Fruitflow attenuates human platelet granule secretion through down-regulating Src/PLCγ2/PKC signaling pathway

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Abstract

Background : Platelet can release lots of active molecules from granules in active state, which contributes to the progress of atherosclerosis and thrombosis. Fruitflow is a water-soluble tomato extract that has been reported to exert protective cardiovascular effects. The present study aims to investigate whether Fruitflow acts on human platelet granule secretion in vitro and further explore its underlying mechanisms.

Methods: Recruit healthy volunteers to collect their peripheral blood and isolate gel-filtered platelets for in vitro experiments. Agonist-induced platelet granule secretion was detected by flow cytometry or ELISA kit. To elucidate the molecular mechanisms, the signaling pathway was tested by western blotting.

Results: Fruitflow inhibited platelet surface expression of CD62P, CD40L, and CD63. Moreover, agonist-induced release of platelet factor 4 (PF4), β-thromboglobulin (β-TG), Regulated on Activation Normal T cell Expressed and Secreted (RANTES), transforming growth factor-β1 (TGF-β1), ATP, Ca 2+ , and serotonin were also significantly attenuated by Fruitflow. Furthermore, Fruitflow down-regulated expression of Lyn and phosphorylation of Src, LAT, Syk, PLCγ2 and PKC. However, PKC inhibitor GF109203X did not show additive effects on platelet granule secretion when combined with Fruitflow.

Conclusion: Fruitflow effectively inhibited platelet granule secretion via down-regulating platelet Src/PLC-γ2/PKC signaling pathway in vitro , which may provide a novel evidence for the cardioprotective benefits of tomato.

Background

Platelets are small, anucleate, versatile cells that contain three specialized secretory organelles, α-granules, dense granules, and lysosomes [1–3]. A large number of active molecules are secreted from platelet granules into the circulation or translocated to the platelet surface after the stimulation of various agonists. These granule contents contribute to the recruitment of circulating leukocytes and platelets to the site of injured endothelium, which play crucial roles in thrombosis and hemostasis and contribute to atherothrombosis [4–6].

Upon platelet activation, the first granules found to secrete their constituents are dense granules, followed by α-granules and lysosomes [7]. Dense granules are found exclusively in platelets, containing extraordinarily high concentrations of non-protein small molecules including ATP, ADP, Ca^{2+}, serotonin and histamine. Once released, these small molecules act back on circulating platelets and contribute to the positive feedback signaling that greatly amplify activation signals [8, 9]. α - granules, the most abundant platelet granule, contain both membrane bound proteins (e.g., CD62P, and CD40L) that are expressed on the platelet surface and soluble proteins (e.g., PF4, β-TG, RANTES, and TGF-β1) that are secreted into the extracellular environment in the platelet active state, which further promote platelet activation and aggregation [10]. The third category of granules involved in the release reaction concern lysosomes. Lysosomes contain many acid hydrolases and cathepsins and express CD63 and lysosome-

associated membrane proteins 2 (LAMP-2) in their membrane, while the function of lysosomes are still not well understood [3, 11].

Platelet granule secretion plays a significant role in the process of thrombus formation and controlling granule secretion is considered as a potential strategy to prevent atherothrombosis [12]. Currently, the signaling pathways leading to platelet granule secretion are not totally clear but are believed that protein kinase C (PKC) and its upstream signaling cascade (Src family kinases, Syk tyrosine kinase, linker for activation of T cells (LAT), and phospholipase C-γ2 (PLC-γ2)) are involved [13–15].

A variety of antiplatelet drugs such as aspirin and clopidogrel can effectively inhibit excessive platelet activation, granule secretion and prevent thrombosis [16–18]. However, studies have shown that these agents have significant side effects, such as increased bleeding risks [19, 20]. It is therefore important to find alternative safe platelet inhibitors to slow or prevent the atherothrombosis. Fruitflow, a water-soluble tomato extract, is the European Food Safety Authorityapproved natural cardioprotective functional ingredient [21]. Previous study have shown that Fruitflow inhibited ADP and collagen-induced healthy human platelet aggregation and GPIIb/IIIa expression in vivo and ex vivo [22]. Besides, in a randomized, double-blinded, placebo-controlled crossover trail in European, platelet aggregation was significantly reduced after supplementation with tomato extract equivalent to 150 mg Fruitflow [23]. Although the effects of Fruitflow on platelet activation and aggregation have been shown in European populations, the effects of Fruitflow on platelet granule secretion and its possible mechanisms have not yet been studied. Our present study therefore aims to investigate the effects of Fruitflow on platelet granule secretion and further explore the underlying mechanisms.

Materials And Methods

Materials

Fruitflow in powder formats was purchased from By-health (Guangdong, China). Thrombin and Fura-2/AM were purchased from Sigma-Aldrich (St Louis, MO, USA). Type I collagen fibrils (equine Horm collagen) and Luciferase-luciferin reagent were purchased from Chrono-log (Havertown, PA, USA). GF109203X was purchased from MedChemExpress (NJ, USA). PE-conjugated mouse anti-human CD40L, CD63 antibody, FITC-conjugated mouse anti-human CD62P antibody, and FITC-conjugated mouse IgG1 isotype control antibody were purchased from BD Biosciences (USA). Goat anti-mouse antibodies to β-actin and goat anti-rabbit antibodies to GAPDH, Lyn, Src, phospho-Src (Ser17), Syk, phospho-Syk (Tyr323), LAT, phospho-LAT (Tyr171), PLC-γ2, phospho-PLCγ2 (Tyr759), phospho-PKC were all purchased from Cell Signaling Technology (Beverly, MA, USA).

Healthy human subject recruitment

Healthy men and women (25–40 years old) who had not taken any antiplatelet agents within the previous two weeks were recruited for the in vitro platelet study. The subjects had no history of serious diseases, such as CVDs, diabetes mellitus, hypertension, hemostatic disorders, or thyroid disorders. This

study was approved in China by the Ethics Committee of Sun Yat-sen University (NO. 2016036) and conformed to the Helsinki Declaration, and all volunteers have signed informed consent.

