# The Effect of the Thai Herbal Wattana Formula on Platelet Aggregation and the Relationship with Innate *Dhatu Chao Ruean*

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## ABSTRACT

**Objective:** To investigate the effects of the Thai Herbal Wattana formula (WNF) on platelet aggregation and find a link between Innate *Dhatu Chao Ruean* (iDCR) factors and platelet aggregation.

**Materials and Methods:** Forty healthy volunteers with different iDCRs (Earth, Water, Wind, and Fire) received a single dose of 1,000 mg WNF. A blood sample was taken before and after the WNF administration at 3, 6, and 24 hours for analysis of platelet aggregation by aggregometry. Epinephrine, adenosine diphosphate (ADP) and collagen were used as platelet agonists.

**Results:** The WNF affects platelet aggregation in some subjects, especially females with an Earth iDCR or Wind iDCR with hyperaggregation patterns at baseline. The result after WNF treatment revealed that the percentage of platelet aggregation significantly changed downward at 3 hours and then recovered to pre-dosing levels after 24 hours. Additionally, it also did not have any relationship to iDCR. There were no reported adverse drug events.

**Conclusion:** WNF should be used with caution in patients with blood diseases and a close eye should be kept on herb-drug interactions such as with aspirin or other NSAIDs.

**Keywords:** Thai Herbal Wattana formula; WNF; Herbal medicine; Platelet aggregation; innate *Dhatu Chao Ruean*
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## INTRODUCTION

New trends regarding good health have generated interest in young people in society. Recently, the use of herbal medicine as an alternative or complementary therapy has increased dramatically in many parts of the world. Thai Traditional medicine refers to the knowledge, skills, and practices passed down from generations of folk healers from many cultures and is used to help maintain health against age-related deterioration, as well as prevent and treat various diseases. As a basic theory, Thai traditional medicine considers the imbalance of four elements (Earth, Water, Wind, and Fire) as an illness. In each person, there will be one element that is more dominant. Innate *Dhatu Chao Ruean* (iDCR) is the dominant body element at birth. It is analyzed by the month of birth and is divided as follows: Earth, from September to November, Water, from June to August, Wind, from March to May, and Fire, from December to February.[1]

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The Thai Herbal Wattana formula (WNF) has been used for over 40 years. It is used to help maintain health, and prevent age-related problems, such as muscle pains, loss of appetite, weaknesses, digestion, and gastrointestinal problems. The 18 medicinal plant components in the formula are *Boesenbergia rotunda* (L.) Mansf, *Saussurea lappa* C.B. Clarke., *Ligusticum sinense* Oliv. cv. Chuanxiong, *Cinnamomum illicioides* A. Chev., *Carthamus tinctorius* L., *Mallotus repandus* (Willd.) Muell. Arg., *Cladogynos orientalis* Zipp. ex Span., *Derris scandens* (Roxb.) Benth., *Cryptolepis buchanani* Roem. & Schult., *Tinospora crispa* (L.) Hook.f. & Thomson, *Caesalpinia sappan* L., *Piper nigrum* L., *Ferula assa-foetida* Regel, *Drypetes roxburghii* (Wall.) Hurusawa, *Aegle marmelos* (L.) Corrêa, *Citrus sinensis* (L.) Osbeck, *Terminalia chebula* Retz. and, *Cyperus rotundus* L. The recommended dose is 3-5 pills (200 mg/pill), 3 times per day before meals. The indication of this drug is older people (aging in Thai traditional medicine is defined as aged 32 and over). Moreover, the WNF has been investigated by many scientific studies to explain its pharmacological activities relating to age degeneration, including anti-oxidation[2], immunomodulatory[3,4] anti-neurodegenerative,[5] and anti-inflammatory properties.[6] Anti-inflammation is one effect linked to WNF's ability to ease muscle pain compared to diclofenac in OA knee patients.[7] The WNF may work similarly to NSAID inhibition. NSAIDs provide a COX inhibition that prevents synthesis of thromboxane A2. It is also effective at inhibiting platelet aggregation.[8] Understanding antiplatelet medications that balance anti-thrombotic potential with the danger of bleeding is still a concern. Therefore, this study aimed to investigate the impact of the Thai Herbal Wattana formula on platelet aggregation in healthy volunteers. This will help understand the safety of the WNF and any potential unwanted side effects. Moreover, it will be interesting to investigate the relationship between factors of innate *Dhatu Chao Ruean* and the impact of platelet aggregation on herbal drugs to better understand specific responses to provide personalized health care.

## MATERIALS AND METHODS

### Study drugs

WNF pills (200 mg) and powder were manufactured under GMP PIC/S (Good Manufacturing Practices) by the Manufacturing Unit of Herbal Medicines and Products, Center of Applied Thai Traditional Medicine (CATTM), Faculty of Medicine Siriraj Hospital, Mahidol University. The experiments used the same batch of WNF pills in the study. All WNF pills were authenticated and qualified by quality control, including the FTIR method, UPLC method, physical properties, and microbial contamination. WNF pills and powder were stored and preserved at room temperature in dry conditions.

