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INHIBITORY EFFECTS OF GREEN TEA EXTRACT ON ADP AND THROMBIN INDUCED PLATELET AGGREGATION

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Abstract: The study was performed to investigate the effects of green tea extract (GTE) on platelet aggregation in Wistar rats. Forty male rats were used in this study and treated with GTE for 35 days. Experimental groups were as follows; control group; distilled water, aspirin 10 mg/kg/day, green tea extract 25, 50 and 100 mg/ kg/day. The platelet aggregation was measured by using microplate reader after inducing platelet aggregation by adenosine diphosphate (ADP) and thrombin. The results showed that green tea extract 25, 50 and 100 mg/kg/day tended to inhibit platelet aggregation induced by ADP and thrombin. However, only green tea 25 mg/kg/day significantly suppressed platelet aggregation when compared with the control group ($p<0.05$). This experiment showed the potential preventive effects of low dose GTE against platelet aggregation via ADP and thrombin pathway. Thus, green tea extract may be beneficial for preventing against cardiovascular diseases.

Keywords: Green tea, platelet aggregation, adenosine diphosphate (ADP), thrombin

บทคัดย่อ: งานวิจัยนี้ทำการศึกษาผลของสารสกัดชาเขียวต่อการเกาะกลุ่มของเกล็ดเลือดในหนูขาวเพศผู้ จำนวน 40 ตัว โดยป้อนสารที่ต้องการทดสอบให้หนูขาวทางปากต่อเนื่องเป็นเวลา 35 วัน หนูขาวที่นำมาใช้ทดลองแบ่งออกเป็น 5 กลุ่ม ได้แก่ กลุ่มควบคุมที่ได้รับน้ำกลั่น กลุ่มที่ได้รับแอสไพริน 10 มิลลิกรัมต่อกิโลกรัมต่อวัน และกลุ่มที่ได้รับสารสกัดชาเขียวขนาด 25 50 และ 100 มิลลิกรัมต่อกิโลกรัมต่อวัน ทำการวัดการเกาะกลุ่มของเกล็ดเลือดด้วยเครื่องไมโครเพลทรีดเดอร์ โดยใช้อะดีโนซีนไดฟอสเฟต และทรอมบิน เป็นสารกระตุ้นให้เกิดการเกาะกลุ่มของเกล็ดเลือด ผลการศึกษาพบว่าสารสกัดชาเขียวทุกขนาดมีแนวโน้มลดการเกาะกลุ่มของเกล็ดเลือดที่กระตุ้นด้วยอะดีโนซีนไดฟอสเฟต และทรอมบินได้ แต่เฉพาะสารสกัดชาเขียวขนาด 25 มิลลิกรัมต่อกิโลกรัมต่อวัน เท่านั้นที่สามารถลดการเกาะกลุ่มของเกล็ดเลือดได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) เมื่อเทียบกับกลุ่มควบคุม จากผลการศึกษานี้แสดงให้เห็นถึงศักยภาพของสารสกัดชาเขียวเมื่อใช้ขนาดต่ำ ในการต้านการเกาะกลุ่มของเกล็ดเลือด โดยมีกลไกการยั้บยั้งการกระตุ้นการเกาะกลุ่มของเกล็ดเลือดผ่านวิถีของอะดีโนซีนไดฟอสเฟต และทรอมบิน ดังนั้นสารสกัดชาเขียวน่าจะมีประโยชน์ในการป้องกันโรคหัวใจและหลอดเลือดได้

