

Inhibitory effect of Fruitflow on platelet function: a randomized placebo-controlled trial in elderly subjects

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Abstract

Background: The elderly has a high risk of cardiovascular disease, which is often accompanied by platelet hyperactivity. Tomato extracts can inhibit platelet activation and have beneficial health effects. We aimed to investigate the effect of Fruitflow® (FF), a water-soluble tomato extract, on platelet function in elderly participants.

Methods: This randomized group study was conducted with people over 50 years old. The participants were randomly divided into four groups: placebo (150 mg/day), FF (150 mg/day), acetylsalicylic acid (ASA; 100 mg/day), and FF (150 mg/day) + ASA (100 mg/day). These groups received the respective supplements after dinner daily for 7 days. Fasting blood was collected from the participants on days 0 and 8 to analyze platelet aggregation and the content of thromboxane B₂ (TXB₂), 6-keto-prostaglandin F_{1α}, and platelet factor 4 (PF4).

Results: One hundred ninety elderly participants were recruited and completed this clinical trial. The results showed that the FF intervention for 7 days decreased platelet aggregation by 7.7% in adenosine diphosphate-stimulated platelets, which was similar to the effect of ASA, which decreased platelet aggregation by 9.4%. FF reduced platelet aggregation by 10.2% in collagen-stimulated platelets, and ASA reduced platelet aggregation by 38.3% in collagen-activated platelets. This suggests that ASA exerts a stronger inhibitory effect than FF on collagen-stimulated platelet aggregation. The combination of FF + ASA did not exert a synergistic inhibitory effect on platelet aggregation. Treatment with FF significantly decreased plasma TXB₂, 6-keto-PGF_{1α}, and PF4 levels, and its effects were similar to ASA.

Conclusion: Fruitflow® suppressed platelet aggregation and decreased TXB₂, 6-keto-PGF_{1α}, and PF4 levels in elderly participants. These findings indicate that FF might reduce the risk of thrombosis in cardiovascular diseases.

Trial registration code: ChiCTR2000034647 at www.clinicaltrials.gov.

1. Introduction

Cardiovascular disease is associated with the highest rates of morbidity and mortality worldwide[1]. Elderly individuals usually have the highest risk of cardiovascular events [2, 3]. Age-related vascular inflammation and platelet hyperactivity are linked to cardiovascular events [4, 5]. Platelet hyperactivity has been associated with several chronic diseases, including hypertension, diabetes, and hyperlipidemia [6-8]. Platelet activation is involved in thrombosis and promotes chronic inflammation and vascular injury in atherosclerosis. Antiplatelet therapy is considered to effectively reduce the risk of cardiovascular events in these patients [9]. Acetylsalicylic acid (ASA; aspirin) is a classic antiplatelet medication that has been used clinically for decades, but its side effects are associated with a risk of bleeding and gastrointestinal damage after long-term intake [10-12]. Therefore, the discovery of new antiplatelet therapies with fewer side effects is still necessary.

Nutritional interventions may be promising treatment alternatives. Some foods have obvious health benefits, such as reducing the incidence of cardiovascular disease in countries with a Mediterranean diet [13-16]. Studies have shown that tomatoes exert protective effects on the cardiovascular system, and the active ingredients of tomatoes can inhibit platelet function [17, 18]. FF is a water-soluble tomato extract that mainly contains flavone, adenosine, and chlorogenic acid [19, 20]. FF was first product in Europe to obtain an approved, proprietary health claim under Article 13(5) of the European Health Claims Regulation 1924/2006 on nutrition and health claims made on foods [17]. The ingredients of FF were safe for human consumption [21-23]. O’Kennedy et. reported that FF have similar effect on inhibition of platelet function compared with aspirin, but the effect is weaker than aspirin. A water-soluble tomato extract was also reported to inhibit angiotensin-converting enzyme and relax the vascular endothelium [24].

The elderly has a high risk of cardiovascular disease that is associated with platelet hyperactivity and the dysfunction of endothelial cells [25]. Whether the FF intervention can improve platelet function in the elderly requires further investigation. The present study used randomized grouping and placebo- and ASA-controlled assessments in elderly participants to evaluate the effects of FF on platelet function.

2. Materials And Methods

2.1. Participant

In the current trial, we included the participants with the age over 50 years old, with no medical history of serious disease, hemostatic disorders, or intolerance to ASA. The platelet aggregation rate in these participants reached higher than 50% in ADP- and collagen-stimulated platelets. The exclusion criteria were blood pressure $\geq 160/100$ mmHg, platelet count $< 100 \times 10^9/L$, prothrombin time outside the normal reference range, the chronic use of medications that may affect platelet function and dietary supplements (e.g., fish oil and evening primrose oil), allergy to tomatoes or their ingredients, intolerance or allergy to ASA, contraindications for ASA, and positive fecal occult blood test. Moreover, according to the researchers’ judgment, individuals with serious hematological diseases, digestive diseases, organic diseases of the heart, liver, or kidneys, severe liver or kidney dysfunction, or metabolic, endocrine, or nervous system diseases were also excluded. Eligible participants were recruited from February to December 2019. Participants undergone clinical examination on days 0 and 8. A flowchart of the recruitment procedure and disposition of the participants is shown in Fig. 1.

