

Effect of Ayurved Siriraj Herbal Recipe Chantaleela on Platelet Aggregation

Rath Itthipanichpong MD*,
Arunee Lupreechaset MD*, Sirikul Chotewuttakorn MSc*,
Pravit Akarasereenont MD, PhD*,**, Tasanee Onkoksoong BSc*,
Titchaporn Palo BSc*, Supornchai Kongpatanakul MD*,
Somruedee Chatsiricharoenkul MD*, Premrutai Thitilertdecha BSc**,
Prasopporn Pungpeng BSc**, Tawee Laohapand MD**

* Department of Pharmacology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok Thailand.

** Center of Applied Thai Traditional Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok Thailand

Introduction: Ayurved Siriraj Chantaleela recipe is a traditional Thai remedy consisting of eight medicinal plants, which is employed for the treatment of fever.

Objective: To investigate the effects of Ayurved Siriraj Herbal recipe Chantaleela on platelet aggregation.

Study design: Clinical research; ex vivo with before and after study design.

Material and Method: Twelve healthy male and female volunteers participated in the present study. Platelet aggregation test before Chantaleela ingestion was done as a control. After administration of 750 mg Chantaleela (3 x 250 mg tablets) every 8 hours for 3 doses, platelet aggregation was measured 8 hours following the first dose using an aggregometer and microplate reader. Adrenaline (Adr) and adenosine diphosphate (ADP) were used as platelet stimulants. Platelet aggregation was measured again at 32 hours and 8-10 days after the first dose.

Results: All of the participants completed the present study without any adverse event. Ayurved Siriraj Chantaleela did not affect platelet aggregation; neither Adr nor ADP were used as platelet agonists in both aggregometer and microplate reader. Subgroup analysis revealed no significant change in platelet aggregation after Chantaleela administration according to the control for both male and female groups. The same results were also obtained in other subgroup analysis including hyperaggregation group, hypo-normal aggregation group.

Conclusion: From the present study, normal dose of Chantaleela for alleviation of fever does not have an effect on either platelet aggregation or platelet numbers. It may conclude that the present study supports the safety use of Chantaleela for relieving fever, as platelet status does not need to be taken into consideration.

Keywords: Siriraj Ayurved recipe, Platelet aggregation, Adrenaline, ADP

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Chantaleela is one of the National List of Herbal Medicinal Products AD 2006. It is indicated for relieving fever. It has long been prescribed in Thai traditional folk medicine. Ayurved Siriraj Herbal recipe Chantaleela is composed of eight kinds of herbal

plants including *Gymnopetalum cochinchinense*, *Myristica fragrans*, *Dracaena loureiri*, *Tinospora crispa*, *Eurycoma longifolia*, *Atractylodes lancea*, *Angelica sylvestris* and *Artemisia vulgaris* Linn. Normal dose of Chantaleela for alleviation of fever is 750 mg orally every 8 hours. Non-clinical studies revealed the antipyretic action of Chantaleela in white rabbits at the dose of 400 mg/kg, and its efficacy and duration of action were equivalent to paracetamol 200 mg/kg⁽¹⁾. Toxicological evaluation of this medicinal

Correspondence to: Akarasereenont P, Pharmacology Department and Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-7569, Fax: 0-2419-7567. E-mail: sipak@mahidol.ac.th

remedy demonstrated a high safety profile. The LD₅₀ of the alcoholic extract of Chantaleela was 13.22 g/kg by oral route. For subchronic toxicity study, Chantaleela was administered to rats as a high dose of 40 mg/kg, which was 80 times higher than the normal dose used in humans. There was no abnormal finding in organ function and clinical laboratory value⁽²⁾. Furthermore, Chantaleela did not affect cell viability of human umbilical vein endothelial cell (HUVEC) at concentrations up to 1 mg/ml⁽³⁾.