คำสำคัญ : ชาเขียว, การเกาะกลุ่มของเกล็ดเลือด, อะดีโนซีนไดฟอสเฟต, ทรอมบิน

INTRODUCTION

Platelets play an important role in the development and progression of atherosclerosis-thrombosis (Weitz, 2011). Platelet aggregation is initiated by various agonists, including adenosine diphosphate (ADP), thrombin, collagen and adrenalin. In addition, secretion of intracellular granule contents may accompany platelet aggregation, which may further recruit additional platelets to induce full aggregation and subsequent gross thrombus formation (Z. M. Ruggeri, 1997). Aberrant platelet activation may either directly or indirectly induce blood clot formation, thrombosis and sustained vascular wall inflammation, resulting in cardiovascular diseases such as atherosclerosis and cardiovascular attack. Green tea is one of the most popular beverages consumed worldwide. It is made from *Camellia sinensis* leaves without undergoing withering and oxidation. Several epidemiological and interventional studies showed that green tea associated with a risk reduction of cardiovascular events and/or modulation of cardiovascular risk factors (Hertog et al., 1993). Green tea has many biological properties particularly in the cardiovascular system, including antioxidant, vasodilation, antihypertensive and antiatherogenic effect (Higdon and Frei, 2003; Kang et al., 1999). In addition, epigallocatechin gallate (EGCG), a major component of green tea catechins from its leaves, has been shown antiplatelet activity (Lill, Voit, Schrör, and Weber, 2003; Mosawy, Gaiz, Karaksha, and Singh, 2016). However, it is unclear whether regular intake of green tea exhibits antiplatelet effect. Therefore, this study aimed to investigate the effects of green tea extract on platelet function.

MATERIALS AND METHODS

Preparation of extract

Green tea leaves were purchased from Choui Fong Tea®. A total of 50 g of green tea leaves were blended into small pieces with the blender and then packed in soxhlet apparatus and extracted. The extraction solvent was separated and concentrated under reduced pressure using rotary evaporator. The semi-solid crude was weighed and stored at 4ºC until used. It was dissolved with distilled water to a required concentrations before using in the experiments.

Animals

Male albino Wistar rats (150-200 g) obtained from the National Laboratory Animal Center, Mahidol University at Salaya, Thailand, were used in the experiments. Rats were housed in an animal room maintained at a constant temperature, 25±2ºC, and humidity with a 12-hour light and dark cycle. They were fed with standard diet and water *ad libitum*. The study protocol was approved by the ethics committee for animal research, Rangsit University (RSEC 01/2558).

Rats were randomly assigned into 5 groups of 8 animals each, as follows; (1) control group; distilled water, (2) aspirin 10 mg/kg BW/day as a standard drug and (3-5) green tea extract 25, 50 and 100 mg/ kg BW/day. All test samples were given orally once daily for 35 days.

Preparation of platelet and platelet aggregation assay

The experiment was performed on platelet derived from rats after treatment for 35 days. Rats were anesthetized with ether and blood was collected from the abdominal aorta. Whole blood was mixed with 3.8% sodium citrate solution (1:9 v/v) as an anticoagulant, and centrifuged at 250 g for 15 minutes at room temperature. Platelet rich plasma (PRP) obtained from supernatant suspension was pooled together. From the same donor, platelet free plasma

(PFP) was obtained from PRP centrifugation at 3000g for 2 minutes. Platelet aggregation was determined by using microplate reader as previously described (Krause, Scholz, Temmler, and Lösche, 2001) with ADP and thrombin as platelet agonists. Reading of optical density (OD) at 650 nm was taken using kinetic mode of Biorad® microplate reader at 30-second intervals for 3 minutes. During the run time, the plate was incubated at 37°C and mixed with the automix function of the reader. Each measurement was performed in duplicate. The decrease in OD of PRP after being mixed with each agonist was considered to indicate platelet aggregation. Percentage of aggregation was then calculated from the following formula:

% of platelet aggregation = (A-B)/(A-C) x 100
A = OD PRP plus normal saline
B = OD PRP plus agonist
C = OD PFP plus normal saline

Statistical analysis

Results were expressed as mean±SEM, statistical significance was calculated by One way ANOVA test. $p<0.05$ was considered as significant difference.