2.2. Sample size calculation

On the basis of the sample size formula suggested for randomized clinical trials and by considering type I error of 5% ($\alpha = 0.05$), type II error of 10% ($\beta = 0.10$, power =90%), and allowing for a 20 % drop-out rate, we determined a sample size of 40 individuals in each group. To get a more confident result, we intended to recruit 100 participants for each group. However, we temporarily terminated the trial after the recruitment of 50 participants in each group because of COVID-19 crisis.

2.3. Ethical consideration

The present study was conducted according to the principles of the Declaration of Helsinki. All procedures that involved human participants were approved by the Ethics Committee of Beijing Hospital (no. 2018BJYYEC-195-02). Written informed consent was obtained from all of the participants. The study was retrospectively registered at the Chinese Clinical Trial Registry (www.clinicaltrials.gov; registration no. ChiCTR2000034647).

2.4. Study supplements and materials

Fruitflow®(FF) and placebo were provided by By-Health Co., Ltd (Zhuhai, Guangdong, China) in tablet formats. Each FF tablet contained 150 mg of water-soluble tomato extract. The main biological ingredients of FF are flavone, adenosine, and chlorogenic acid (total > 8 mg/g). Each aspirin tablet contained 100 mg ASA (Bayer, purchased from Beijing Hospital, Beijing, China). The packages of FF and placebo tablets were same in the terms of color, appearance, and taste and were given to the participants at the study baseline.

Collagen and adenosine diphosphate (ADP) were purchased from Chrono-Log (Havertown, PA, USA). The human PF4 enzyme-linked immunosorbent assay (ELISA) kit, TXB₂ ELISA kit, and 6-keto-PGF_{1α} ELISA kit were purchased from Abcam (Boston, MA, USA).

2.5. Intervention

All study activities were carried out in the Beijing Hospital, Beijing, China. The study followed a randomized placebo-controlled trial. The participants were randomly allocated in a manner of 1:1:1:1 to four groups: placebo (150 mg/day), FF (150 mg/day), ASA (100 mg/day), and FF (150 mg/day) + ASA (100 mg/day). Simple randomization sequence was created using SPSS 25.0 (Armonk, NY, USA) statistical software. The participants in the placebo group did not know the aim of the study. The participants were asked to take their respective intervention after dinner daily for 7 days. The participants were asked to record their intervention form every day and to ensure their adherence in the present study. The duration of the interventions was based on O’Kennedy, who showed that the FF intervention for 7 days is sufficient to evaluate its effectiveness [17]. The primary outcomes were post-supplement changes in platelet aggregation response to agonist, changes in TXB₂, 6-keto-prostaglandin F_{1α}(6-keto-PGF_{1α}) and platelet factor 4 (PF4) generation by platelets. Secondary outcomes included post-supplement changes in plasma clotting times and fecal occult blood test. Outcome variables were assessed at the study baseline and end of the trial (days 0 and 8). Fasting blood from participants was collected by a second researcher who was unaware of assignment of the groups.

2.6. Platelet aggregation rate measurement

Venous blood was collected from the participants in the placebo, FF, ASA, and FF + ASA groups pre-intervention and post-intervention. The blood samples were immediately mixed with 3.2% sodium citrate.

The blood samples were centrifuged at 200 ´ g for 10 min to obtain platelet-rich plasma and then centrifuged again at 1500 ´ g for 10 min to obtain platelet-poor plasma. Platelet-rich plasma (300 µl) was used to analyze platelet aggregation using a Chrono-Log aggregometer. Platelet-rich plasma was set in the platelet aggregation assay channel with stirring for 1 min, and then ADP (2 µM) or collagen (2 µg/ml) was added to induce platelet aggregation. The rate of platelet aggregation was recorded using a Chrono-Log aggregometer.

2.7. Assessment of TXB₂, 6-keto-PGF1α and PF4

Venous blood was collected from the participants on days 0 and 8. After centrifugation at 1500 ´ g for 15 min, plasma was collected and stored at -80°C. The levels of TXB₂, 6-keto-PGF1α, and PF4 were determined using ELISA kits (Abcam, Boston, MA, USA) according to the manufacturer's instructions.

2.8. Biochemical determination

Biochemical testing was performed using a LABOSPECT 008 AS automatic biochemical analyzer (Hitachi, Tokyo, Japan).

2.9. Coagulometry and Fecal occult blood test

Prothrombin time, fibrinogen, activated partial thromboplastin time, and thrombin time were measured using an ACL-TOP-700 coagulometer (Werfen, Barcelona, Spain). Fecal occult blood testing using tetramethylbenzidine (Wan Hua Pu Man Biological Engineering Co., Ltd., Beijing, China).

2.10. Assessment of other variables

Required information on age, gender, having a history of other diseases, medication, body mass index (BMI), and supplement use was collected using a researcher-made questionnaire. BMI was calculated as weight in kilograms divided by height in square meters (kg/m²). Height was measured in a standing position without shoes. Weight was determined with minimal clothing and without shoes by an analog scale.

2.11. Statistical analysis

The data are expressed as mean ± SD. All of the variables were tested for a normal distribution using the Kolmogorov-Smirnov test. Two-tailed paired Student's *t*-test was used to compare platelet aggregation and TXB₂, 6-keto-PGF1α, and PF4 levels between pre- and post-intervention. The Kruskal-Wallis H test was used to analyze differences between groups. The statistical analyses were performed using SPSS 25.0 software (Armonk, NY, USA). Values of *p* < 0.05 were considered statistically significant.