Up to now, the exact mechanism of Chantaleela is still not clear despite the fact that it produces fever relief throughout the body. Prostaglandins may play an important role in its antipyretic mechanism, as in other non-steroidal anti-inflammatory drugs (NSAIDs). Since the production of prostaglandins is part of the body's inflammatory response to injury and fever, the inhibition of prostaglandin production by blocking the cyclooxygenase enzymes, COX-1 and COX-2, has long been known to be the mechanism of action of aspirin and other NSAIDs such as ibuprofen. However, their action in blocking COX-1 is also known to be responsible for inhibiting platelet aggregation causing unwanted side effects such as GI bleeding. The prescription of NSAIDs is, therefore, prohibited in dengue hemorrhagic fever. This should be the precaution for the prescription of Ayurved Siriraj Chantaleela as well. Some unwanted side effects of Ayurved Siriraj Chantaleela as in the NSAIDs cannot be ignored unless there is any supporting information. Thus, the present study was designed to evaluate the effect of Ayurved Siriraj Chantaleela on platelet aggregation and the safety profile of this remedy in normal Thai volunteers.

Material and Method

Materials

Ayurved Siriraj Chantaleela in the dosage of 250 mg tablets was obtained from Herbal Medicines and Products Manufacturing Unit, manufactured under GMP by Ayurved Siriraj, Center of Applied Thai Traditional Medicine (CATTM), Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The layout of Chantaleela fingerprint was shown in Fig. 1 and 2. Adrenaline (Adr) and Adenosine diphosphate (ADP) were purchased from Sigma® (USA).

Chantaleela solution preparation for chromatogram fingerprinting

Each batch of Siriraj Chantaleela recipe was accurately weighed and dissolved with 80% ethanol or

50% methanol, mixed and centrifuged at 15,000 rpm for 10 minutes at 4°C. After precipitation, each supernatant was filtered through a 0.2 µm membrane filter and used for thin layer chromatography (TLC) or ultra performance liquid chromatography (UPLC) analysis.

TLC chromatogram fingerprinting

Some filtrate of supernatant as mentioned above, was loaded using sample applicator (Camag Linomat 5, Switzerland) to TLC plates coated with silica gel 60 F₂₅₄ on aluminium sheets (Merck, Germany). Solvent system of hexane: ethyl acetate: acetic acid (31: 14: 5 v/v) was used as mobile phase for phenolic separation. The detection was examined under UV 254 nm, 366 nm and visible light after spraying with fast blue salt (FBS).

UPLC chromatogram fingerprinting

Another filtrate was injected into the UPLC with photodiode array detector (Water, Milford, MA, USA). The separation was performed on an Acquity UPLC column, 100 mm x 2.1 mm ID; particle size 1.7 µm (Water, Milford, MA, USA). The experiment was performed at a wavelength of 280 nm for 17 minutes.

Subjects

Twenty four participants were recruited (twelve males and twelve females) in the present study at Siriraj clinical study unit. Their mean age was 24.5 years (ranging from 19 to 30 years). The average body mass index was 20.6 kg/m² (ranging from 20.13 to 23 kg/m²). They were in good health on the basis of medical history, physical examination and laboratory value including complete blood count (CBC), fasting plasma glucose (FBS), blood urea nitrogen (BUN), creatinine, aspartate transaminase enzyme (AST), alanine transaminase enzyme (ALT), lipid profiles. Negative urine pregnancy test was required in female volunteers. For each participant, platelet aggregation test before Chantaleela administration was performed as a baseline control platelet aggregability.

Experimental design

The study protocol was approved by The Ethic Committee for Human Research, Faculty of Medicine Siriraj Hospital. The protocol is a before-after study design in normal volunteers who gave informed consent about the objective, method complication and benefit of the research.