Human blood collection and gel-filtered platelet preparation

Whole human blood was collected into a Vacutainer tube containing 3.8% sodium citrate (1/9, v/v) via venipuncture and was allowed to rest at 37ºC for 10 min. The blood sample was then centrifuged at 300 g for 7 min at 22ºC to obtain platelet-rich plasma (PRP). Gel-filtered platelets were isolated from PRP with a Sepharose 2B column in PIPES buffer(5 mM PIPES, 1.37 mM NaCl, 4 mM KCl, 0.1% (w/v) glucose, pH7.0) as we previously described [24].

Detection of platelet surface P-selectin, CD40L and CD63 expression by flow cytometric analysis

Gel-filtered platelets (5×10^6 platelets/mL) were incubated with different concentrations of Fruitflow (20, 40, or 80 mg/L) or control buffer (PBS) for 10 min at 37°C. Platelets were labeled with FITC-conjugated anti-human CD62P antibody, PE-conjugated anti-human CD63 antibody, PE-conjugated anti-human CD40L antibody or FITC-conjugated anti-human IgG1 antibody (isotype control) at room temperature for 20 min, followed by the stimulation of thrombin (0.5 U/mL) or collagen (10 μg/mL) for 3 min in the presence of 1 mM Ca^{2+}. Samples were then fixed with 1% paraformaldehyde and analyzed via a CytoFLEX flow cytometer (Beckman Coulter, CA, USA) and data was analyzed by using CytExpert 2.0 (Beckman Coulter, CA, USA).

Measurement of platelet PF4, β-TG, RANTES, TGF-β1 and serotonin secretion

Gel-filtered platelets ($2–3 \times 10^8$ platelets/mL) were initially treated with fruitflow or vehicle control (PBS) for 10 min at 37 °C and were then stimulated by 0.1 U/mL of thrombin in the presence of 1 mM Ca^{2+}, followed by centrifugation at 10,000 × g for 5 min at 4 °C. The cell-free supernatant was transferred into another tube and stored at -80 °C until use. The levels of PF4 (Abcam, UK), β-TG (Abcam, UK), RANTES (Raybiotech, UK), TGF-β1(Raybiotech, UK) and serotonin (IBL, Hamburg, Germany) in platelet supernatant were tested by ELISA kit following the manufacturer's instructions.

Assay of platelet ATP release

The ATP release of gel-filtered platelets was determined in Chrono-log lumi-aggregometer. The platelets ($2–3 \times 10^8$ platelets/mL) were incubated with fruitflow or vehicle control (PBS) for 10 min at 37 °C. Luciferin-luciferase reagent was added directly to platelet suspensions followed by the stimulation of thrombin (0.1 U/mL) or collagen (2 μg/mL) in the presence of 1 mM Ca^{2+}, and then real-time ATP secretion was recorded.

Measurement of platelet Ca^{2+} mobilization

The concentrations of intracellular calcium ion were measured by Fura-2/AM. Briefly, gel-filtered platelets ($2–3 \times 10^8$ platelets/mL) was incubated with 5 μM Fura-2/AM for 60 min at 37 °C. The Fura-2-loaded platelets were preincubated with different concentration of Fruitflow or control buffer (PBS) for 10 min at 37 °C, and then stimulated with thrombin(0.1 U/mL)in the presence of 1 mM Ca^{2+}. Fura-2 fluorescence in the cytosol was measured with a CytoFLEX flow cytometer and the data was analyzed by using CytExpert 2.0.

Lactate dehydrogenase (LDH) release assay

To explore the cell toxicity of Fruitflow, leakage of LDH from platelets was determined by spectrophotometric analysis. In brief, gel-filtered platelets (1×10^7 platelets/mL) were incubated with Fruitflow or solvent control (PBS), LDH activity was determined by using an LDH cytotoxicity detection kit (Beyotime institute of biotechnology, Jiangsu, China) according to the manufacturer's instructions. LDH leakage was expressed as a percentage of total enzyme activity.

Western blotting

Gel-filtered platelets were initially treated with fruitflow or vehicle (PBS) for 10 min at 37 °C followed the stimulation with 0.1 U/mL of thrombin for 5 min in an aggregometer, platelets were then collected and lysed. Western blotting were performed using previously described methods [25–27]. Solubilised platelet protein (20 μg) was separated by sodium dodecyl sulphate polyacrylamide gelelectrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% skim milk power in TBST (20 mM Tris, 137 mM NaCl, and 0.1% (v/v) Tween 20, pH 7.6), then were probed with primary and secondary antibodies respectively. The bands were detected with an enhanced chemiluminescence (ECL) reagent (Thermo Scientific).

Statistical analysis.

SPSS 20.0 software was used for statistical analysis. Data were expressed as mean ± SD of at least three independent experiments. Statistical significance was determined by one-way ANOVA followed by Dunnett's t-test or followed by Bonferroni post hoc analysis was used for multiple comparisons. Differences were considered statistically significant at $P < 0.05$.

Results

Fruitflow inhibits platelet dense granule secretion in vitro

To identify the effects of Fruitflow on platelet dense granule secretion, the gel-filtered platelets were isolated from PRP and incubated with various concentrations of Fruitflow (20, 40, 80 mg/L). ATP, Ca^{2+} and serotonin were the primary dense granule content, we therefore detected the ATP secretion, Ca^{2+} fluxes and serotonin release in gel-filtered platelets. We found that ATP secretion was significantly inhibited dose-dependently by Fruitflow after the stimulation of thrombin(Fig. 1A) and collagen (Fig. 1B).

Moreover, the inhibitory effect of Fruitflow on thrombin-induced Ca^{2+} fluxes was observed (Fig. 1C). Additionally, serotonin release was also suppressed by Fruitflow after thrombin stimulation (Fig. 1D).