3. Results

3.1. Subject characteristics and routine examination

The baseline characteristics of the 190 participants are presented in Table 1. Blood pressure in participants with hypertension was not higher than 160/100 mmHg. All of the smokers smoked no more than 5 cigarettes per day. A small number of participants had hypertension and diabetes and were taking related medications, but the platelet aggregation rate in these participants was higher than 50%, suggesting that platelet function in these participants was unaffected by their medications. No significant difference was found between groups in the baseline characteristics.

Plasma lipid levels, fasting plasma glucose (FPG), high-sensitivity C-reactive protein (HsCRP), and homocysteine (HCY) were evaluated pre-intervention and post-intervention. As shown in Table 2, no changes in plasma lipid levels, FPG, HsCRP, or HCY were found between pre- and post-intervention in any of the groups. Moreover, no significant differences were found in the biochemical testing indicators between groups.

Fecal occult blood and coagulation were evaluated pre- and post-intervention. As shown in Table 3, no changes in fecal occult blood were found between pre- and post-intervention in any of the groups. The coagulation tests **indicated that** prothrombin time, fibrinogen, activated partial thromboplastin time, and thrombin time were not different between pre- and post-intervention in any of the groups. None of these parameters were significantly different between groups or between pre- and post-intervention. Therefore, short-term (7 days) interventions with FF and ASA did not find bleeding trend.

3.2. Effect of FF on ADP- and collagen-induced platelet aggregation

To determine the effect of FF on platelet aggregation *in vivo*, blood was collected from the participants in the placebo, FF, ASA, and FF + ASA groups. The platelet aggregation rate was compared between pre- and post-intervention for 7 days. Platelet aggregation was induced by ADP (2 mM) and collagen (2 mg/ml). As shown in Fig. 2A, placebo treatment for 7 days did not alter the aggregation rate. The aggregation rate that was induced by ADP was $76.2\% \pm 16.6\%$ and $76.2\% \pm 13.4\%$ pre- and post-intervention, respectively. Treatment with FF (150 mg/day) for 7 days decreased the rate of ADP-induced platelet aggregation by 7.7%, and the aggregation rate was $73.7\% \pm 13.8\%$ and $66.0\% \pm 16.3\%$ pre- and post-intervention, respectively. A significant difference was found between pre- and post-intervention in the FF group ($p < 0.01$). A similar trend was found over 7 days in the ASA group, in which the rate of ADP-induced platelet aggregation decreased by 9.4%, and the aggregation rate was $74.3\% \pm 14.9\%$ and $64.9\% \pm 18.9\%$ pre- and post-intervention, respectively ($p < 0.01$). We then determined whether FF and ASA exert synergistic inhibitory effects on ADP-stimulated platelet aggregation. Treatment with the combination of FF + ASA for 7 days decreased the rate of ADP-induced platelet aggregation by 9.3% ($72.8\% \pm 16.5\%$ and $63.5\% \pm 19.7\%$ pre- and post-intervention, respectively; $p < 0.01$). We analyzed differences in platelet aggregation between the placebo, FF, ASA, and FF + ASA groups. No difference was found between groups pre-intervention. Post-intervention, a difference was found between the FF, ASA, and FF + ASA groups and placebo group. Treatment with the combination of FF + ASA did not exert a synergistic inhibitory effect on ADP-induced platelet aggregation.

We also evaluated the effects of placebo, FF, ASA, and FF + ASA on collagen-induced platelet aggregation. As shown in Fig. 2B, the rate of platelet aggregation did not change between pre- and post-intervention in the placebo group ($80.4\% \pm 16.0\%$ pre-intervention vs. $81.4\% \pm 13.3\%$ post-intervention). Treatment with FF for 7 days decreased collagen-induced platelet aggregation by 10.2% ($79.1\% \pm 12.4\%$ pre-intervention vs. $68.9\% \pm 16.5\%$ post-intervention; $p < 0.001$). Treatment with ASA decreased the rate of platelet aggregation by 39% ($74.3\% \pm 14.9\%$ pre-intervention vs. $35.3\% \pm 26.8\%$ post-intervention; $p < 0.001$). Treatment with FF + ASA decreased the rate of platelet aggregation by 38.3% ($75.3\% \pm 15.3\%$ pre-intervention vs. $37.0\% \pm 29.9\%$ post-intervention; $p < 0.001$). No differences were found between the four groups before treatment, whereas significant differences were found between the FF, ASA, and FF + ASA groups and the placebo group after treatment. Moreover, no difference in platelet aggregation was found between the ASA group and FF + ASA group. This suggests that FF did not enhance the inhibitory effect of ASA on collagen-induced platelet aggregation.