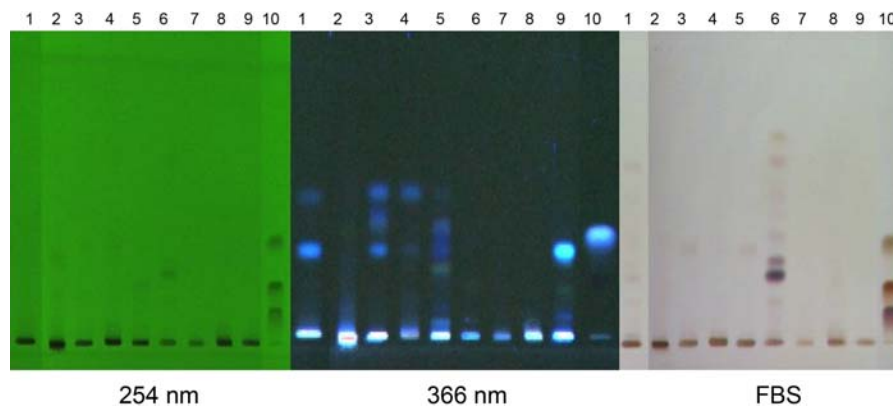
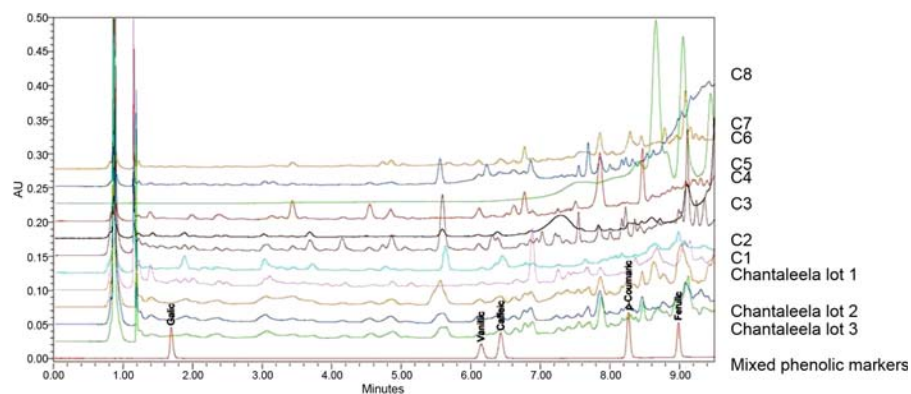
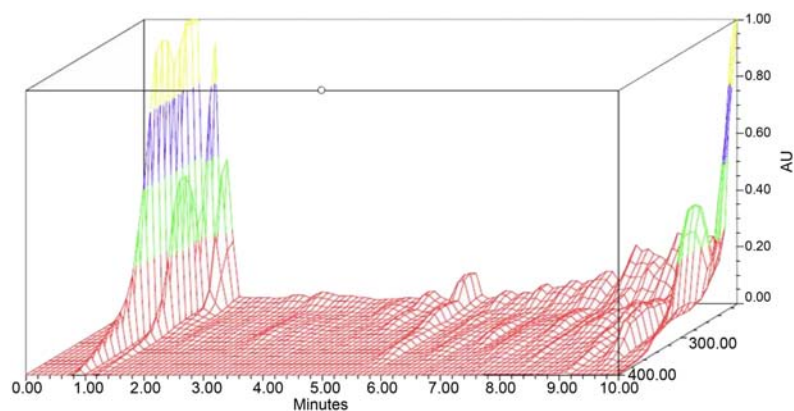


Fig. 1 TLC fingerprints of Ayurved Siriraj Chantaleela and its eight components visualized under UV 254, 366 nm and visible light (after spraying with fast blue salt: FBS). Lane1: Chantaleela; lane2-9: component no. 1-8; lane10: mixed phenolic markers (from bottom to top): kojic acid, gallic acid and caffeic acid



A) Ayurved Siriraj Chantaleela lot1-3 and its 8 components: C1 = *Gymnopetalum cochinchinense* (Lour.) Kurz (Ka-Dom), C2 = *Atracylodes lancea* (Thunb) DC. (Koad-Kamao), C3 = *Artemisia vulgaris* Linn. (Koad-Chulalumpa), C4 = *Angelica sylvestris* Linn. (Koad-So), C5 = *Dracaena loureiri* Gagnep. (Chan-Dang), C6 = *Myristica fragrans* Houtt. (Chan-Thet), C7 = *Tinospora crispa* (Linn) Miers ex.Hook.f.& Thoms. (Bor-Ra-Pet), C8 = *Eurycoma longifolia* Jack. (Pra-Lai-Peark)