Fruitflow attenuates platelet α-granule secretion in vitro

As Fruitflow were found to effectively inhibit platelet dense granule secretion, we further examined its effects on platelet α-granule secretion. We observed that expression of platelet surface CD62P was inhibited by different doses of Fruitflow after thrombin (Fig. 2A) and collagen (Fig. 2B) stimulation, while platelet CD40L expression was attenuated by Fruitflow only at concentrations of 40 and 80 mg/L with the stimulation of thrombin (Fig. 2C) and collagen (Fig. 2D). Furthermore, Fruitflow also attenuated platelet soluble proteins secreted into extracellular matrix induced by thrombin, including PF4 (Fig. 2E), β-TG (Fig. 2F), RANTES (Fig. 2G), TGF-β1 (Fig. 2H).

Fruitflow attenuates platelet lysosome secretion in vitro

CD63, the lysosome membrane glycoprotein, expressed on platelet surface after the the stimulation of various kinds of agonist [28]. To investigate the effects of Fruitflow on platelet lysosome secretion, we detected the CD63 expression on platelet surface by flow cytometry. Compared to the control group, different doses of Fruitflow (20, 40, 80 mg/L) reduced the expression level of CD63 after stimulated by thrombin (Fig. 3A), while CD63 expression induced by collagen was decreased by Fruitflow only at the concentrations of 40 and 80 mg/L, there is no significant effect at 20 mg/L (Fig. 3B).

Fruitflow has no cytotoxicity to platelets

Cytotoxicity of Fruitflow were evaluated by cytosolic LDH leakage. The gel-filtered platelets were incubated with various concentrations of Fruitflow (0, 20, 40, 80 mg/L) for 40 min at room temperature. Compared to the resting platelet, the platelet LDH leakage at 20, 40, 80 mg/L have no statistic difference (P□0.05)(Fig. 4). These findings demonstrated that Fruitflow did not affect platelet cytolysis or permeability.

Fruitflow downregulates platelet Src/PLCγ2/PKC signalling

We have identified the inhibitory effects of Fruitflow on the platelet granule secretion, and further investigated the potential intracellular signalling targets in platelets. Src family members, PLCγ2, PKC have been proved to play an important role in platelet granule secretion [15, 29]. In our study, we found that Fruitflow (40, 80 mg/L) significantly suppressed thrombin-induced platelet Lyn expression (Fig. 5A). A marked reduction of phosphorylated Syk (Fig. 5D) and PLCγ2 (Fig. E) were also exhibited in 40 and 80 mg/L doses of Fruitflow. Furthermore, In comparison with the control, Fruitflow significantly decreased the phosphorylation of Src (Fig. 5B), LAT (Fig. 5C) and PKC (Fig. 5F) at all three doses (20, 40, 80 mg/L). These data indicated that the inhibitory effects of Fruitflow on platelet granule secretion might be related to a concomitant inhibition of Src/PLCγ2/PKC signalling.

Fruitflow inhibits platelet granule secretion involving PKC signalling

As our study demonstrated Fruitflow downregulated platelet PKC signalling, we further identified whether the reduction of platelet granule secretion is related to PKC signaling pathway. As shown in Fig. 6A-6D, both Fruitflow (80 mg/L) and GF109203X (a highly selective PKC inhibitor) significantly inhibited the release of PF4, β-TG, RANTES, TGF-β1. However, we did not find any significant additive effects of Fruitflow on platelet α-granule secretion when combined with GF109203X. Similar to α-granule secretion, both Fruitflow and GF109203X alone effectively reduced platelet ATP release (Fig. 6E) and CD63 expression (Fig. 6F), and shown no synergetic effects when used in combination. These data suggested that PKC signalling pathway inhibition maybe one of the major mechanisms underlying the inhibition of Fruitflow on platelet granule secretion.

Discussion

A number of epidemiological studies have shown that tomato consumption was positively correlated with cardioprotection [30]. Some studies suggested that the cardioprotective effects of tomatoes may arise in part due to lycopene [31]. However, other studies have reported that dietary lycopene is not associated with the risk of cardiovascular disease (CVDs) [32]. Therefore, there may be unidentified components in tomatoes that possess cardiovascular protective effects. In vitro studies have found that platelet aggregation was inhibited after the treatment of tomato juice, the main components of its anti-platelet function are water-soluble [33]. In order to further clarify the protective effect of non-fat-soluble components in tomatoes on cardiovascular system, Fruitflow was obtained by physically removing fat-soluble components from tomatoes [22]. Fruitflow was shown to possess the inhibitory effects on platelet aggregation and platelet glycoprotein IIb/IIIa expression in vitro and in vivo [22, 23]. However, the effects of Fruitflow on platelet granule secretion have not yet been investigated. Our present study demonstrated that Fruitflow inhibited the thrombin/collagen-induced platelet α-granule, dense granule, and lysosome secretion in human gel-filtered platelets in vitro. To the best of our knowledge, this study provides the first link between tomato extract Fruitflow and platelet granule secretion.

Platelet granule secretion was activated after the stimulation of different agonists, e.g., thrombin and ADP. Collagen is a common sub-endothelial matrix protein that is exposed to platelets when the vessel endothelium is damaged [34], thrombin is a physiological platelet agonist that exist in the circulation [35]. Collagen and thrombin can bind to the platelet surface receptors GPVI and PARs, respectively, which initiates the activation of sarcoma tyrosine-protein kinase (Src) family members Lyn, Fyn and Src, and further phosphorylates Syk [14, 36]. Activation of Syk, in turn, promotes an intracellular signaling cascade that eventually leads to phosphorylation and activation of the LAT [37], resulting in the phosphorylation of PLCγ2 [38]. PLCγ2 then acts as an adapter protein for PKC, which finally leading to the activation of downstream effectors and contribute to the platelet granule secretion [39]. In order to clarify the mechanism of Fruitflow inhibiting platelet granule release, we investigated the activation of

Src/PLCγ2/PKC signaling pathway. Our immunoblotting results showed that Fruitflow markedly inhibited Lyn expression and Src, Syk, LAT, PLCγ2, PKC phosphorylation. Moreover, we showed that GF109203X, a PKC inhibitor, has no significant additive effects on thrombin-induced platelet granule secretion when combined with Fruitflow, suggesting that PKC signaling pathway inhibition is probably one of the major mechanisms underlying the inhibition of Fruitflow on platelet granule secretion.