### Subject design

This controlled pre-post intervention study was conducted at the Faculty of Medicine Siriraj Hospital, Bangkok, Thailand. The protocol was approved by the Siriraj Institutional Review Board (COA no. Si 756/2019) and registered in the Thai Clinical Trials Registry (TCTR20221213002). Before enrolling in the study, participants were provided necessary information, including the risks and benefits and signed an informed consent form. For the pilot study, 40 healthy volunteers with four different innate *Dhatu Chao Ruean* (Earth, Water, Wind and Fire) were recruited. The following criteria was used for inclusion: 1) Thai male or female ≥ 32 years old at time of enrollment; 2) body mass index (BMI) between 18-24 kg/m$^2$ ; 3) in good health as confirmed by blood chemistry, or an AST ≤ 40 U/L (male), ≤ 32 U/L (female); ALT ≤ 41 U/L (male), ≤ 33 U/L (female); ALP ≤ 141 U/L (male), ≤ 105 U/L (female) and GFR ≥ 60 ml/min/1.73m$^2$. The exclusion criteria were: 1) evidence of allergic reactions from herbal medicine; 2) habitual smoking with no ability to abstain from cigarettes during the study; 3) history of excess alcohol ingestion without ability to abstain from alcohol during the study or drug abuse; 4) pregnant or breastfeeding; 5) history of blood donation or transfusion within 3 months of the study. The volunteers who had an adverse event caused by WNF or believed to have had an event as were withdrawn as per physician agreement. All participants received advice to prepare themselves prior to the study and were informed to abstain from caffeine, alcohol and intake of vitamins, dietary supplements, and foods containing any of the 18 components of the WNF for at least two weeks. Moreover, other information or concerns from volunteers was followed up on and proper advice provided. On experiment day, blood samples were drawn and kept in sodium citrate vacutainer (Greiner Bio-one GmbH, Austria) at pre-dose for baseline, 3, 6, and 24 hours after administration of 1,000 mg WNF. Moreover, vital signs and all reported adverse events were evaluated and recorded by study physicians.

### Platelet aggregation assay

Platelet aggregation was determined using light transmission (LTA) and Born's technique in an aggregometer (AggRAM, Helena, USA). LTA is the gold standard for determination of platelet aggregation by measuring the change in absorbance as platelet-rich plasma (PRP) is

agitated with reagents. Epinephrine (Epi), adenosine diphosphate (ADP), and collagen (Col) were used as a panel of platelet agonists.[9] All platelet aggregation assays were run within 3 hours of blood collection. Citrated whole blood was centrifuged at 250g for 10 minutes at 25°C to prepare PRP. Some PRP was centrifuged at 4500g for two minutes at 25°C to prepare platelet-free plasma (PFP) to set as a blank. PRP was incubated at 37°C for 3 minutes before being induced with 1μM Epi, 25 μM Epi, 5 μM ADP and 1 μg/ml Col while stirring at 600 rpm. The reaction was allowed to proceed for 5 minutes. The difference between light transmissions of aggregated PRP and PFP was used to calculate the maximal amplitude of platelet aggregation as a percentage. Moreover, the platelets count was measured by using a non-metalized haemocytometer (Helena, USA).

### Platelet status classification

There is still a need for a formally accepted standard for measuring platelet function. The previous method classified the function into 3 categories of aggregation based on how platelets responded to various epinephrine concentrations.[10,11] The primary phase of platelet aggregation, caused by 25 μM epinephrine, is defined by the "disaggregation pattern". In contrast, platelet aggregation caused by 1 μM epinephrine and expressed as the secondary phase of aggregation is known as the "hyperaggregation pattern". Therefore, the concentration-dependent response of platelets to 1 μM and 25 μM epinephrine was labeled as the "normal aggregation pattern".[10,11]

### Statistical analysis

All data was presented as mean ± standard deviation (SD). Statistical analyses were performed with SPSS, version 18 (SPSS Inc., Chicago, IL, USA). Platelet aggregation data was evaluated for normality using the Kolmogorov–Smirnov and Shapiro-Wilk tests. Group comparisons were performed by a nonparametric Mann-Whitney U test (between gender) and the Kruskal-Wallis test (between iDCR). Time-dependent changes (before and after WNF administration) in groups were assessed by the nonparametric Friedman test; pairwise post hoc comparisons. Moreover, the chi-square statistic was used for categorical variables. Analyses were declared significant for a *P-value* <0.05.

## RESULTS

### Demographic characteristics

Forty healthy volunteers were enrolled in this study from March 2020 to August 2020. All subjects completed the study. The average age of the male group and female group was 36.3±3.9 and 39.1±5.9, respectively. The baseline characteristics, clinical chemistry, and hematologic screening of all subjects were normal. All data was homogeneous at the baseline. There was no difference between the sex groups (Table 1).

### Platelet status pattern before dosing

Before WNF treatment, 47% of the total 40 subjects exhibited the hyperaggregation pattern while 40% and 13% exhibited the normal and disaggregation pattern (Fig 1a). A comparison of, male and female platelet aggregation status before dosing did not significantly differ between the two groups (Figs. 1b1-1b2). Among the iDCR group, more than half of the subjects in the Wind group (70%) exhibited the hyperaggregation pattern (Fig 1c3). However, platelet status patterns of subjects in the Wind, Earth, Water and Fire group were not significantly different (Figs 1c1-c4).