B) Ayurved Siriraj Chantaleela lot 1 (3 dimension)

Fig. 2 UPLC-PDA chromatogram of Ayurved Siriraj Chantaleela was shown in 2 and 3 dimension (Panel A and Panel B)

Participants were not allowed to take any medication that might affect platelet aggregation for at least two weeks before the present study. After a screening test for baseline control, they were admitted at Siriraj Clinical Research Center to receive Chantaleela 750 mg (three 250 mg tablets) orally every 8 hours for 3 doses. They fasted overnight, then, their platelet aggregation was measured 8 hours following the first dose and at 32 hours after the first dose. At 8-10 days of follow-up, platelet aggregation was measured again in participants whose platelet aggregation was affected after Chantaleela administration. The time of blood sampling for each platelet aggregation test was the same time (± 1 hour) in order to reduce diurnal variation⁽⁴⁾. The adverse events were recorded throughout the present study and the assessment of casualty was done.

Platelet aggregation study

Whole blood was collected from antecubital vein and mixed with 3.8 percent sodium citrate 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⁽⁵⁾. Aggregation was determined by Born's technique⁽⁶⁾ using aggregometer with Adr and ADP as platelet agonists. Platelet aggregation was recorded as an increase in the percentage of light transmission and was evaluated by comparing the amplitudes of the aggregation curves. Each experiment was repeated twice.

Platelet aggregation was also detected by using microplate reader as previously described^(7,8) with some modification⁽⁹⁾. Reading of optical density (OD) at 595 nm was taken using kinetic mode of

microplate reader (Synergy HT, Biotex, USA) at 1 minute intervals for 8 minutes. 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: Mean values for each point were plotted against time.

$$\text{Percentage of aggregation} = (A-B)/(A-C) \times 100$$

A = OD PRP plus normal saline

B = OD PRP plus agonist

C = OD PFP plus normal saline

Statistical analysis

Results were shown as means \pm sem from the analysis group on separate experimental times. The difference between each group was analyzed by ANOVA using statistical software GraphPad Prism 5 and p-values less than 0.05 were accepted as statistically significant.

Results

The data obtained from the present study revealed that, the vital signs and laboratory value were not significantly different for participants after completion of the present study (Table 1) except for the decrease of mean hemoglobin value from 13.6 to 13.1 g/dL ($p < 0.0001$) and the reduction of mean hematocrit level from 41.0 to 39.5% ($p = 0.0018$). However, there was no clinical significance. No change in platelet numbers was detected following Chantaleela administration. All of the participants accomplished the present study. No adverse event was found throughout the present study.

Average percentage of maximum aggregation in aggregometer before and at 8 hours, 32 hours

Table 1. Comparing the values of different laboratory parameter following Chantaleela administration in 24 healthy volunteers. Values are given as mean (\pm SEM), CI 95% confidence intervals of difference

	Before		After		95% CI of difference	p-value
	Mean	SEM	Mean	SEM		
Hb (g/dL)	13.6	0.2	13.1	0.2	0.3-0.8	<0.0001
Hct (%)	41.0	0.6	39.5	0.7	0.6-2.4	0.0018
WBC ($\times 10^3/\mu\text{L}$)	6.3	0.4	6.6	0.4	-1.0-0.6	0.5666
Platelets ($\times 10^3/\mu\text{L}$)	257.0	8.0	263.0	10.0	-21.8-9.2	0.4084
BUN (mg/dL)	12.4	0.8	11.9	0.5	-1.2-2.2	0.5537
Creatinine (mg/dL)	0.78	0.04	0.8	0.04	-0.01-0.05	0.2031
AST (U/L)	19.7	0.7	18.5	0.7	-0.1-2.3	0.0654
ALT (U/L)	16.0	1.2	15.1	1.3	-1.4-3.1	0.4334

including 8-10 days after the first dose, demonstrated no significant changes, with neither Adr (Fig. 3) nor ADP (Fig. 4) as an agonist. The result was confirmed by using a microplate reader. For subgroup analysis of this data, the result also showed no significant change in percent of maximum aggregation in both male and female group (Fig. 5, 6).