A lot of natural bioactive compounds exhibited platelet protection and each possesses multiple targets with pleiotropic and synergistic effects. For example, anthocyanins attenuated platelet activation and aggregation involving the signaling pathways of NF-κB, GPVI, PI3K/Akt, MAPK et al [40–42]; polyphenols improved the platelet function by suppressing ROS, PLCγ2/PKC, AMPK signaling pathways [43, 44]; Curcumin inhibited platelet reactivity through inhibition of thromboxane formation, Ca^{2+} and GPVI signaling [45, 46]. In our study, we only found Fruitflow may affect platelet granule secretion through platelet Src/PLCγ2/PKC signaling pathway, whether platelet granule release affected by Fruitflow through other signaling pathways remains unknown. We therefore need to explore multiple mechanisms that Fruitflow act on platelet granule release in our future study.

According to the HPLC results, Fruitflow contains three main active ingredients including nucleoside derivatives, phenolic conjugates, and flavonoid derivatives, the most representative among the three components are adenosine, chlorogenic acid, rutin respectively [21]. The three substances can be used to quantify and evaluate the quality of Fruitflow and ensure that Fruitflow is as close to fresh tomatoes as possible. A large numerous of studies have already confirmed that nucleoside, polyphenols, flavonoid possessed the effects of platelet protection and improved platelet activation, adhesion, aggregation [47–49]. It is therefore reasonable to believe that Fruitflow's effect on platelet granule secretion may be due to these three components, which components play the most important role remains to be further studied in the future.

Fruitflow is now authorized by EFSA for daily consumption with 150 mg in the format of powder, tablet or capsule. According to the results of pharmacokinetics, the maximum theoretical circulating concentration after ingestion of a single 150 mg dose of Fruitflow is 43 mg/L [21], we therefore used the dose of 20, 40, 80 mg/L in vitro experiment. These doses did not exert cytotoxic effects on platelets as measured by cytosolic LDH leakage (Fig. 4). As Fruitflow possessed a significant anti-platelet ability, care was taken to determine whether the intrinsic or extrinsic clotting pathways could be effected by Fruitflow alongside antiplatelet effects. Large numbers of trials proved that Fruitflow does not directly affect prothrombin time (PT) and thrombin clotting time (TCT) at any dose tested [50, 51]. Unlike many anti-platelet drugs, which irreversibly inhibit platelet function and affected the life span of circulating platelets, the antiplatelet effects of Fruitflow are not irreversible, or cumulative, and does not bring extra bleeding risks [21]. In summary, Fruitflow is safe and reliable as a food supplement.

Conclusion

Taken together, Fruitflow significantly inhibited platelet α-granule, dense granule, and lysosome secretion in a concentration-dependent manner. Furthermore, Fruitflow effectively inhibited platelet granule secretion via inhibiting platelet Src/PLC-γ2/PKC signaling pathway, which provides a novel mechanism as to why tomato is a kind of important protective food for cardiovascular diseases.

Abbreviations

PF4
platelet factor 4; β-TG:β-thromboglobulin; RANTES:Regulated on Activation Normal T cell Expressed and Secreted; TGF-β1:transforming growth factor-β1; PKC:protein kinase C; LAT:linker for activation of T cells; PLCγ2:phospholipase C γ2; LDH:Lactate dehydrogenase.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DF have carried out experiments with the assistance from XM; XG and SW were involved in the analysis of data; DF, FY, ZT and YS wrote the manuscript; WL and FS revised and edited the draft manuscript; YY participated in the study design and had primary responsibility for the final content. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

Consent for publication

All authors provide consent for publication of this paper.

Ethical approval and consent to participate

The study was approved by the ethics committee of Sun Yat-sen University, and all volunteers have signed informed consent.