### Effect of WNF on platelet aggregation

To assess the impact of the WNF on each subject, each individual subject's pattern of platelet aggregation stratified to sex, and iDCR is shown in Fig 2. The total result after WNF treatment revealed that the pattern of platelet aggregation status changed at 3 and 6 hours, and then recovered to pre-dosing levels after 24 hours. In the female group, especially those in the Earth or Wind iDCR, the hyperaggregation pattern was almost downward (Fig 2). The percentage of platelet aggregation for each agonist is shown as a heat map in Fig 3. The average of the percentages of aggregation decreased at 3 and 6 hours and then reverted to pre-dosing levels after 24 hours (Fig 4). However, the WNF also revealed an interesting trend of increased platelet aggregation classified as disaggregation status (Figs 4c1-3). In this study, platelet aggregation did not have any link to gender (Figs 4b1-4b2). Additionally, it also did not have any relationship to iDCR even if platelet aggregation in the Earth and Fire group significantly decreased after WNF treatment (Figs 4d1-4).

At each investigation time point, the average platelet counts were within an acceptable range (150-750 x $10^9$ cells/L) and did not impair LTA experiments.[12] The average platelet count of 40 PRP subjects at pre-dose, 3, 6, and 24 hours after WNF administration were 366 x $10^9$ cells/L, 298 x $10^9$ cells/L, 307 x $10^9$ cells/L, and 369 x $10^9$ cells/L, respectively. The results showed a decreasing trend at 3 and 6 hours of dosing before reverting to pre-dosing levels after 24 hours.

**TABLE 1.** Demographic data and baseline laboratory values with reference range criteria.

| Topic | Mean + SD | | Reference range | |
|---|---|---|---|---|
| | Male (n=20) | Female (n=20) | Male | Female |
| Age (years) | 36.3±3.9 | 39.1±5.9 | ≥ 32 | |
| iDCR (4 elements) | | | | |
| EARTH (Sep-Nov) | 5 | 5 | | |
| WATER (Jun-Aug) | 5 | 5 | - | |
| WIND (Mar-May) | 5 | 5 | | |
| FIRE (Dec-Feb) | 5 | 5 | | |
| Vital sign | | | | |
| Temp (°C) | 36.5±0.3 | 36.3±0.3 | - | |
| Pulse rate (/min) | 69.0±7.6 | 73.1±7.4 | - | |
| Respiratory rate (/min) | 18.3±0.7 | 18.1±0.4 | - | |
| Blood pressure (mmHg) | | | | |
| Systolic | 112.8±10.4 | 110.7±11.6 | - | |
| Diastolic | 70.7±11.6 | 68.9±9.5 | - | |
| Body weight (kg) | 65.1±8.3 | 53.8±5.5 | - | |
| Height (cm) | 171±0.1 | 159±0.05 | - | |
| Body mass index (kg/m$^2$) | 22.1±1.9 | 21.2±1.7 | 18-24 | |
| Hb (g/dL) | 14.9±0.9 | 12.5±1.1 | 12.70-16.90 | 12.0-14.90 |
| Hct (%) | 44.7±2.3 | 38.2±2.8 | 40.30-51.90 | 37.0-45.70 |
| WBC (x 10$^3$/uL) | 5.7±1.3 | 6.0±1.5 | 4.50-11.30 | 4.40-10.30 |
| Platelet (x10$^3$/uL) | 270.2±49.8 | 246.2±42.1 | 160-356 | 179-435 |
| FBS (mg/dL) | 86.7±6.0 | 87.2±5.1 | 74-99 | |
| BUN (mg/dL) | 12.2±2.9 | 10.7±2.8 | 6-20 | |
| Creatinine (mg/dL) | 1.0±0.1 | 0.7±0.1 | 0.67-1.17 | 0.51-0.95 |
| eGFR | 98.9±13.2 | 109.9±12.4 | ≥60 | |
| Total cholesterol (mg/dL) | 186±28.9 | 193.7±29.5 | <200 | |
| Triglyceride (mg/dL) | 96.7±37.8 | 77.2±50.7 | <150 | |
| HDL (mg/dL) | 54.5±11.9 | 65.5±14.9 | >40 | |
| LDL (mg/dL) | 112.3±27.8 | 112.8±27.5 | <130 | |
| Total bilirubin | 0.9±0.4 | 0.5±0.3 | 0.00-1.20 | |
| AST (U/L) | 21.2±6.6 | 17.5±3.1 | 0-40 | 0-32 |
| ALT (U/L) | 19.4±6.6 | 12.8±4.4 | 0-41 | 0-33 |
| Alkaline (ALP) | 66.3±17.9 | 57.4±13.8 | 40-129 | 35-105 |