3.3. Effect of FF on TXB₂ and 6-keto-PGF_{1α} levels

To investigate the effect of FF on TXB₂ and 6-keto-PGF_{1α} production, we measured plasma TXB₂ and 6-keto-PGF_{1α} levels using ELISA kits. As shown in Fig. 3A, TXB₂ levels were not different between pre-intervention and post-intervention in the placebo group (799.5 ± 82.9 pg/ml vs. 835.5 ± 81.6 pg/ml, respectively; $p > 0.05$). In contrast, TXB₂ levels were significantly lower pre-intervention in the FF group after 7 days. TXB₂ content was 738.5 ± 70.4 pg/ml and 579.3 ± 37.1 pg/ml pre- and post-intervention, respectively ($p < 0.01$). TXB₂ levels also decreased post-intervention in the ASA group after 7 days (777.4 ± 93.1 pg/ml vs. 542.7 ± 55.6 pg/ml pre- and post-intervention, respectively; $p < 0.01$). Similarly, TXB₂ levels decreased post-intervention in the FF + ASA group after 7 days (704.1 ± 70.5 pg/ml vs. 531.6 ± 47.3 pg/ml pre- and post-intervention, respectively; $p < 0.01$). As shown in Fig. 3B, the analysis between groups showed no significant difference between groups pre-intervention. Post-intervention, the FF, ASA, and FF + ASA groups were significantly different from the placebo group. No significant difference was found between the FF, ASA, and FF + ASA groups.

As shown in Fig. 3B, 6-keto-PGF_{1α} levels were not different between pre- and post-intervention in the placebo group (1053.1 ± 111.2 pg/ml vs. 1164.1 ± 132.7 pg/ml, respectively; $p > 0.05$). In contrast, 6-keto-PGF_{1α} levels were significantly lower post-intervention in the FF group after 7 days. The levels of 6-keto-PGF_{1α} were 956.1 ± 84 pg/ml and 701.8 ± 68 pg/ml pre- and post-intervention, respectively ($p < 0.001$). 6-Keto-PGF_{1α} levels also decreased post-intervention in the ASA group after 7 days (970.2 ± 104.1 pg/ml vs. 743.1 ± 88.7 pg/ml pre- and post-intervention, respectively; $p < 0.001$). Similarly, 6-keto-PGF_{1α} levels decreased post-intervention in the FF + ASA group after 7 days (920.4 ± 97.5 pg/ml and 739.3 ± 95.7 pg/ml pre- and post-intervention, respectively; $p < 0.001$). No significant difference was found between groups pre-intervention. The FF, ASA, and FF + ASA groups were significantly different from the placebo group post-treatment.

3.4. Effect of FF on plasma PF4 levels

We investigated the effect of FF on PF4 levels, which is released from α -granules of platelets. Plasma PF4 levels were detected using an ELISA kit. As shown in Fig. 4A, PF4 levels did not significantly change in the placebo group between pre- and post-intervention. PF4 levels were 1012.2 ± 98.8 ng/ml and 984.7 ± 93.8 ng/ml in the placebo group pre- and post-intervention, respectively. Post-intervention, PF4 levels significantly decreased in the FF group. PF4 levels were 1028.0 ± 70.1 ng/ml and 656.9 ± 54.3 ng/ml pre- and post-intervention, respectively ($p < 0.001$). PF4 levels also decreased in the ASA group post-intervention. Before and after treatment with ASA, PF4 levels were 963.8 ± 73.6 ng/ml and 658.8 ± 35.4 ng/ml, respectively ($p < 0.001$). PF4 levels were 1003.5 ± 84.0 ng/ml and 638.9 ± 46.1 ng/ml in the FF + ASA group pre- and post-intervention, respectively ($p < 0.001$). Before the intervention, PF4 levels were not significantly different between groups. After the intervention, significant differences were found between the FF, ASA, and FF + ASA groups and the placebo group.

4. Discussion

Nutritional interventions can provide beneficial protection for health. This strategy has been widely accepted in the field of disease prevention. In the present study, we first investigated the effects of FF in a sample of elderly individuals in China. We conducted a randomized, placebo-controlled trial to evaluate the inhibitory effect of FF on platelet function in aging individuals and compared the effects of FF with ASA and the combination of FF + ASA. Our results showed that FF (150 mg/day) reduced ADP-induced platelet aggregation after the intervention, and the effect was compared with ASA (100 mg/day). The ASA group exhibited more potent suppression of collagen-stimulated platelet aggregation, and the effectiveness of ASA was four-times stronger than FF. However, the results did not show that FF acted synergistically with ASA to inhibit platelet aggregation. This suggests that the mechanism of the inhibitory effect of FF on platelet aggregation might be distinct from ASA. Moreover, our results showed that treatment with 150 mg/day FF or 100 mg/day ASA for 7 days significantly reduced TXB₂ and 6-keto-PGF_{1 α} levels.

TXB₂ is a stable product of the platelet aggregation mediator TXA₂. 6-Keto-PGF_{1 α} is a stable product of the endogenous cyclooxygenase metabolite prostaglandin I₂. Both TXB₂ and 6-keto-PGF_{1 α} are metabolites of arachidonic acid. TXB₂ levels positively correlate with cardiovascular disease and thrombosis, whereas 6-keto-PGF_{1 α} plays a negative role in anti-platelet activation [26, 27]. Aspirin inhibits platelet function by inhibiting the arachidonic acid-metabolizing enzyme cyclooxygenase-1. Interestingly, although FF and ASA had different abilities in inhibiting collagen-stimulated platelet aggregation, FF effectively reduced plasma TXB₂ and 6-keto-PGF_{1 α} levels post-intervention, and the effectiveness of FF was the same as ASA. This suggests that FF might reduce the risk of thrombosis by lowering TXB₂ levels, but the mechanism of action of FF on TXB₂ is still unclear. A previous study reported that FF had no cumulative effect on cyclooxygenase-1 in platelets [19].