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Figures

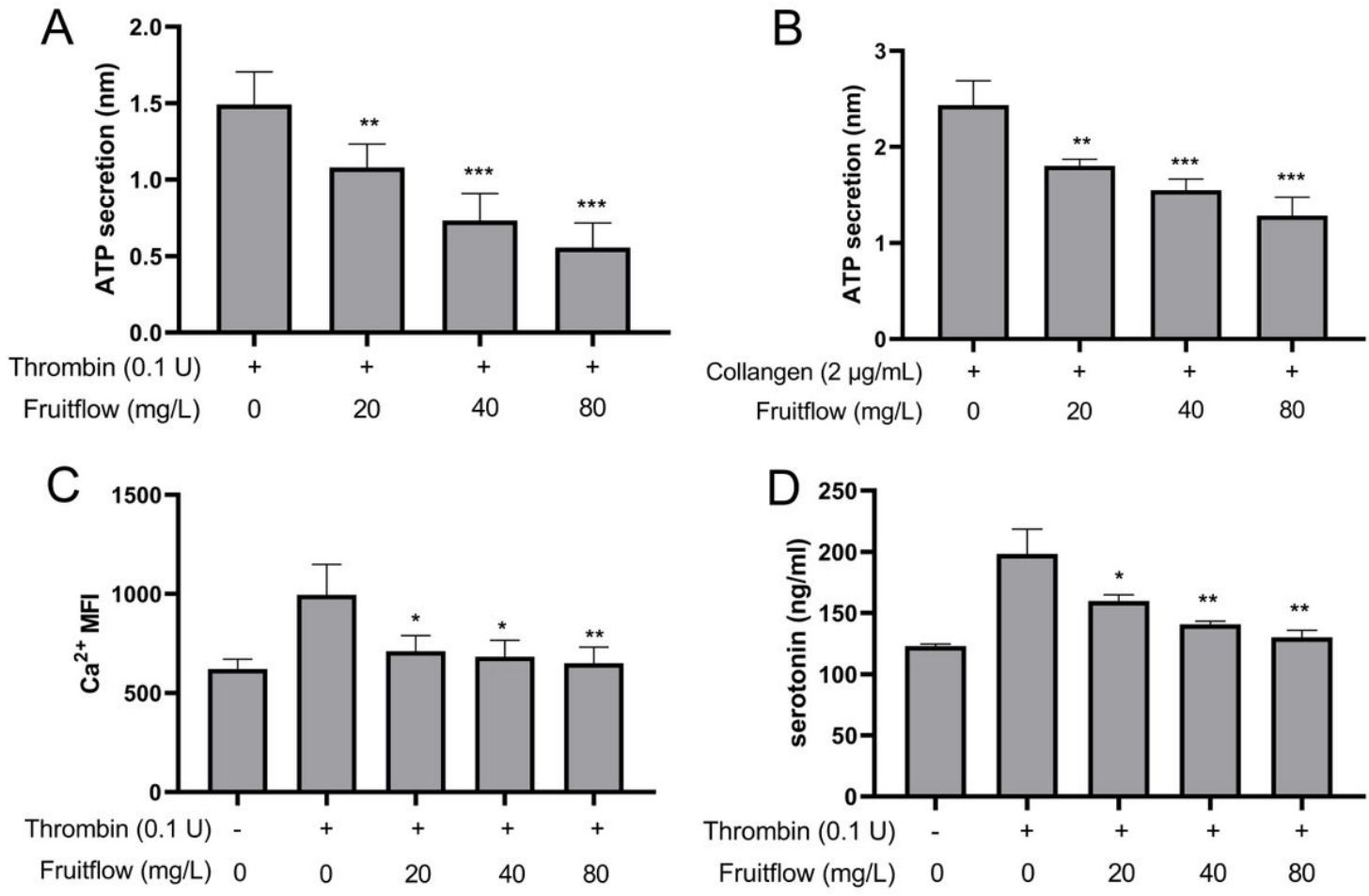

Figure 1

Fruitflow inhibits platelet dense granule secretion in vitro Gel-filtered platelets from healthy subjects were pre-incubated with various concentrations (20, 40, or 80 mg/L) of Fruitflow or solvent control for 10 min before stimulation with agonist. ATP secretion from platelet dense granules was determined in a Chrono-log lumiaggregometer and expressed as nmol/mL. Ca2+ concentration in platelets were measured by flow cytometry, Serotonin secretion were tested with commercial ELISA kits. ATP secretion were attenuated by Fruitflow after the stimulation of A) 0.1 U thrombin and B) 2 µg/mL collagen. 0.1 U Thrombin-induced C) Ca2+ fluxes and D) Serotonin secretion were attenuated by Fruitflow. Data are presented as mean ± SD (n=5). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus Control as assessed by ANOVA followed by Dunnett's t-test.