Data were determined by Mean±SD

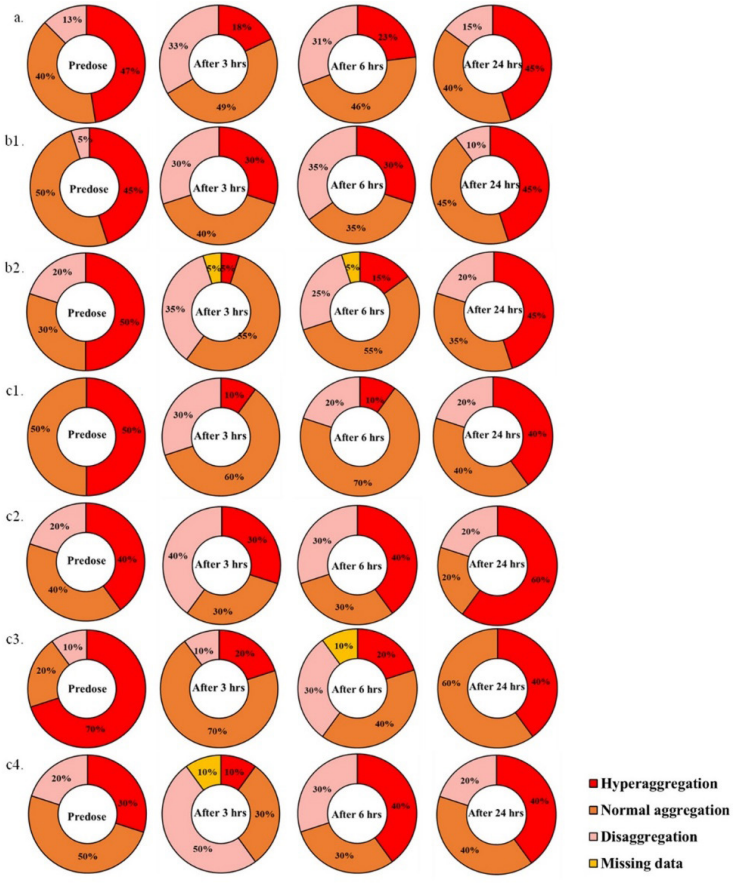


**Fig 1.** Platelet aggregation pattern before and after 3, 6 and 24 hours of WNF dosing in 40 healthy volunteers (a), grouping with sex (Male: b1, Female: b2) and iDCR (Earth: c1, Water: c2, Wind: c3 and Fire: c4)