According to the sensitivity of their platelets, the participants were also classified into hyper-aggregation group and hypo-normal group. The hyperaggregation group refers to the subjects whose platelet has biphasic or irreversible aggregation in response to only a small amount of an agonist (0.5-1 μM Adr). The hypo-normal group is defined as monophasic or reversible aggregation when exposed to a high

concentration of an agonist (25 μM Adr) or platelet aggregation pattern is dose response relationship with an agonist. The result revealed that platelet aggregation seemed to decrease after Chantaleela administration in hyperaggregation group and increased slightly in hypo-normal group. However, there was no statistical significant change of platelet aggregation after Chantaleela administration in each group using both Adr (Fig. 7) and ADP (Fig. 8) as an agonist.

Discussion

At present, commonly used antipyretic drugs are acetaminophen, aspirin and some NSAIDs. Although NSAIDs and aspirin are good antipyretic

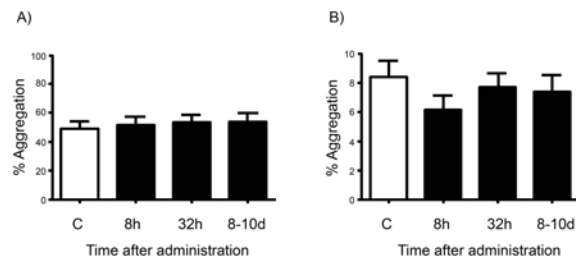


Fig. 3 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 25 μM adrenaline. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in aggregometer study, $p = 0.93$. Panel B showed percent of platelet aggregation in microplate reader study, $p = 0.45$

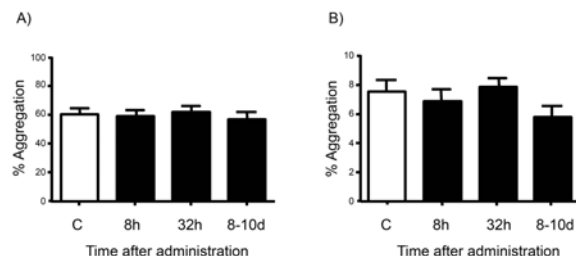


Fig. 4 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 5 μM ADP. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in aggregometer study, $p = 0.89$. Panel B showed percent of platelet aggregation in microplate reader study, $p = 0.34$

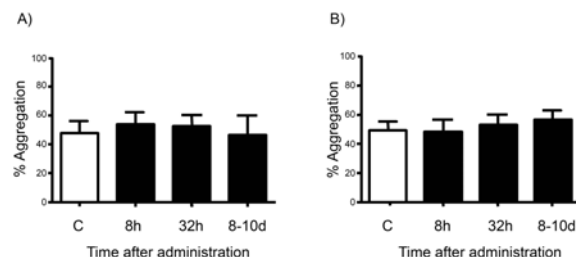


Fig. 5 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 25 μM adrenaline. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in male subjects ($n = 12$, $p = 0.93$). Panel B showed percent of platelet aggregation in female ($n = 12$, $p = 0.83$)

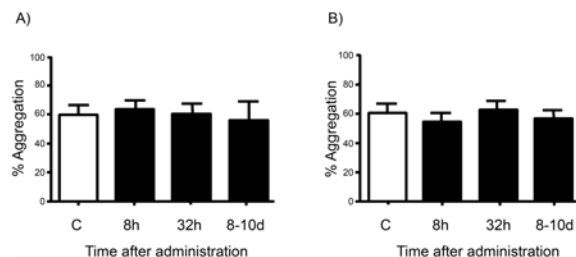


Fig. 6 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 5 μM ADP. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in male subjects ($n = 12$, $p = 0.93$). Panel B showed percent of platelet aggregation in female ($n = 12$, $p = 0.70$)

