A Short-term Safety Assessment of Herbal Medicine Combination: A Randomized Cross-over Controlled Trial in Healthy Volunteer

การประเมินความปลอดภัยระยะสั้นของสารสกัดสมุนไพรผสม แบบสุ่มและควบคุมเปลี่ยนจากยาจริงเป็นยาหลอกในอาสาสมัครสุขภาพปกติ

Anan Udombhornprabha[1,2], Naowarat Kanchanakhan[2] and Pichai Phongmanjit[3]
อนันต์ อุดมพรประภา[1,2], เนาวรัตน์ กาญจนาคาร[2] และพิชัย พงศ์มั่นจิต[3]

[1]Faculty of Pharmacy, Eastern Asia University
[1]คณะเภสัชศาสตร์ มหาวิทยาลัยอีสเทิร์นเอเชีย
[2]College of Public Health Sciences, Chulalongkorn University
[2]วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
[3]Chiang Rai Prachanukroh Hospital
[3]โรงพยาบาลเชียงรายประชานุเคราะห์

Abstract

We investigated short-term adverse drug events (ADEs) of 2% Asiaticoside and 1% Acemannan in 2% acetyl-salicylic-acid base external administration in 20 healthy volunteers. Data collection was conducted in hospital setting by using (i) a spontaneous safety report (ii) a weekly-diary summary of participants' self-reporting (iii) a Naranjo's algorithm assessment with physical examinations and alanine aminotransferase blood level test. A descriptive statistic with likelihood ratio Chi-square test for ADEs comparing active with placebo was analyzed. We found: (1) The outstanding ADEs for all participants reported for three weeks were 50 (40.3%) pruritus/itching sensation (ICD-10 L29), 45(36.2%) rash/other nonspecific skin eruption (ICD-10 R21), and 15 (12.1%) non-specified contact dermatitis/uncomfortable skin sensations (ICD-10 L25). (2) There were no significant changes for physical examination . During the first two weeks. (3) For the adjusted standardized likelihood ratio Chi-square test for active as compared with placebo, there was a significant different risk proportion of 1.113, 95% CI, 0.955 to 1.296, p-value < 0.001, attributable risk of 0.057, 95% CI, -0.025 to 0.136. However, during the second to the third week, (4) There was no significant different risk proportion of 1.164, 95% CI, 0.924 to 1.467, p-value of 0.28 for placebo vs active, attributable risk of 0.080, 95% CI, -0.042 to 1.999. In conclusion, the ADE findings were (1) Localized skin reactions at application site were similar between drug and placebo (2) There were no different changes on physical function and liver enzymes. (3) Other formulation adjuvant should be further established if this may cause ADE.

Keywords: herbal medicine combination 2% asiaticoside and 1%acemannan external gel, venous disease, adverse drug events, healthy volunteers

บทคัดย่อ

การทดลองเพื่อศึกษาผลข้างเคียงระยะสั้นจากการทายาสมุนไพรผสมเอเซียติโคไซด์ 2% เอซแมนแนน 1% กรดอะเซทิลซาลิไซลิก 2% เปรียบเทียบยาจริงกับยาหลอกชนิดไม่มีสารสกัดสองชนิดแรก วิจัยในอาสาสมัครปกติจำนวน 20 รายโดยรวบรวมข้อมูลจากอาสาสมัครปกติในโรงพยาบาลโดยใช้ (1) รายงานอาการสำคัญจากการทายา (2) รายงานสรุปจากบันทึกประจำสัปดาห์ของอาสาสมัคร และ (3) สรุปแนวทางนารันโจและการตรวจร่างกายและระดับเอนไซม์อะลานีนอะมิโนทรานเฟอร์เรสในเลือดก่อนและหลังสิ้นสุดการศึกษา วิเคราะห์ข้อมูลสถิติแบบพรรณนาด้วยการทดสอบ Likelihood ratio Chi-square test เปรียบเทียบอุบัติการณ์สัดส่วนอาการไม่พึงประสงค์จากยาจริงกับยาหลอก ผลการวิจัย (1) พบว่ายาจริงและยาหลอกทั้งหมดทำให้เกิดอาการผื่นคันผิวหนัง (ICD-10 L29) 50ครั้ง (40.3%) อาการปื้นบวมแดง (ICD-10 L21) 45ครั้ง (36.2%) อาการผื่นคันผิวหนังแบบอื่นและไม่เฉพาะเจาะจง (ICD 10L25) 15ครั้ง (12.1%) และ (2) การตรวจร่างกายทั่วไปไม่พบการเปลี่ยน แปลงอย่างมีนัยสำคัญ ระหว่างสองสัปดาห์แรก พบว่า (3) จากค่าสถิติ adjusted standardized likelihood ratio Chi-square test ยืนยันว่าสัดส่วนความเสี่ยงแตกต่างกันยาจริงมีความเสี่ยงสัมพันธ์มากกว่ายาหลอกที่ 1.113, 95% CI, 0.955 to 1.296 p-value < 0.001 ความเสี่ยงจากการทายาจริง ที่ 0.057, 95% CI,-0.025 to 0.136 แต่ระหว่างสัปดาห์สองและสาม (4) กลับพบความเสี่ยงไม่แตกต่างกันที่ 1.164,95% CI, 0.924 to 1.467 p-value < 0.283 และความเสี่ยงจากยาหลอกที่ 0.080, 95% CI,0.042 to 1.999 ผลการวิจัยสรุปว่า (1) อาการไม่พึงประสงค์ที่ผิวหนังเกิดเฉพาะที่บริเวณทายาไม่แตกต่างกันระหว่างยาจริงกับยาหลอก (2) ความดันโลหิตและระดับเอนไซม์ตับปกติ และ (3) ควรทำการศึกษาสารผสมอื่นในตำรับในการนำมาพัฒนาหากเป็นสาเหตุของการแพ้