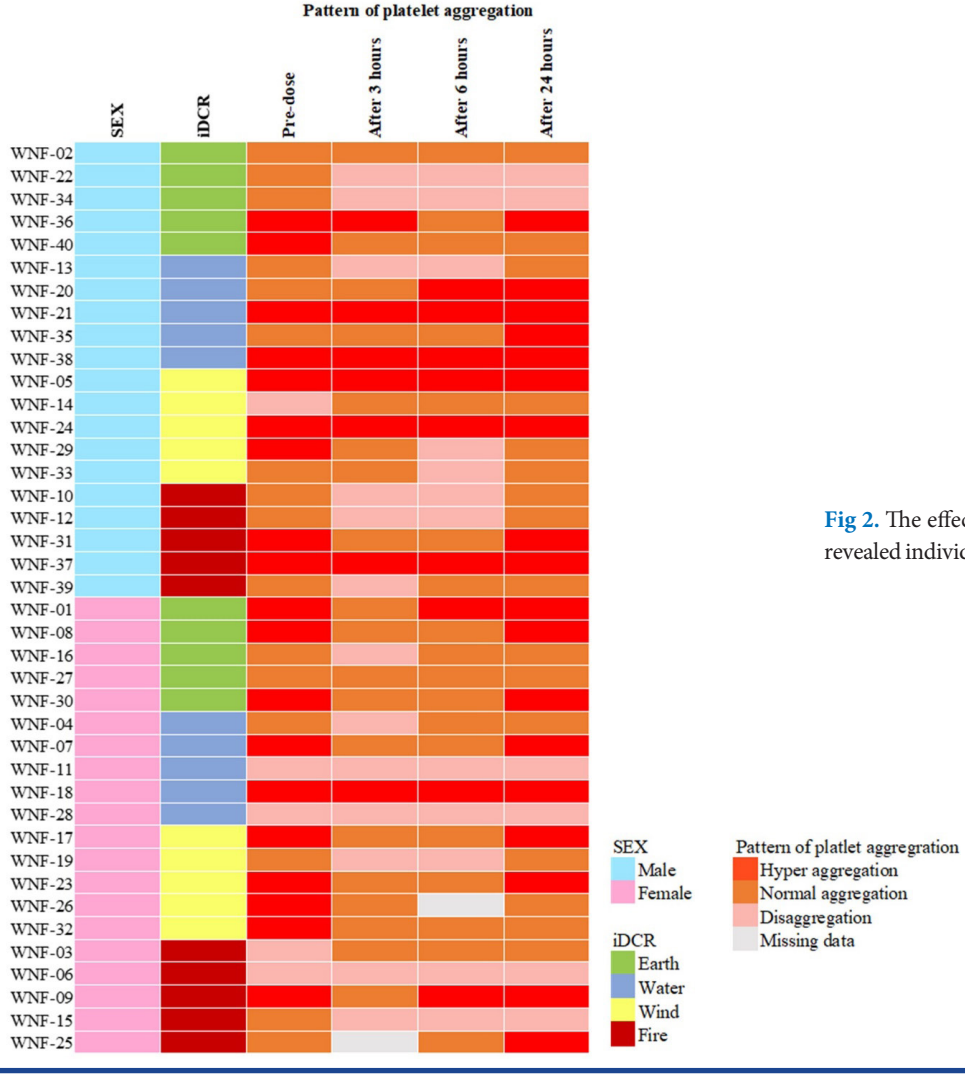


**Fig 2.** The effect of WNF on platelet aggregation revealed individual patterns after 3, 6 and 24 hours.

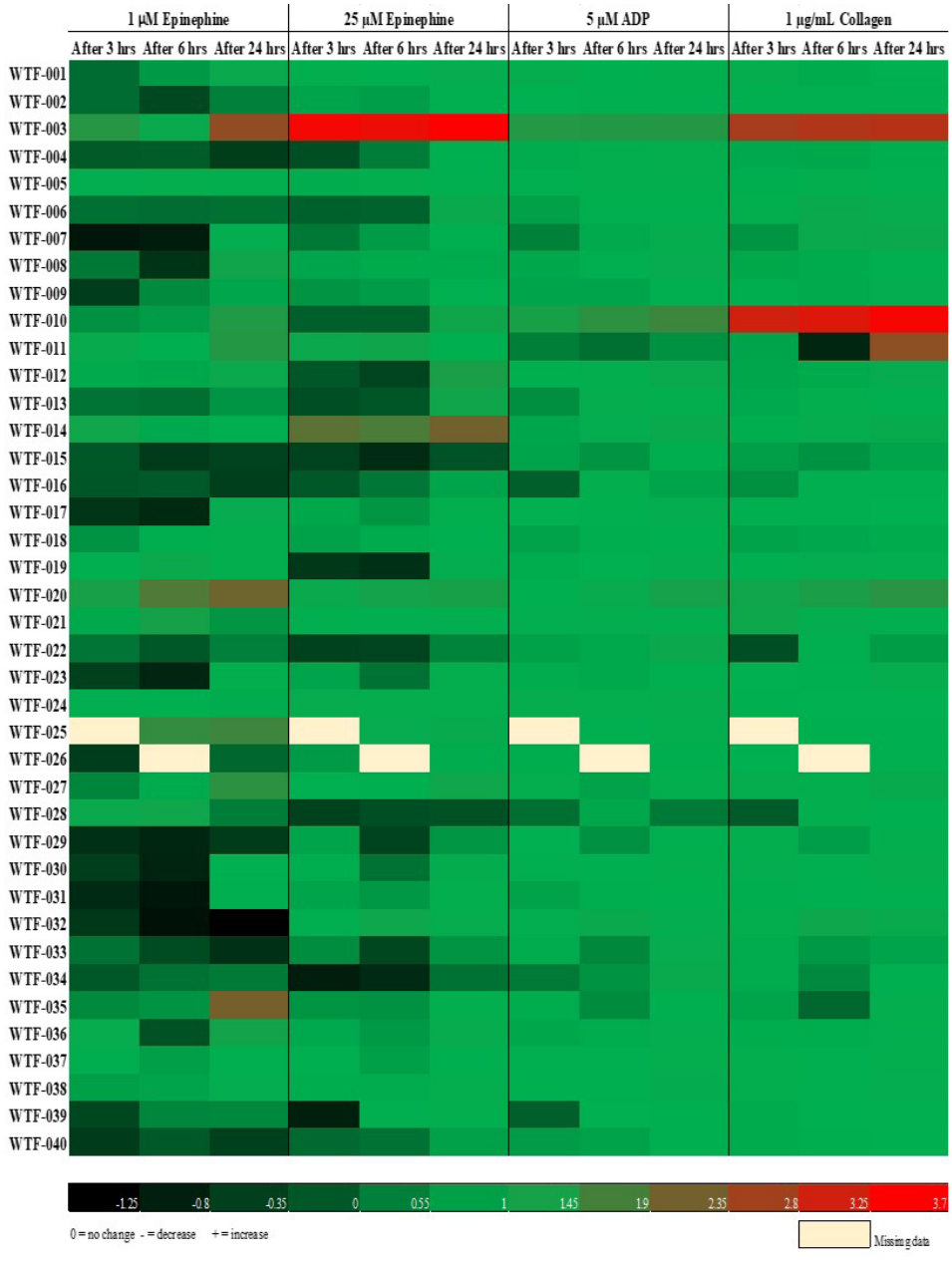


**Fig 3.** Heatmap of percent platelet aggregation in each subject for each agonist after WNF administration

## DISCUSSION

The Thai Herbal Wattana Formula is indicated for pain relief and anti-inflammation like NSAIDs in OA knee patients.[7] A study on the WNF also revealed an anti-inflammatory impact that may be attributed to an enzyme's COX-specific inhibitory activity.[6] It is well-known that reducing thromboxane A2 production by suppressing COX enzymes affects platelet aggregation and raises the risk of bleeding.[8] However, the lack of an anti-platelet aggregation study is a cause of concern about the safety of older people or blood disorders using WNF.

The aggregation pattern observed in the 40 participants of this study before treatment is similar to one observed previously in healthy subjects and is known as hyperaggregation.[13,14] According to Thai traditional medicine theory, which supports these findings, the participants' Earth iCDR is the heaviest, slowest-changing element. Due to these reasons, no disaggregation pattern was observed. Additionally, the subjects' Wind iCDR, which is stimulated more quickly than other iCDR components is intrinsically reflective of motion movements. Hyperaggregation may be present in the Wind element more than others.[1] In our study, the WNF siginifcantly inhibited aggregatation by Epi, ADP and collagen. According to previous studies, many receptors and signaling pathways are involved in the anti-platelet aggregation impact of WNF components. PAF-induced platelet aggregation, 5-HT release by platelets, and an increase in free calcium in platelets can all be inhibited by safflower aqueous extract.[15] Additionally, hydroxysafflor yellow A, safflower yellow A, luteolin and carthamin, the