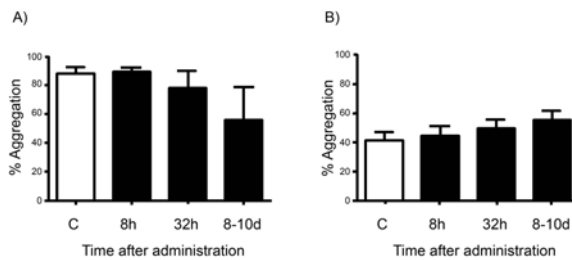


Fig. 7 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 25 μ M adrenaline. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in hyper aggregation group (n = 6, p = 0.18). Panel B showed percent of platelet aggregation in hypo and normal aggregation group (n = 18, p = 0.47)

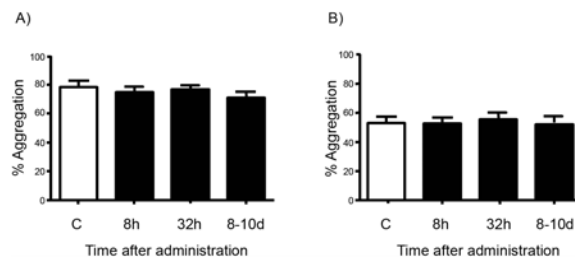


Fig. 8 The effects of Chantaleela on platelet aggregation induced *ex vivo* by 5 μ M ADP. Platelet aggregation after the first dose of 750 mg Chantaleela for 8 hours, 32 hours and 8-10 days were not significant difference from platelet aggregation of control. Panel A showed percent of platelet aggregation in hyper aggregation group (n = 6, p = 0.67). Panel B showed percent of platelet aggregation in hypo and normal aggregation group (n = 18, p = 0.96)

agents, they cause undesired side effects on platelet and gastrointestinal tract. In some diseases, *e.g.* dengue hemorrhagic fever, if the patient receives antipyretic drugs with antipyretic effect, their condition could worsen. So, it might be necessary to investigate the effect on platelet aggregation of an antipyretic drug.

In order to investigate the safe use of Ayurved Siriraj Chantaleela recipe, the authors examined its effect on human platelet aggregation induced by Adr or ADP. The present *ex vivo* study did not demonstrate any significant changes in the aggregation of platelets in all groups of the volunteers regardless of gender or baseline platelet aggregation. In consideration for each of Chantaleela components, the previous *in vitro* studies found that 3 stibenoids obtained from *Dracaena loureiri*, one of eight components of Chantaleela recipe, exhibited potent non specific COX inhibitor⁽¹⁰⁾. In contrast to this finding, Kaewkamson (2006) found that no platelet aggregation effect was observed in ethanolic extract of *Tinospora crispa*, the other component of Chantaleela recipe⁽¹¹⁾. At present, there is still no evidence in platelet aggregation effect for the rest of Chantaleela's components. Therefore, this was the first *ex vivo* study that investigated the effect of Chantaleela on human platelet aggregation. From this result, there was no significant change in platelet aggregation after Chantaleela administration even though one component of Chantaleela (*Dracaena loureiri*) exhibited potent nonspecific COX inhibitor *in vitro*. This may be explained by the following reasons. Firstly, dosage of *Dracaena loureiri* in Chantaleela recipe is not sufficient to exhibit the activity.

In the present *ex vivo* study, the pharmacokinetic factors including absorption, biotransformation and distribution could alter the action of the drug. Secondly, other components of Chantaleela may antagonize its effect on platelets. Finally, there may be change in active substances which affect on platelet functions by mixture of eight components.

The difficulty for evaluation of a traditional medicine like Chantaleela was finding the correct dose. The dosage regimen was not directly specified, as it was traditional used to relieve fever for a long time. The result obtained from the present study seems to support the safety use of Chantaleela as an antipyretic drug in which there is no effect on platelet aggregation, platelet numbers and no other serious adverse effect observed from the present study.