RESULTS AND DISCUSSION

Effects of GTE on ADP induced platelet aggregation

Platelet aggregation was induced by 3 μM and 10 μM of ADP. Platelet aggregation of aspirin and GTE 25, 50 and 100 mg/kg BW/day induced by 3 μM ADP were 65.89±6.82%, 62.79±14.53%, 85.00±7.543% and 85.78±16.69%, respectively (Figure 1) and those induced by 10 μM ADP were 74.16±7.09%, 67.65±13.14%, 92.09±7.83% and 109.38±18.61%, respectively (Figure 2). The ability of GTE to inhibit platelet aggregation did not depend on concentration of green tea. Aspirin and GTE 25 mg/kgBW/day significantly decreased platelet aggregation when compared to control. Surprisingly, no difference in the platelet aggregation response between GTE 50, 100 mg/kg BW/day treated groups and control was observed.

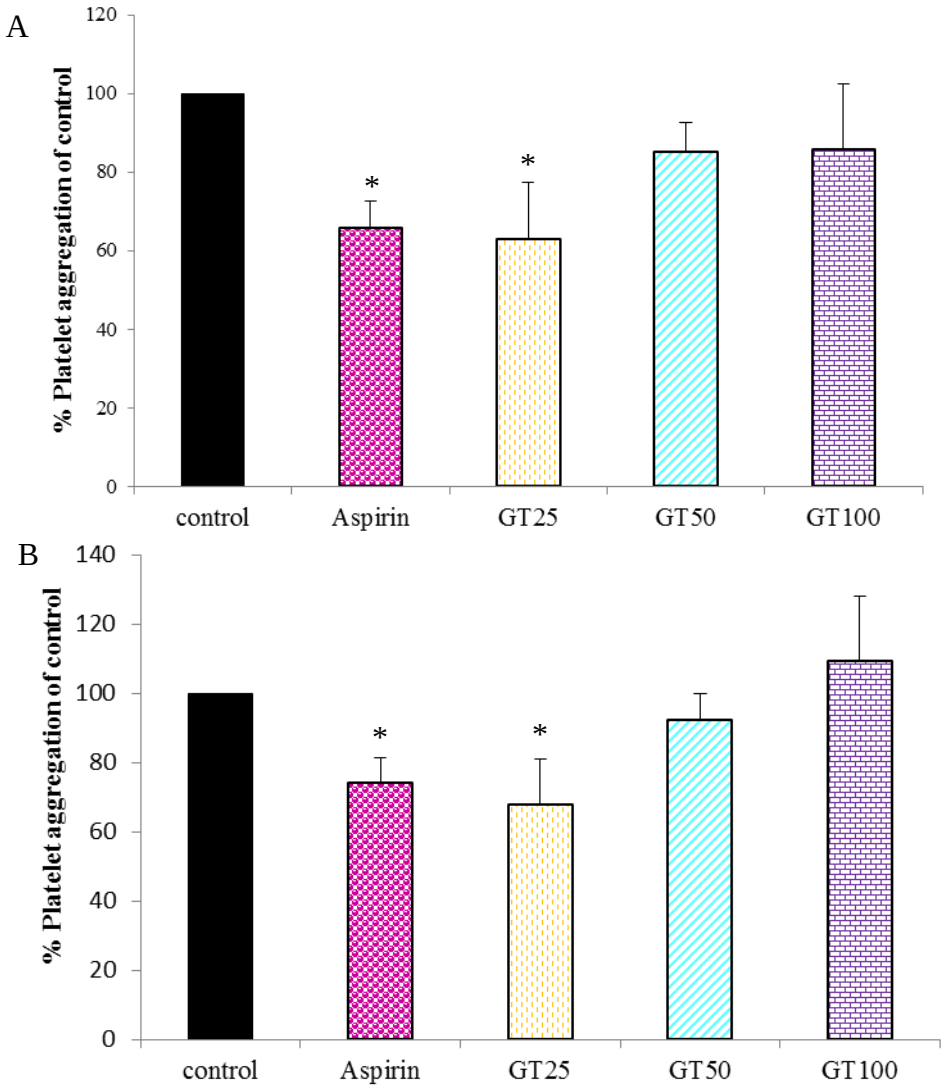

Figure 1. The effects of green tea extract on 3 μM ADP (A) and 10 μM ADP (B) induced platelet aggregation as shown in percent platelet aggregation of control. Data was shown as mean±SEM (n=8). *p<0.05 compared to control.