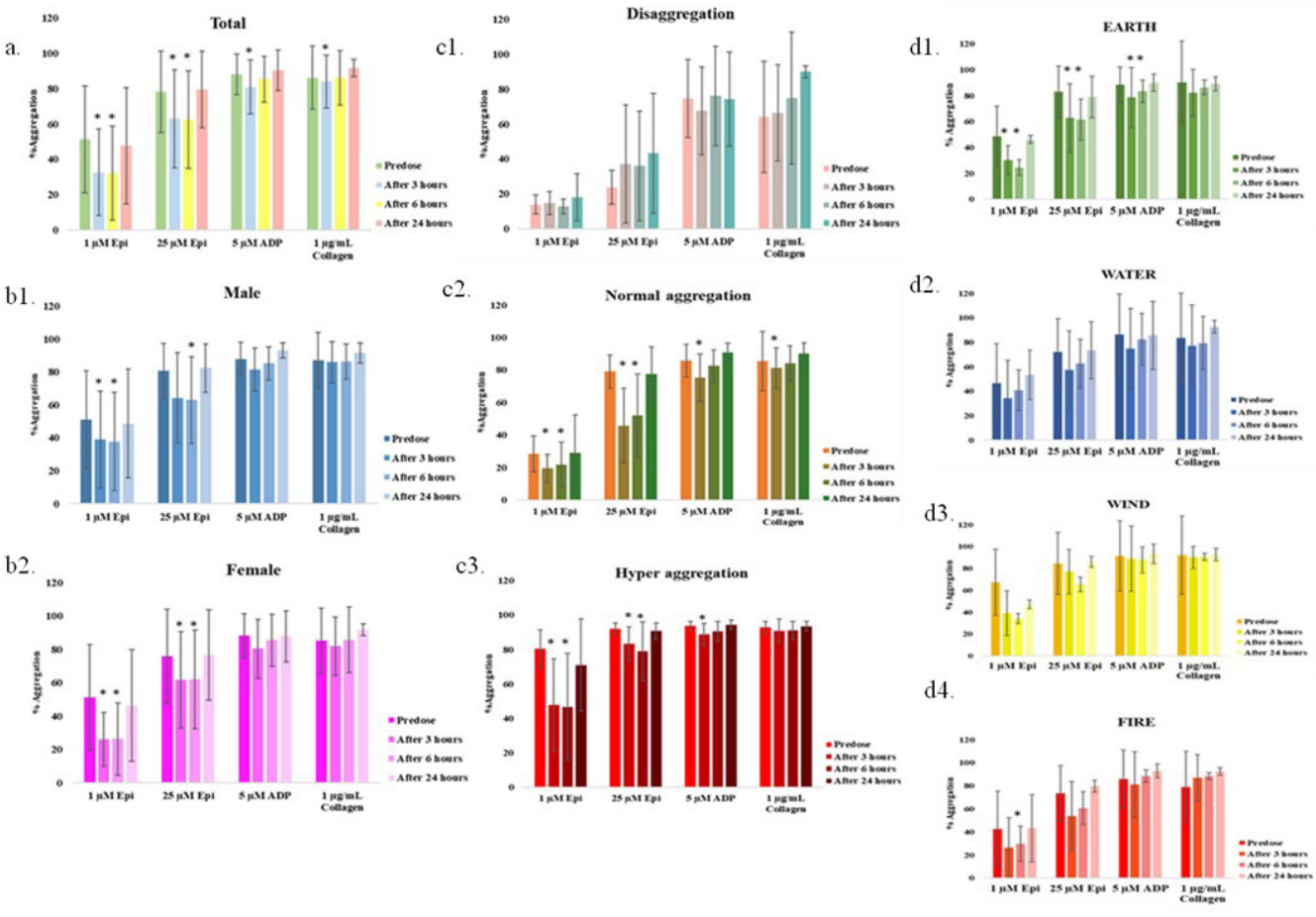


**Fig 4.** The average of platelet aggregation of 40 subjects after WNF administration (a) sub analysis with sex (b1-2), pattern (c1-3) and iDCR (d1-4). Aggregation of platelet was induced by 1 µM Epi, 25 µM Epi, 5 µM ADP and 1 µg/ml Col. Percent aggregation of platelet after 3, 6 and 24 hours of WNF administration were compare to predose and interpreted as percent of control. Data was shown as mean ± SD. *Post-hoc Wilcoxon test by Friedman test, Significance level was set at *P-value* < 0.05

safflower's active marker, can prevent ADP-controlled aggregation of human platelets.[16,17] (Brazilin, isolated from heartwood of *Caesalpinia sappan* acts as a collagen receptor agonist.[18] An extract of *Cyperus rotundus* in ethanol demonstrated effective inhibition of thrombin, collagen, or AA-induced platelet aggregation.[19] The major active component of *piper nigrum*, piperine, significantly reduced AA liberation by attenuating cPLA2 activity in collagen-stimulated platelets.[20] A dose-dependent reduction of AA-induced human platelet aggregation was shown with *T. chebula* fruit extract.[21] Interpreting the effects of the WNF on platelets has been a challenge due to significant inter-individual variability in aggregation between various agonists. This could be the result of multiple pathways that lead to platelet aggregation and the involvement of numerous platelet receptors in each pathway. Additionally, each subject's specific genetic diversity significantly impacts changing platelet responsiveness and function. In any case, additional research is still required to comprehend the mechanism. We must be aware of platelet aggregation's effects on the WNF in individual patients.