PF4, also referred to as CXCL4, belongs to the chemokine family. It is stored in α -granules of platelets and is the most abundant protein in α -granules. PF4 is a pleiotropic inflammatory chemokine that has been

implicated in various inflammatory diseases, including atherosclerosis [28]. Endothelial cell damage occurs in early stages of atherosclerosis. PF4 promotes the local vascular inflammatory response by recruiting inflammatory cells to damaged endothelial cells. Our previous study found that PF4 levels significantly increased in apolipoprotein E-deficient mice, which is a model of atherosclerosis [29]. The present results showed that PF4 levels were higher before the intervention in elderly individuals, and treatment with FF, ASA, and FF + ASA decreased PF4 levels. This indicates that FF may reduce PF4 levels to improve the inflammatory state in the elderly body.

Epidemiological studies have found that the Mediterranean diet is beneficial for reducing the risk of cardiovascular disease [30, 31]. Tomatoes are the main vegetable component in the Mediterranean diet. A European study reported that a daily intake of 65 mg FF can also partially inhibit platelet aggregation in health participants [17]. The present results are consistent with this previous study. Tomato extracts include various beneficial ingredients, such as lycopene, which has been considered to protect against benign prostatic hyperplasia [32]. This suggests that a tomato extract can provide various beneficial biological effects.

One limitation of the present study was the relatively short intervention time (i.e., 7 days). The side effects of ASA may only appear only after months or longer. Unknown is whether long-term FF administration has undesirable side effects in humans.

Conclusion

The present study found that FF inhibited platelet aggregation and reduced TXB₂, 6-keto-PGF_{1α}, and PF4 levels in elderly participants. The combination of FF + ASA did not affect coagulation. Overall, the present results suggest that FF may be a suitable nutritional intervention strategy to reduce the risk of cardiovascular disease.

Abbreviations

FF: Fruitflow®; ASA: acetylsalicylic acid (aspirin); TXB₂: thromboxane B₂; 6-keto-PGF_{1α}: 6-keto-prostaglandin F_{1α}; ADP: adenosine diphosphate; PF4: platelet factor 4; FPG: fasting plasma glucose; HsCRP: high sensitivity C reactive protein; HCY: homocysteine.

Declarations

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Authors' contributions

QRM designed the experiments. CH, ZS, BL, WH, and WW performed the experiments. CH and JY analyzed the data. QRM wrote the manuscript. All of the authors read and approved the final manuscript.

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Availability of data and materials

The datasets that were used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Beijing Hospital (no. 2018BJYYEC-195-02). Written informed consent was obtained from all of the participants in the present study.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare no conflicts of interest.

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Tables

Table 1 Baseline characteristics of the subjects

Variable	Placebo (n=46)	FF (n=49)	ASA (n=48)	FF + ASA (n=47)
Age (years)	60.8 ± 4.3	59.0 ± 4.6	60.6 ± 5.6	60.4 ± 5.9
BMI	25.3 ± 3.3	25.0 ± 2.6	25.9 ± 4.9	24.9 ± 2.8
SBP (mmHg)	132.1 ± 12.0	127.0 ± 15.6	130.7 ± 15.2	128.6 ± 13.8
DBP (mmHg)	82.7 ± 6.8	80.2 ± 7.8	81.4 ± 7.6	80.8 ± 6.8
Females	84.8% (39)	89.8% (44)	81.2% (39)	95.7% (45)
Diabetes	13.0% (6)	4.1% (2)	10.4% (5)	8.5% (4)
Hypertension	17.4% (8)	12.2% (6)	18.8% (9)	6.4% (3)
Smoking	6.5% (3)	0.0% (0)	4.2% (2)	6.4% (3)
Medication	26.1% (12)	12.2% (6)	27.1% (13)	14.9% (7)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. There was no difference between groups.

Table 2 Biochemical test in the subjects pre- and post-intervention

Variable	Placebo (n=46)		FF (n=49)		ASA (n=48)		FF+ASA (n=47)	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
TG (mmol/L)	1.7 ± 1.7	1.6 ± 1.1	1.5 ± 1.0	1.6 ± 1.1	1.5 ± 0.9	1.4 ± 0.7	1.4 ± 0.7	1.5 ± 0.7
TC (mmol/L)	4.9 ± 0.8	4.9 ± 0.8	5.1 ± 0.9	5.2 ± 0.8	5.2 ± 1.1	5.2 ± 1.1	5.2 ± 1.1	5.1 ± 0.9
LDL-C (mmol/L)	3.3 ± 0.8	3.4 ± 0.9	3.4 ± 0.9	3.4 ± 0.7	3.4 ± 1.1	3.5 ± 1.0	3.5 ± 0.9	3.4 ± 0.8
HDL-C (mmol/L)	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.3	1.3 ± 0.3
FPG (mmol/L)	6.0 ± 1.4	5.9 ± 1.3	6.1 ± 1.7	5.9 ± 1.9	5.9 ± 1.4	5.8 ± 1.4	6.1 ± 2.3	6.2 ± 2.4
HsCRP (mg/L)	1.9 ± 2.5	1.5 ± 0.8	2.0 ± 2.8	1.6 ± 1.5	1.3 ± 1.5	1.3 ± 1.6	1.2 ± 1.2	1.1 ± 0.9
HCY (mmol/L)	11.0 ± 3.7	11.6 ± 4.5	10.9 ± 6.4	11.3 ± 6.9	12.3 ± 8.1	12.2 ± 6.8	12.4 ± 8.4	12.4 ± 7.5

TG: triglyceride; TC: total cholesterol; LDL-C low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; FPG: fasting plasma glucose; HsCRP: high sensitivity C reactive protein; HCY: homocysteine. There was no statistical difference between the groups before and after the intervention, and no difference was found between the groups.