Effects of GTE on thrombin induced platelet aggregation

Platelet aggregation was induced by 0.1 U/mL and 0.5 U/mL of thrombin. Platelet aggregation of aspirin and GTE 25, 50 and 100 mg/kg BW/day induced by 0.1 U/mL thrombin were 53.55±19.00%, 23.84±15.83%, 78.84±15.83% and 91.51±18.11%, respectively (Figure 3), and those induced by 0.5 U/mL of thrombin were 24.12±11.61%, 28.30±15.93%, 88.91±12.86% and 89.35±11.22%, respectively (Figure 4). Platelet aggregation induced by thrombin was significantly decreased by aspirin and GTE 25 mg/kg BW/day. There was no difference in the maximum aggregation response between control and GTE 50 and 100 mg/kg BW/day treated groups.

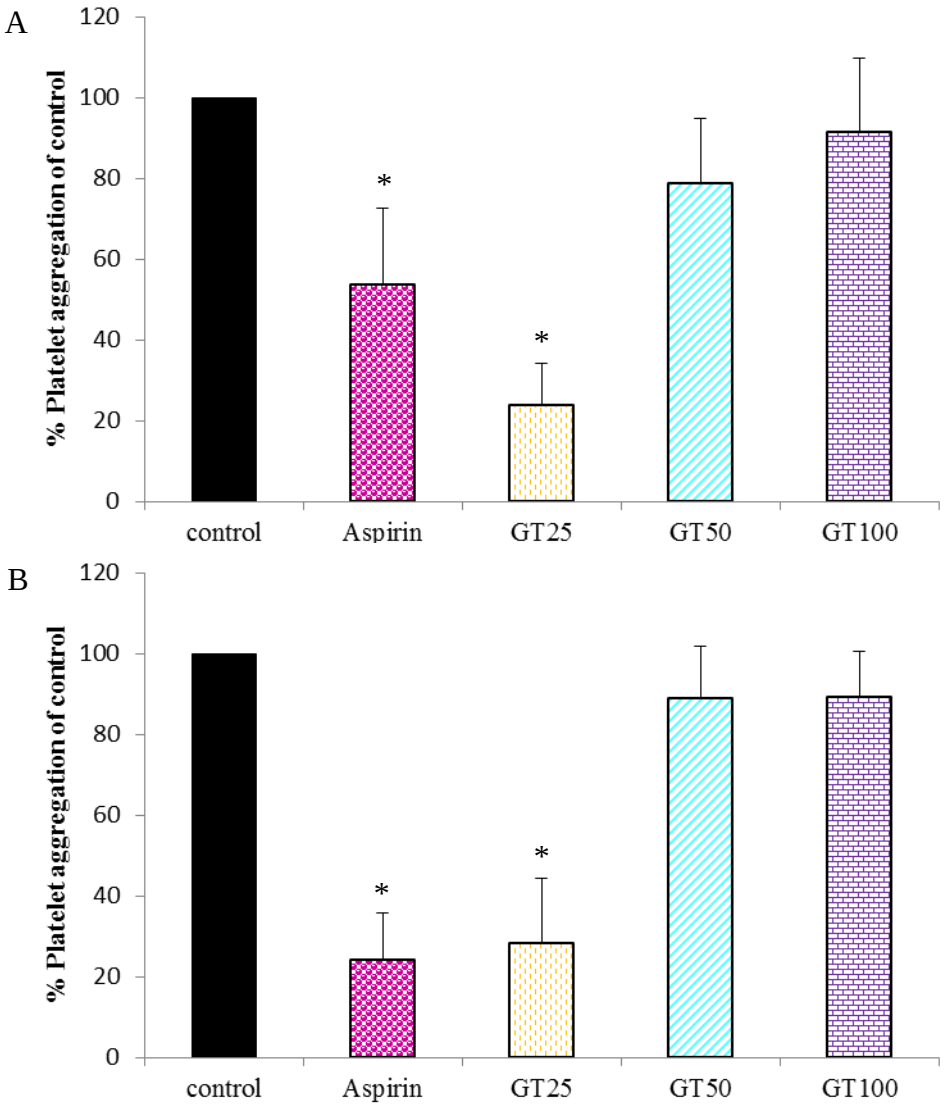

Figure 2. The effects of green tea extract on 0.1 U/mL (A) and 0.5 U/mL (B) induced platelet aggregation as shown in percent platelet aggregation of control. Data was shown as mean±SEM (n=8). *p<0.05 compared to control.

Platelet aggregation plays a key role in thrombus formation at the damaged blood vessels (Packham and Mustard, 1986). Interfering the process of platelet aggregation is one of the therapeutic strategies for the treatment of platelet-related thrombosis (Z. Ruggeri, 1997). Our results showed that GTE significantly inhibited ADP and thrombin induced platelet aggregation in non dose-dependent manner. GTE at low concentration, 25 mg/kg, significantly reduced platelet aggregation when compared to control while higher concentration (more than 25 mg/kg) did not affect platelet aggregation. These results indicated that GTE at low dose was effective for prevention of platelet aggregation. This finding is in accordance with the previous study, which showed that EGCG inhibited agonist induced platelet function at low concentration. EGCG inhibited collagen induced platelet aggregation by inhibiting both $[Ca^{+}]_i$ mobilization and TXA_2 production (Ok et al., 2012). In addition, tea leave extracts showed effective antiplatelet activity induced by ADP in a reverse dose-dependent manner (Yadav and Mendhulkar, 2015). In *in vitro* and *ex vivo* studies, there