## CONCLUSION

This study is the first to examine how the WNF affects platelet aggregation in healthy volunteers with various innate *Dhatu Chao Ruean* (iDCR) groups. In this investigation, there were no drug-related adverse events related to the WNF. The results suggest that 1,000 mg of WNF could impact platelet aggregation (Fig 5). Out of 40 subjects, 60% displayed a downward trend of platelet aggregation patterns (Fig 5a). Similar results were revealed by an analysis based on gender, but the female group had a trend to greater downward platelet pattern than the male group (Fig 5b). Earth, Wind, and Fire iCDR groups under investigation also showed a downward trend, while the Water iCDR group showed no change (Fig 5c). Within 24 hours, the patterns of platelet status and percent platelet aggregation changed. After being stimulated with 1 µM Epi, 25 µM Epi, 5 µM ADP and

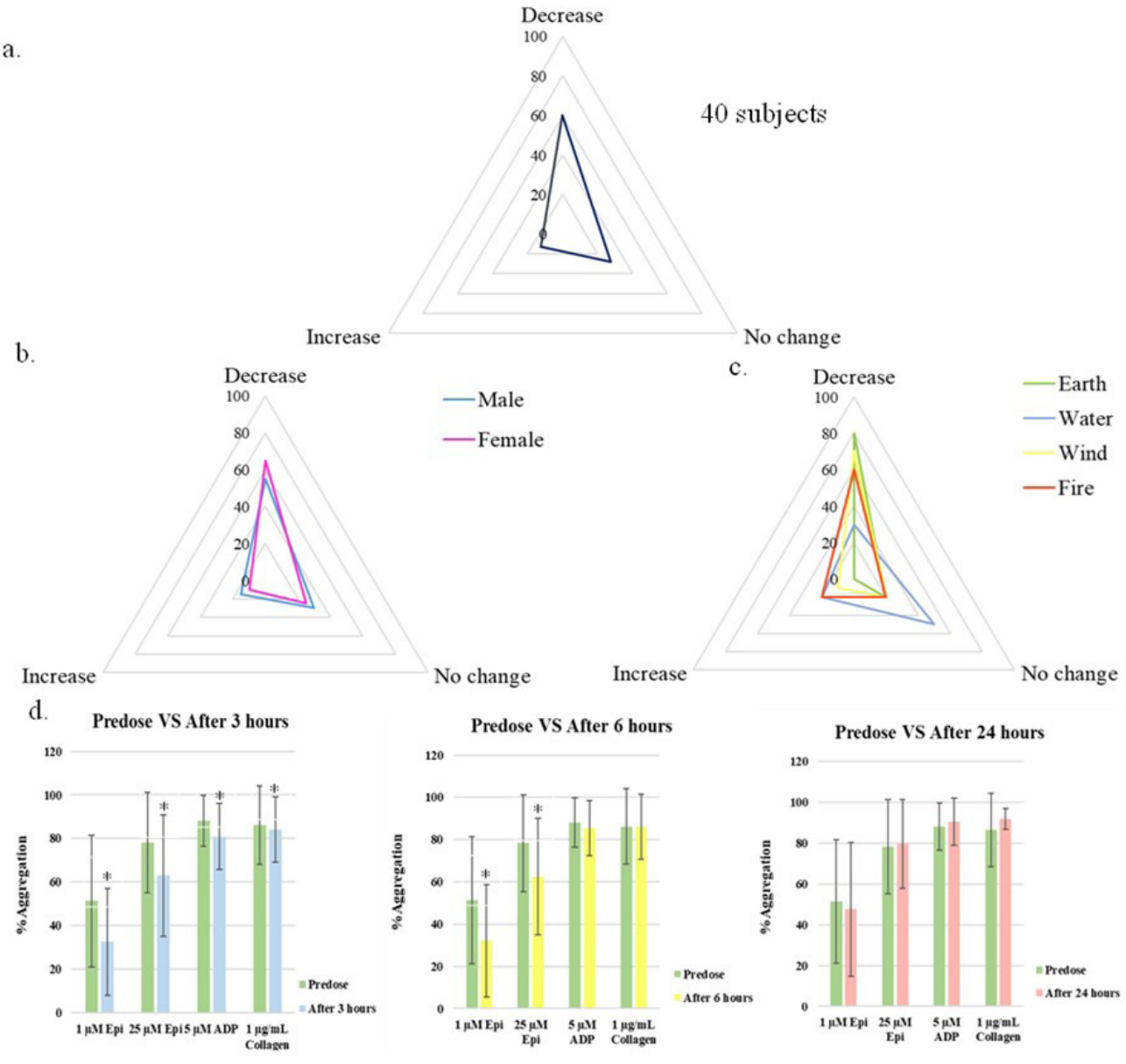


**Fig 5.** The effect of the WNF on platelet aggregation is defined as the percentage of platelet aggregation pattern changing (a: 40 subjects, b: different sex, c: different iCDR) and the effect of WNF on platelet aggregation agonists of 40 subjects (d), Data is shown as mean ± SD. *Post-hoc Wilcoxon test by Friedman test, Significance level was set at *P-value* < 0.05

1 µg/ml Col at 3 hours, the WNF significantly reduced the percentage of platelet aggregation (Fig 5d). Females with Earth iDCR or Wind iDCR with hyperaggregation patterns should use the WNF with caution. The effect of the WNF on platelet aggregation in an individual may be due to intrinsic influences, including iDCR and genetic variations in drug metabolizing enzymes of each volunteer. This supports calls for the concept of personalized medicine based on platelet reactivity. It should be used with caution in older adults and patients with a history of blood disorders, with special attention on herb-drug interactions, such as aspirin or other NSAIDs.

