioactive compounds, such as gingerols, that inhibit fibrin formation.

Ginger-Derived Nanoparticles: The fibrin level in the PRP group treated with ginger-derived Nanoparticles was 36.44 ± 7.73 , which is similar to the level seen in the ginger extract group but significantly higher ($p \leq 0.05$). In the same row (comparing fibrin levels between PPP and PRP for each treatment), different lowercase letters (e.g., *a* and *b*) indicate significant differences in fibrin levels between the two platelet types.

Discussion

Alteplase: As expected, Alteplase showed the most pronounced effect in increasing fibrin levels, particularly in PPP preparations. Alteplase is a recombinant tissue plasminogen activator (rtPA) that works by converting plasminogen into plasmin, which in turn degrades fibrin. This fibrinolytic activity explains the elevated fibrin levels in both PPP and PRP, especially in the PPP preparation, where the increase is more substantial. These findings align with other studies showing Alteplase's powerful fibrinolytic properties (Marder et al., 2009).

Ginger Extract: The Ginger Extract treatment exhibited a marked reduction in fibrin levels in PRP compared to the control, suggesting that ginger has fibrinolytic properties in platelet-rich environments. Ginger's active compounds, such as gingerols and shogaols, have been reported to modulate platelet aggregation and fibrinogen activity (Fakhri et al., 2017). The lower fibrin levels in PRP could be attributed to these bioactive compounds enhancing fibrinogen breakdown or inhibiting its conversion to fibrin.

Ginger-Derived Nanoparticles: The Ginger-Derived Nanoparticles showed a fibrin level between the ginger extract and Alteplase groups. The nano-lipid formulation is designed to improve the bioavailability

and efficacy of ginger's active components. The enhanced activity of nano-lipids in PPP suggests that the nano-encapsulation of ginger's active compounds may improve their ability to modulate fibrin formation and dissolution (Ali et al., 2021). The fact that the effect was more pronounced in PPP than in PRP could be due to the better bioavailability of the nano-lipids in the plasma-poor environment.

Control: In both PPP and PRP, the **positive Control** exhibited relatively low fibrin levels, as expected, since no fibrinolytic agents were applied. The fibrin levels in the control group were slightly higher in PPP than in PRP, which is consistent with the generally higher protein concentrations found in PRP preparations.

Several clinical trials have investigated the effects of ginger on platelet aggregation. Three studies found that ginger can significantly reduce platelet aggregation (Bordia et al., 1997, Young et al., 2006 and Verma et al., 1993). Additionally, one study demonstrated that ginger can inhibit the production of thromboxane B₂, a potent platelet activator (Xia et al., 2019).

(Young et al., 2006) reported and attributed an interesting finding regarding ginger's effect on platelet aggregation. They observed that ginger, when combined with nifedipine, exhibited anti-platelet activity. However, when ginger was administered alone, no significant effect was noted. Nifedipine, a calcium channel blocker, is well known to inhibit intracellular calcium influx, leading to reduced platelet activation and aggregation (Srivastava 1989). Presumably is hypothesized that ginger might potentiate the anti-platelet effect of nifedipine by a synergistic mechanism. Second, (Verma et al., 1993) attributed the effect of ginger on platelet aggregation in individuals consuming a high-calorie diet. The experiment demonstrated that ginger supplementation significantly reduced the diet-induced increase in platelet aggregation compared to a placebo group. However, it is important to note that the high-calorie diet used in this study exceeded normal dietary intake by approximately 1600 kcal/day, as estimated by USDA food data (U.S. Department of Agriculture 2014). That not simulated in vitro impact out comes (Srinivasan et al., 2011 and Marx et al., 2015)

A primary efficient of the experimental in vitro study was reviewed screened is the active entities and metabolomics of the ginger preparations used. The concentration of bioactive compounds, gingerols and shogaols, Zingerone, Paradols can vary significantly depending on factors like the origin, processing, and storage of the ginger (Verma et al., and Schwertner et al., 2006). For instance, 6-shogaol, a potent bioactive compound, is primarily found in dried or heated ginger, whereas fresh ginger contains higher levels of 6-gingerol (Schwertner 2007). These variations in the composition of ginger preparations can influence the results of studies and contribute to the observed inconsistencies.

Ginger-derived nanoplastics may have biocompatibility, antioxidative properties, and potential medicinal uses due to the inherent bioactive compounds of ginger, such as gingerol, shogaol, and zingerone.

The use of such biofunctionalized nanoparticles for medical purposes, including clot lysis, leverages the

therapeutic properties of ginger compounds while enhancing the drug delivery or targeting capabilities of nanotechnology. Clot lysis, or thrombolysis, is a biological process where fibrin clots are dissolved to restore normal blood flow. Enzymes like plasmin, activated from plasminogen, play a critical role in this process (Xia *et al.*, 2019).

Nanotechnology offers significant advantages in this context: **Targeted Drug Delivery:** Nanoparticles can deliver thrombolytic agents like tissue plasminogen activator (tPA) directly to clots and **Bioactivity Enhancement:** Ginger-derived nanoparticles might enhance clot lysis by combining ginger's inherent anti-inflammatory and antioxidative effects with active thrombolytic mechanisms. For example, **gingerol** has shown potential in modulating platelet aggregation and reducing oxidative stress, which might synergize with clot breakdown processes. Ginger's components, when nanoformulated, can influence clot lysis in several ways: **Anti-Thrombotic Effects:** Ginger compounds can inhibit platelet aggregation, preventing excessive clot formation and facilitating natural clot lysis, **Enhanced Plasminogen Activation:** Nanoparticles functionalized with ginger extracts could improve plasminogen activation, speeding up fibrin degradation and **Reduction in Oxidative Stress:** Ginger's antioxidative properties help mitigate oxidative damage around thrombotic sites, improving clot dissolution. (Afzal *et al.*, 2001).

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