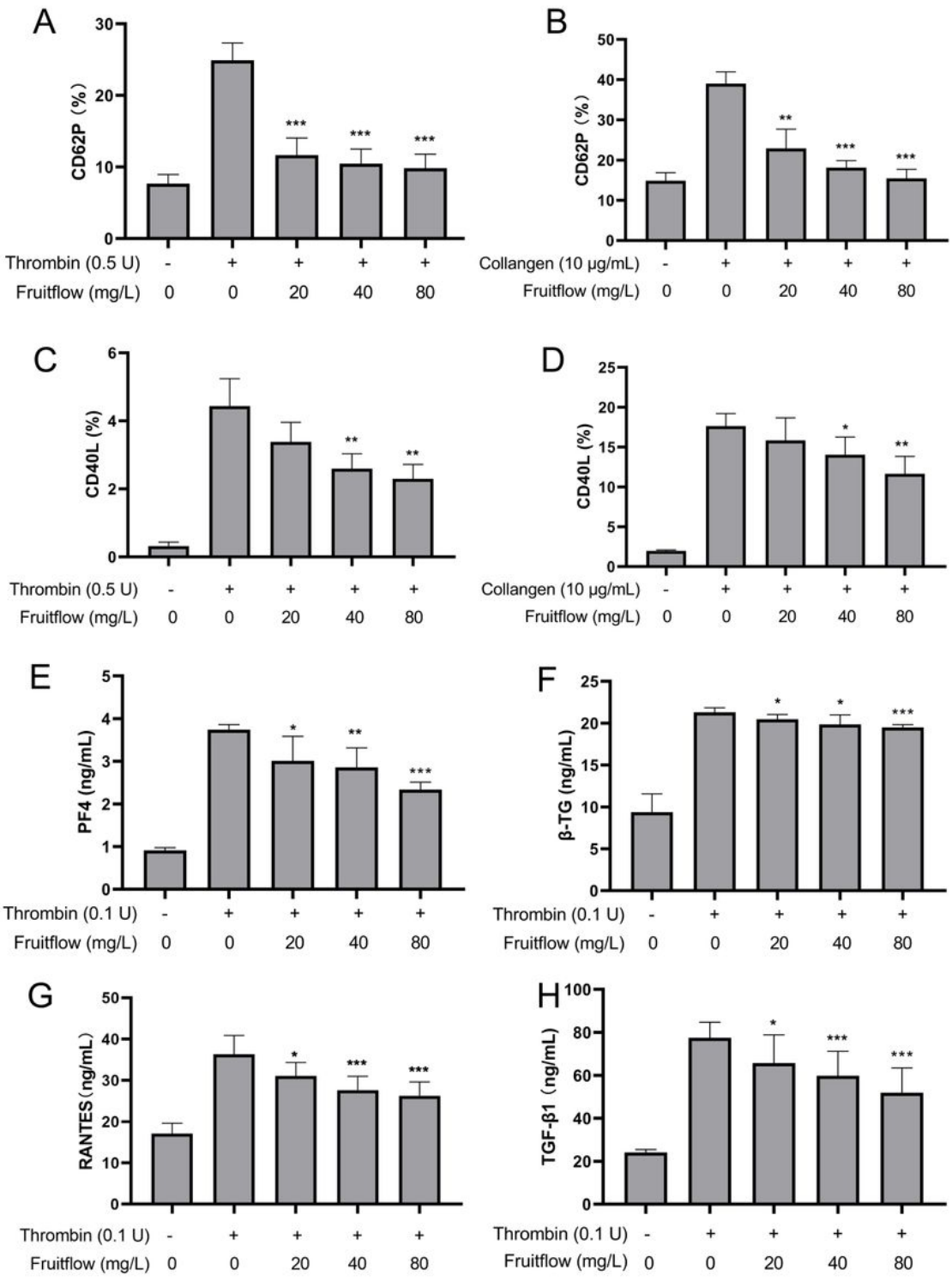

Figure 2

Fruitflow attenuates platelet α-granule secretion in vitro Gel-filtered platelets from healthy subjects were pre-incubated with various concentrations (20, 40, or 80 mg/L) of Fruitflow or solvent control for 10 min before stimulation with thrombin or collagen. P-selectin and CD40L expression on platelets were measured by flow cytometry. PF4, β-TG, RANTES and TGF-β1 were tested with commercial ELISA kits. P-selectin expression on platelets was inhibited by Fruitflow after the stimulation of A) 0.5 U thrombin and

B) 10 μg/mL collagen. CD40L expression on platelets was reduced by Fruitflow after the stimulation of C) 0.5 U thrombin and D) 10 μg/mL collagen. E) PF4, F) β-TG, G) RANTES and H) TGF-β1 secretion in platelets were inhibited by Fruitflow after induced by 0.1U thrombin. Data are presented as mean ± SD (n=5). * P < 0.05, ** P < 0.01 and *** P < 0.001 versus Control as assessed by ANOVA followed by Dunnett's t-test.

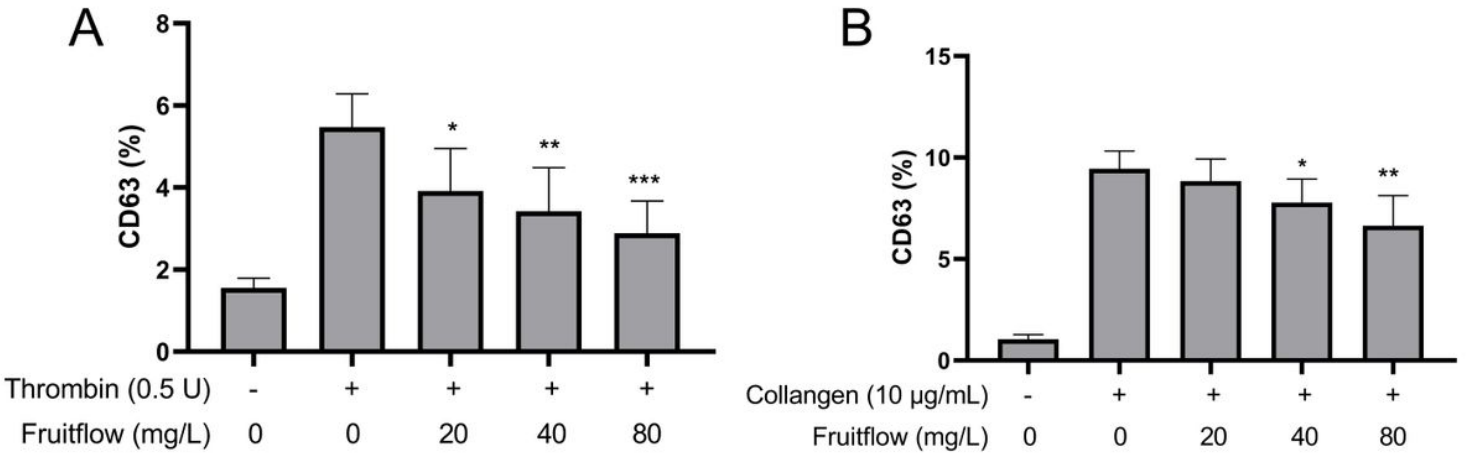

Figure 3

Fruitflow attenuates platelet lysosome secretion in vitro Isolated platelets were pretreated with with various concentrations (20, 40, or 80 mg/L) of Fruitflow or solvent control for 10 min and activated with thrombin or collagen. CD63 expression on platelets were tested by flow cytometry, Fruitflow reduced CD63 expression on platelets after the stimulation of A) 0.5 U thrombin and B) 10 μg/mL collagen. Data are presented as mean ± SD (n=5). * P < 0.05, ** P < 0.01 and *** P < 0.001 versus Control as assessed by ANOVA followed by Dunnett's t-test.