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## REFERENCES

1. Laohapand T and Jaturatamrong U, Thai Traditional Medicine in the Faculty of Medicine Siriraj Hospital, Ayurved Thamrong School, Center of Applied Thai Traditional Medicine, Faculty of

Medicine Siriraj Hospital, Mahidol University. Bangkok: Supavanich Press; 2009.
2. Panich U, Pluemsamran T, Tangsupa-a-nan V, Wattanarangsan J, Phadungrakwittaya R, Akarasereenont P, et al. Protective effect of AVS073, a polyherbal formula, against UVA-induced melanogenesis through a redox mechanism involving glutathione-related antioxidant defense. BMC Complement Altern Med. 2013;13:159.10 page.
3. Duangsa-ard S, Wongkajornsilp A, Akarasereenont P, Huabprasert S, Chongputtharaksa T, Laohapand T. The effects of Ayurved Siriraj Wattana recipe on splenocytes in Wistar rat. Siriraj Med J. 2013;65(3):73-6.
4. Wongkajornsilp A, Numchaisermsuk N, Sa-ngiamsuntorn K, Akarasereenont P, Wamanuttajinda V, Kasetsinsombat K, et al. Effects of the Ayurved Siriraj Wattana recipe on functional and phenotypic characterization of cytokine-induced killer cells and dendritic cells in vitro. BMC Complement Altern Med. 2016;16(489):11 page.
5. Htoo HH, Limsuvan S, Thamsermsang O, Hernandez JF, Checler F, Govitrapong P, et al. The Polyherbal Wattana Formula Displays Anti-Amyloidogenic Properties by Increasing α-Secretase Activities. PLos One. 2017;1-17.
6. Charoenkij P, Palo T, Chotewuttakorn S, Limsuvan S, Laohapand T, Akarasereenont P. The effect of Ayurved Siriraj Wattana Recipe (AVS073) on LPS induced COX-2 expression in human PBMC. Siriraj Med J. 2016;68(2):90-6.
7. Pengkhum T, Chatsiricharoenkul S, Akarasereenont P, Charoencholvanich K. Phase II clinical trial of Ayurved Siriraj Wattana recipe for symptomatic relief in patients with osteoarthritis of the knee. J Med Assoc Thai. 2012;95(3):452-60.
8. Schafer AI. Effects of nonsteroidal antiinflammatory drugs on platelet function and systemic hemostasis. J Clin Pharmacol. 1995 Mar;35(3):209-19.
9. Cattaneo M. Light Transmission Aggregometry and ATP Release for the Diagnostic Assessment of Platelet Function. Semin Thromb Hemost. 2009;35:158–67.
10. Ketsa-Ard K, Poungvarin N, Juengchareon M, Jarerat S, Kittigul L. Clinical study on antithrombotic effects of ticlopidine in ischemic stroke. J Med Assoc Thai. 1991;74(6):331-9.
11. Akarasereenont P, Tripatara P, Chotewuttakorn S, Palo T, Thaworn A. The effects of estrone, estradiol and estriol on platelet aggregation induced by adrenaline and adenosine diphosphate. Platelets. 2006;17(7):441-7.
12. Femia EA, Scavone M, Lecchi A, Cattaneo M. Effect of platelet count on platelet aggregation measured with impedance aggregometry (multiplate analyzer) and with light transmission aggregometry. J Thromb Haemost. 2013;11:2193–6.
13. Yee DL, Bergeron AL, Sun CW, Dong JF, Bray PF. Platelet hyperreactivity generalizes to multiple forms of stimulation. J Thromb Haemost. 2006;4(9):2043-50.
14. Sirikarin T, Palo T, Chotewuttakorn S, Chandranipapongse W, Limsuvan S, Akarasereenont P. The Effects of *Andrographis paniculata* on Platelet Activity in Healthy Thai Volunteers. Evid Based Complement Alternat Med. 2018;2018:2458281.
15. Huang D, Lu Y, Shi L, Zhang J, Shen J, Bao M, et al. Effect of safflower yellow on platelet activating factor mediated platelet activation in patients with coronary heart disease. Bangladesh J Pharmacol. 2012;7:140-4.
16. Lu PH, Kuo CY, Chan CC, Wang LK, Chen ML, Tzeng IS, et al. Safflower Extract Inhibits ADP-Induced Human Platelet Aggregation. Plants (Basel). 2021;10(6):1192.
17. Li HX, Han SY, Wang XW, Ma X, Zhang K, Wang L, et al. Effect of the carthamins yellow from *Carthamus tinctorius* L. on hemorheological disorders of blood stasis in rats. Food Chem Toxicol. 2009;47(8):1797-802.
18. Chang Y, Huang SKH, Lu WJ, Chung CL, Chen WL, Lu SH, et al. Brazilin isolated from *Caesalpinia sappan* L. acts as a novel collagen receptor agonist in human platelets. J Biomed Sci. 2013;20(4):11 page.
19. Seo EJ, Lee DU, Kwakf JH, Lee SM, Kimg YS, Jung YS. Antiplatelet effects of *Cyperus rotundus* and its component (+)-nootkatone. J Ethnopharmacol. 2011;135(1):48-54.
20. Son DJ, Akiba S, Hong JT, Yun YP, Hwang SY, Park YH, et al. Piperine Inhibits the Activities of Platelet Cytosolic Phospholipase A2 and Thromboxane A2 Synthase without Affecting Cyclooxygenase-1 Activity: Different Mechanisms of Action Are Involved in the Inhibition of Platelet Aggregation and Macrophage Inflammatory Response. Nutrients. 2014;6:3336-52.
21. Haq MZU, Shahid SA, Ahmed S, Ahmad S, Qayum M, Khan I. Anti-platelet activity of methanolic extract of *Grewia asiatica* L. leaves and *Terminalla chebula* Retz. fruits. J Med Plants Res. 2012;6(10):2029-32.