were observed that green tea inhibited platelet aggregation induced by collagen, platelet activating factor (PAF) and thrombin (Deana et al., 2003; Sagesaka-Mitane, Miwa, and Okada, 1990). However, our finding disagreed with the study reporting that EGCG inhibited thrombin induced platelet aggregation in a concentration dependent manner (Lill et al., 2003). These results harmonized to those of other studies demonstrating that flavonoid compounds isolated from plants inhibited platelet aggregation (Durairaj and Dorai, 2010). After comparing antiplatelet aggregation of low dose GTE (25 mg/kg) to aspirin, it was found that the potency of green tea is comparable to that of aspirin. It might be implied that the suitable dose of green tea consumption is required for platelet aggregation inhibition. This is supported by the study demonstrating that EGCG has a stronger effect than that of aspirin on inhibition of cyclooxygenase-1 activity (Lee et al., 2013). In addition, it was found in many studies that antioxidant effect can prevent atherosclerosis. It was reported that green tea possess antioxidant effect. This property may be involved in the prevention of cardiovascular risk (Frei and Higdon, 2003; Khalesi et al., 2014). These results support the hypothesis stating that the intake of green tea could be beneficial for prevention of diseases caused by thrombus formation such as cardiovascular diseases.

CONCLUSION

This study concluded that green tea extract could be able to inhibit platelet aggregation. The mechanism by which green tea inhibits platelet aggregation is mediated through ADP and thrombin pathways. However, the ability to inhibit platelet aggregation did not correlate with the concentration of green tea. This finding showed the possibility of green tea in the prevention of thrombosis by its antiplatelet activity when taken in the appropriate dose.

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