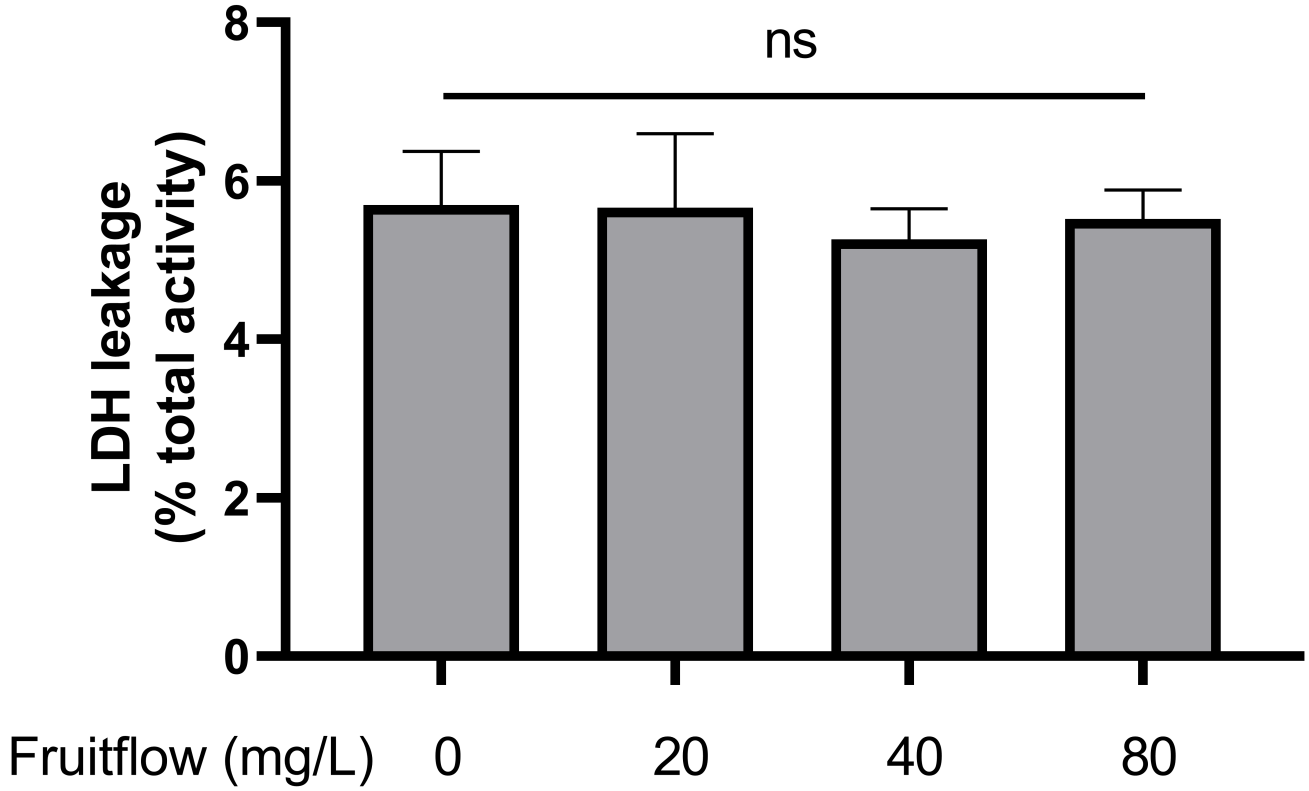

Figure 4

The cytotoxicity of Fruitflow on platelet The gel-filtered Platelets were pre-incubated with Fruitflow or solvent control (PBS) and cytotoxicity was determined by the leakage of LDH. LDH leakage was expressed as % of total enzyme activity (n=5). Fruitflow has no effects on LDH leakage in platelets (P >0.05).