Table 3 Fecal occult blood and coagulation assay in the subjects pre- and post-intervention

Variable	Placebo (n=46)		FF (n=49)		ASA (n=48)		FF + ASA (n=47)	
	Pre- intervention	Post- intervention	Pre- intervention	Post- intervention	Pre- intervention	Post- intervention	Pre- intervention	Post- intervention
Positive FOB	0	0	0	0	0	0	0	0
PT (s)	10.6 ± 0.5	10.6 ± 0.4	10.6 ± 0.5	10.5 ± 0.6	10.6 ± 0.6	10.9 ± 1.3	10.6 ± 0.5	10.6 ± 0.6
FIB (g/L)	3.1 ± 0.5	3.1 ± 0.4	3.1 ± 0.5	3.1 ± 0.5	3.0 ± 0.4	3.0 ± 0.4	3.1 ± 0.4	3.1 ± 0.4
APTT (s)	32.5 ± 2.9	32.0 ± 3.0	33.1 ± 3.8	32.4 ± 3.3	33.3 ± 4.1	33.0 ± 4.1	32.8 ± 3.2	32.7 ± 3.4
TT (s)	13.5 ± 1.1	13.6 ± 0.9	13.6 ± 0.9	13.7 ± 0.9	13.5 ± 0.9	13.6 ± 0.8	13.6 ± 0.8	13.8 ± 0.9

FOB: fecal occult blood; PT: prothrombin time; FIB: fibrinogen; APTT: activated partial thromboplastin time; TT: thrombin time. No significant difference in FOB and coagulation assay between pre-intervention and post-intervention.

Figures

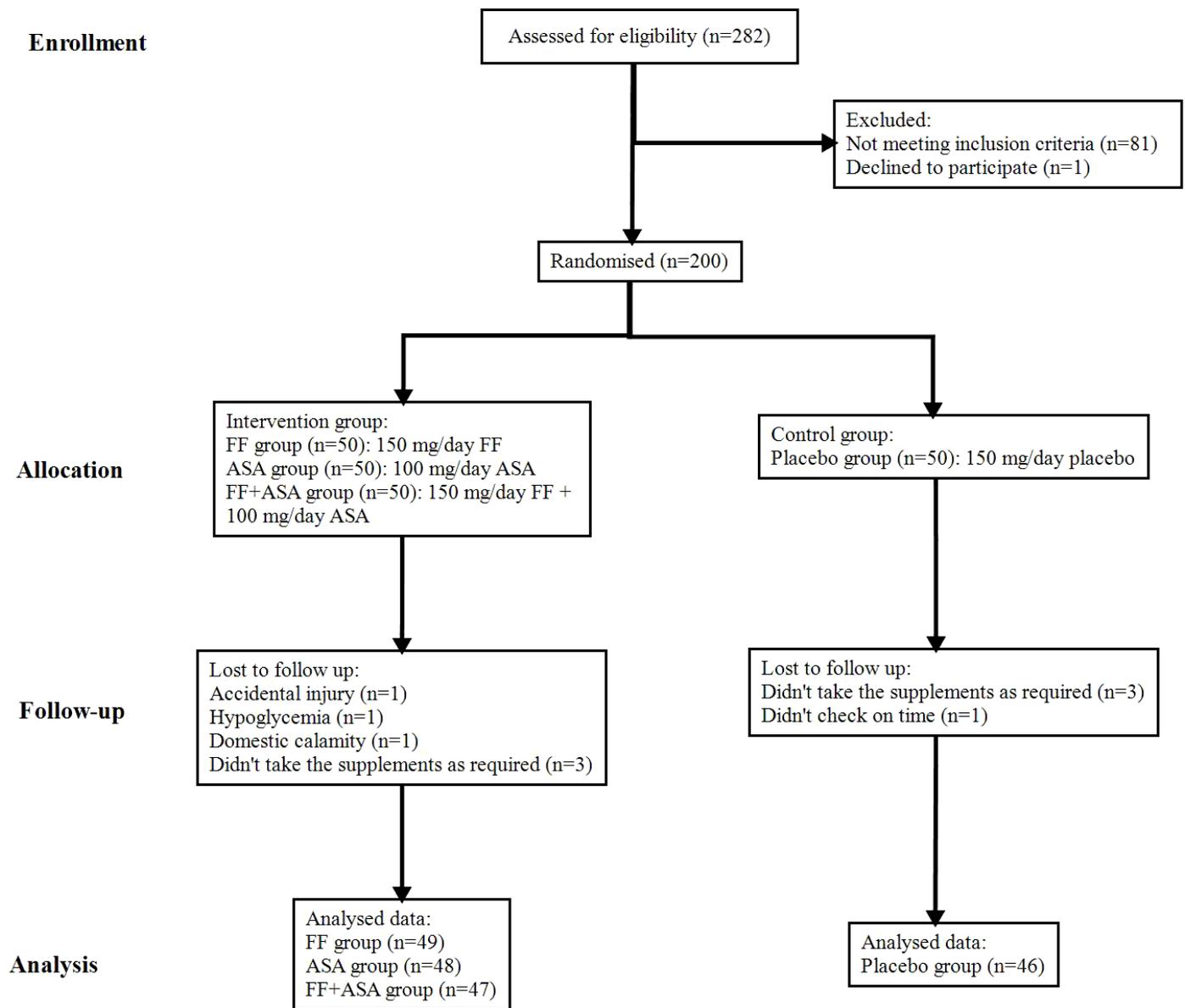


Figure 1

Flow chart.