Acknowledgement

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การศึกษาฤทธิ์ยาจากสมุนไพรอายุรเวทศิริราชตำรับจันทลีลาต่อการจับกลุ่มของเกล็ดเลือด

รัฐ อธิพานิชพงศ์, อรุณี หลงปริษาเศรษฐ์, ศิริกุล โชติวุฒฒนกร, ประวิทย์ อัครเสรินนท์, ทศนี อ่อนโคกสูง, ทิชาภรณ์ ปาโล, สุพรชัย กองพัฒนากุล, สมฤดี ฉัตรศิริเจริญกุล, เปรมฤทัย ธิติลิศเดชา, ประสพพร พันธุ์เพ็ง, ทวี เลหาพันธ์

บทนำ: สมุนไพรอายุรเวทศิริราชตำรับจันทลีลาเป็นยาแผนโบราณที่ใช้รักษาอาการไข้ ซึ่งมีที่มาของตำรับจากโรงเรียนอายุรเวทศิริราช โดยมีพืชสมุนไพรเป็นส่วนประกอบทั้งหมด 8 ชนิด

การออกแบบการศึกษา: การวิจัยทางคลินิก แบบ ex vivo โดยเปรียบเทียบก่อนและหลังการได้รับยา

วัตถุประสงค์: เพื่อศึกษาผลของสมุนไพรอายุรเวทศิริราชตำรับจันทลีลา ต่อการจับกลุ่มของเกล็ดเลือด

วัสดุและวิธีการ: ทำการศึกษาอาสาสมัครสุขภาพดี จำนวน 24 คน เจาะเลือดเพื่อวัดระดับการจับกลุ่มของเกล็ดเลือดเป็นพื้นฐานก่อนการศึกษา สองสัปดาห์ต่อมา ให้อาสาสมัครกินยาเม็ดอายุรเวทศิริราชตำรับจันทลีลา 750 mg (เม็ดละ 250 mg จำนวน 3 เม็ด) ทุก 8 ชั่วโมง 3 ครั้งติดต่อกัน แล้วเจาะเลือดเพื่อวัดการจับกลุ่มของเกล็ดเลือดที่เวลา 8 ชั่วโมงภายหลังการกินยาครั้งแรกโดยใช้ aggregometer และ microplate reader สารกระตุ้นการจับกลุ่มของเกล็ดเลือดที่ใช้คือ adrenaline และ adenosine diphosphate (ADP) ต่อมาทำการวัดการจับกลุ่มของเกล็ดเลือดอีกครั้งที่ 32 ชั่วโมง และ 8-10 วัน ภายหลังการให้ยาครั้งแรก เพื่อติดตามผลที่อาจจะเกิดจากการกินยาจันทลีลา

ผลการศึกษา: อาสาสมัครทุกคนเข้าร่วมการศึกษาจนเสร็จสมบูรณ์ โดยไม่พบอาการไม่พึงประสงค์จากการใช้สมุนไพรอายุรเวทศิริราชตำรับจันทลีลา และจันทลีลาไม่มีผลต่อการจับกลุ่มของเกล็ดเลือดของอาสาสมัครไม่ว่าจะใช้ adrenaline หรือ ADP เป็นสารกระตุ้นการจับกลุ่มของเกล็ดเลือดทั้งโดยวิธีที่วัดด้วย aggregometer และ microplate reader และเมื่อแยกทำการวิเคราะห์ระหว่างเพศ หรือแยกวิเคราะห์ตามกลุ่มที่มีความไว ในการจับกลุ่มของเกล็ดเลือดที่ต่างกัน ทั้งในกลุ่มที่เกล็ดเลือดมีความไวมาก, ความไวปกติ หรือ ความไวน้อย ก็ได้ผลเช่นเดียวกันคือ สมุนไพรอายุรเวทศิริราชตำรับจันทลีลา ในขนาดปกติที่ใช้ลดไข้ ไม่มีผลต่อการจับกลุ่มของเกล็ดเลือดเมื่อเทียบกับก่อนกินยา

สรุป: จากการศึกษาพบว่า ยาจากสมุนไพรอายุรเวทศิริราชตำรับจันทลีลาในขนาดที่ใช้รักษาอาการไข้ไม่มีผลทั้งต่อการจับกลุ่มของเกล็ดเลือด และจำนวนเกล็ดเลือด จึงอาจสรุปได้ว่าจันทลีลาสามารถใช้ลดไข้ในผู้ป่วยได้โดยไม่ต้องคำนึงถึงภาวะการจับกลุ่มและจำนวนเกล็ดเลือดของผู้ป่วย