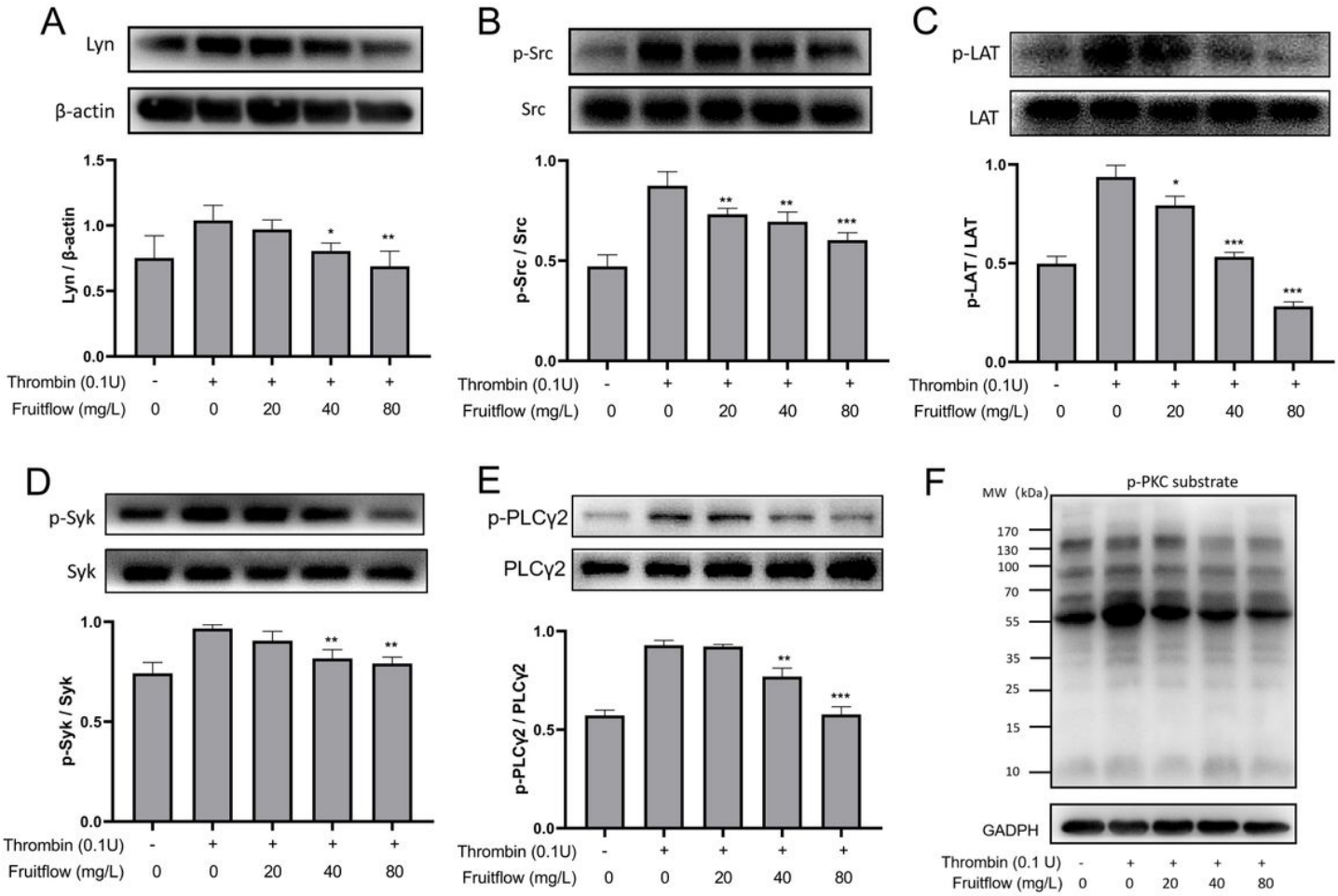

Figure 5

Fruitflow downregulates platelet Src/PLCγ2/PKC signalling Gel-filtered platelets from healthy subjects were pre-incubated with various concentrations (20, 40, or 80 mg/L) of Fruitflow or solvent control for 10 min before stimulation with 0.1 U thrombin. The collected aggregated platelet lysates were separated by SDS-PAGE, and immunoblotted to detect the expression of Lyn and phosphorylation levels of Src, LAT, Syk, PLCγ2, PKC. Fruitflow decreased he expression of A) Lyn, reduced the phosphorylation levels of B) Src, C) LAT, D) Syk, E) PLCγ2, F) PKC. Data are presented as mean ± SD (n=5). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus Control as assessed by ANOVA followed by Dunnett's t-test.

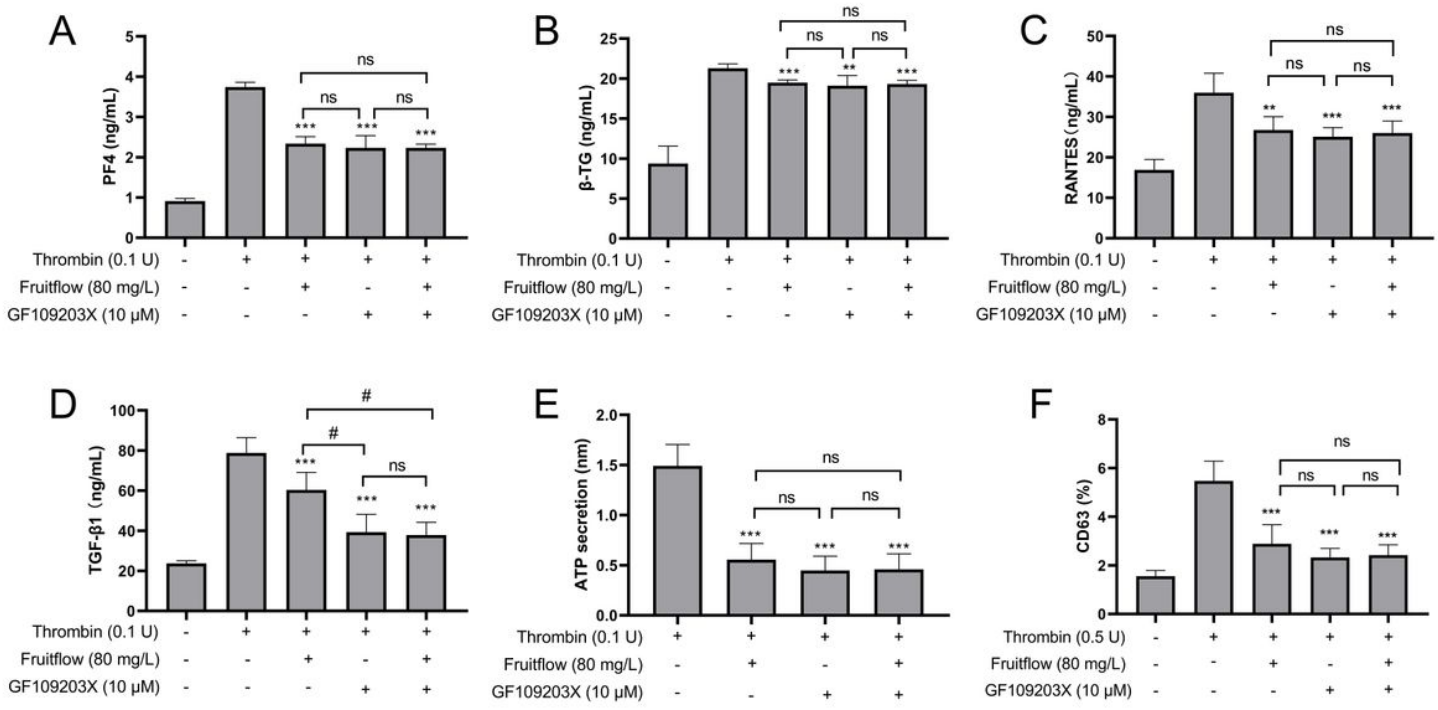

Figure 6

Fruitflow inhibits platelet granule secretion involving PKC signalling Gel-filtered platelets were pre-incubated with Fruitflow (80mg/L) for 10 min or GF109203X (10 μM) for 30 min respectively, or platelets were pre-incubated with GF109203X before the Fruitflow treatment. Fruitflow inhibited A) PF4, B) β-TG, C) RANTES, D) TGF-β1, E) ATP secretion and F) CD63 expression on platelets in the presence of GF109203X. Data are presented as mean ± SD (n=5). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus Control as assessed by ANOVA followed by Dunnett's t-test. # $P < 0.05$ as analyzed by by ANOVA followed by Bonferroni post hoc analysis. ns, not significant difference.