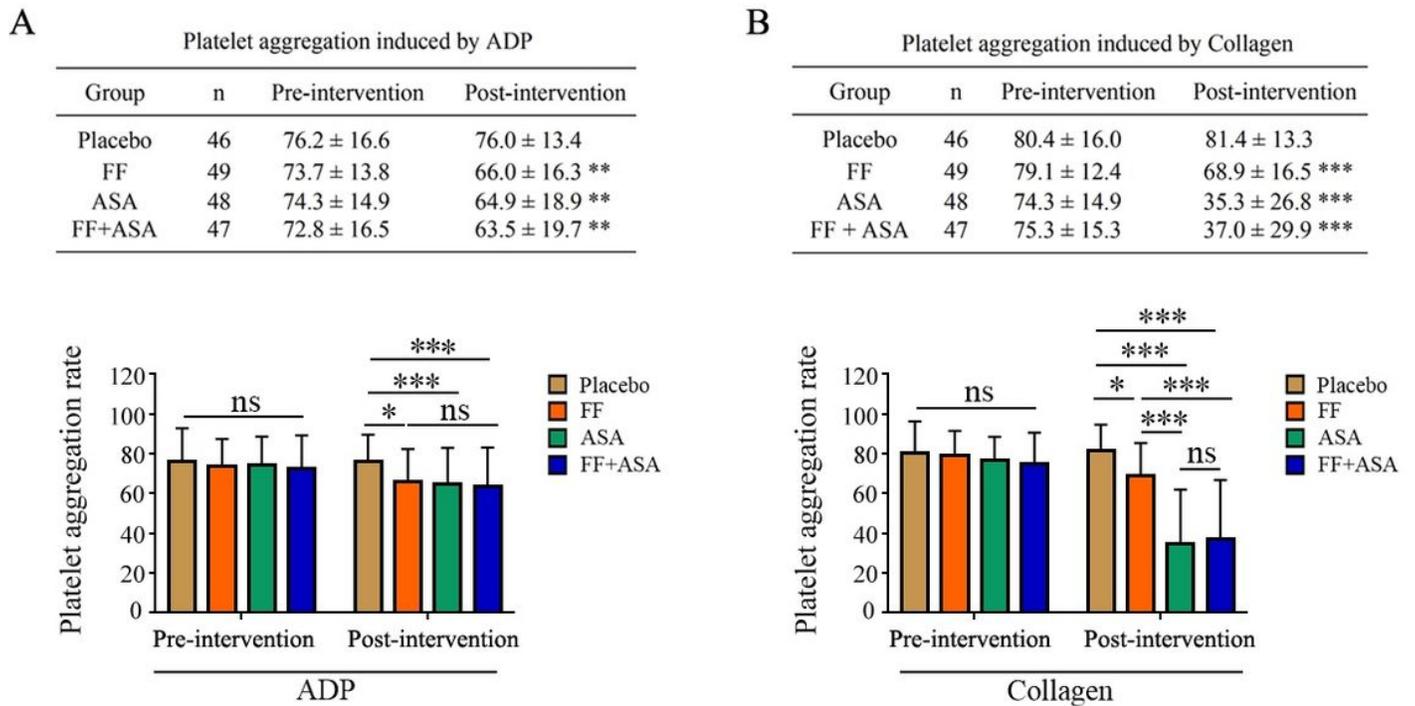


Figure 2

Fruitflow® (FF) inhibited ADP- and collagen-induced platelet aggregation ex vivo. Blood was collected pre- and post-intervention for 7 days. Platelet-rich plasma was used to measure platelet aggregation. (A) The number of participants and comparison of ADP-stimulated platelet aggregation rate pre- and post-intervention in each group. The histogram shows trend changes in platelet aggregation pre- and post-intervention in each group and an analysis between groups. (B) The number of participants and comparison of collagen-stimulated platelet aggregation rate pre- and post-intervention in each group. The histogram shows trend changes in platelet aggregation pre- and post-intervention in each group and an analysis between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significant difference between pre- and post-treatment or between groups.

A

The content of TXB₂ (pg/ml) in plasma

Group	n	Pre-intervention	Post-intervention
Placebo	46	799.5 ± 82.9	835.5 ± 81.6
FF	49	738.5 ± 70.4	579.3 ± 37.1**
ASA	48	777.4 ± 93.1	542.7 ± 55.6**
FF + ASA	47	704.1 ± 70.5	531.6 ± 47.4**

B

The content of 6-keto-PGF_{1α} (pg/ml) in plasma

Group	n	Pre-intervention	Post-intervention
Placebo	46	1053.1 ± 111.2	1164.1 ± 132.7
FF	49	956.1 ± 84.0	701.8 ± 68.0***
ASA	48	970.2 ± 104.1	743.1 ± 88.7***
FF + ASA	47	920.4 ± 97.5	739.3 ± 95.7***

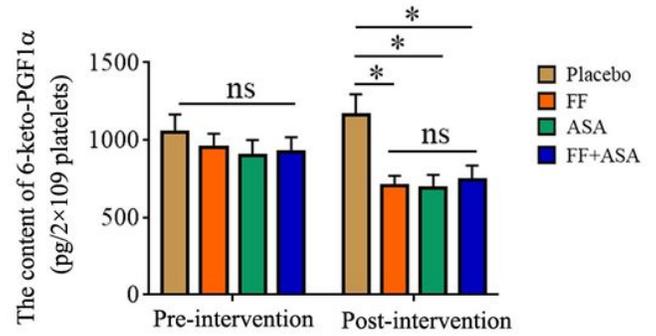
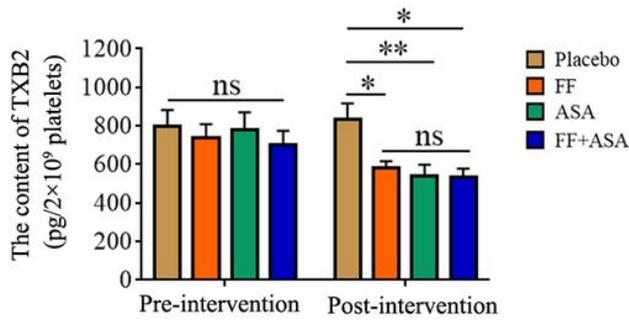


Figure 3

Fruitflow® reduced plasma TXB₂ and 6-keto-PGF_{1α} levels. Blood was collected pre- and post-intervention for 7 days. TXB₂ and 6-keto-PGF_{1α} levels were measured using ELISA kits. (A) Treatment with FF, ASA, and FF + ASA decreased TXB₂ levels post-intervention compared with pre-intervention. The histogram shows trend changes in TXB₂ levels in each group and an analysis between groups. (B) Treatment with FF, ASA, and FF + ASA decreased 6-keto-PGF_{1α} levels post-intervention compared with pre-intervention. The histogram shows trend changes in TXB₂ levels in each group and an analysis between groups. *p < 0.05, **p < 0.01, ***p < 0.001, significant difference between pre- and post-intervention or between groups.

The content of PF4 (ng/ml) in plasma

Group	n	Pre-intervention	Post-intervention
Placebo	46	1012.2 ± 98.8	984.7 ± 93.8
FF	49	1028.0 ± 70.1	656.9 ± 54.3***
ASA	48	963.8 ± 73.6	658.8 ± 35.4***
FF + ASA	47	1003.5 ± 84.0	638.9 ± 46.1***

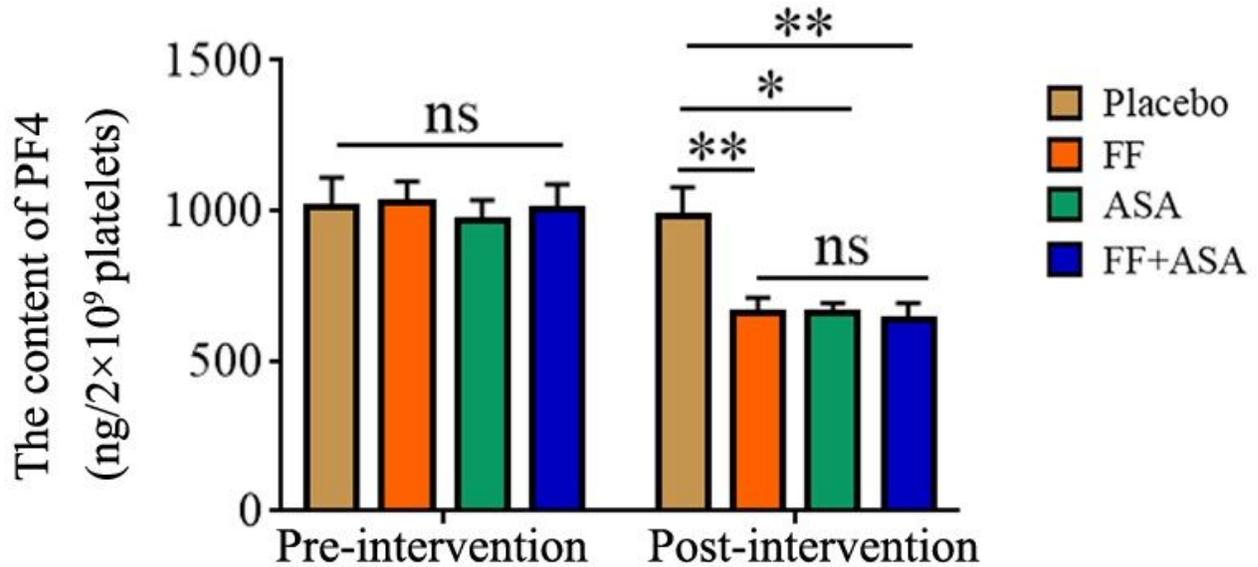


Figure 4

Fruitflow® reduced plasma PF4 levels. Blood was collected pre- and post-intervention for 7 days. PF4 levels were measured using ELISA kits. Treatment with FF, ASA, and FF + ASA decreased PF4 levels post-intervention compared with pre-intervention. The histogram shows trend changes in PF4 levels in each group and an analysis between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significant difference between pre- and post-intervention or between groups.

Supplementary Files

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- [CONSORT2010Checklistrecheck.pdf](#)