คำสำคัญ: ยาสมุนไพรผสมเอเซียติโคไซด์ 2% เอซแมนแนน 1% เจลทาภายนอก, โรคหลอดเลือดดำหย่อนสมรรถภาพ, อาการไม่พึงประสงค์จากยา, อาสาสมัครปรกติ

Introduction

Chronic venous disease (CVD) is known for significant healthcare burden in the long-term among general population. High risk individual could end up with diagnosis of chronic venous insufficiency (CVI) as reported previously (Criqui, et al., 2003; Evans, et al., 1999; Graham, et al., 2003). High incidence of superficial varicose vein of 32.99% (95% CI, 27.99 to 37.99) with early leg symptoms had been reported among female factory workers. Previous investigation had confirmed that over 70% of hospitalized leg and foot ulcer patients were earlier diagnosed with venous origin. Meanwhile, the investigations by groups of vascular specialists, looking at different clinical presentations and patterns of patients with CVD had concluded that reflection on leg complaints symptoms varied differently among young patients from symptoms-free to aching and pain in the legs. During usual activities, the venous disease is progressively deteriorated as ongoing process. Some patients' complaints of leg symptoms have even been opted as major reasons for both job absentee and hospital admission (Eberhardt & Raffetto, 2005; Kanchanabat, et al., 2010). Currently, there is no standard medical treatment to inhibit the progression of disease especially during the early stage of disease (Bergan, et al., 2006). Although bandaging management remains major option, some degree of low patient adherence due to inconvenient and high long-term cost may probably render controversial for its effectiveness (Kolluri, 2011; Raju, Hollis & Neglen, 2007). A safe and effective medical treatment is therefore alternative option during early stage of disease. A basic literature search through electronic databases

PubMed, Medline and the Cochrane Library was initiated. The search query began with "drug treatment of chronic venous disease" filtered with subjected to clinical trial, available as at least abstracts and investigation only in human since 1960 to 2015. There were only 356 articles founded. Subsequently, three independent researchers screened through each of the abstracts to exclude the overlapping CVD reported within either the domain of other cardiovascular disease or CVD mimic with venous leg ulcers. Subject to data exclusion, a Delphi approach with consultations and agreements among researchers for the search data with a vascular specialist group were conducted. However, conflicting evidences of true efficacy of herbal drugs, and limitations of study designs is urgently need clarification. The major active herbal extracts (i) Asiaticoside (*Centella asiatica* (L.)) Urban., Asiatic Pennywort, Gotu Kola, Pegaga) (Bylka, et al., 2013; Gohil, Patel & Gajjar, 2010), (ii) Acetyl Salicylic Acid (Willow Bark Extract) (Del Río Solá, et al., 2012; Layton, et al., 1994; Tilbrook, et al., 2015) and (iii) Acemannan from Aloe vera (*A. barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam) were identified and selected based on possible synergistic of different mechanisms of action combined as intended for anti-inflammation, anti-fibrosis and anti-coagulation, antibacterial and immunomodulation. (Hausen, 1993; Sierra-Garcia, et al., 2014). The preparation of herbal medicine combinations external gel formulation was thus formulated and a pilot scale finished preparation for clinical investigation was carried out and completed.

Research Objective

To investigate short-term safety of an exposure to herbal extract combination as a pilot test for an external application by examining incidence of adverse drug events as compared with non-exposure in a three weeks cross-over trial among healthy volunteers.

Methodology

Preparation of the herbal medicine combinations

The herbal extracts of the HMC were acquired through reliable herbal extracts sources supplied globally. This active gel-bases HMC contains 1% Acemannan, 2% Asiaticosides in 2% acetyl-salicylic-acid base as part of ingredients. Specifically, Acemannan was a US organic Aloe vera in freeze dried powder with high contents of Acemannan (18%) with Acemannan short chain (< 50 KDa) as high as 4%. In the process of pre-formulation, formulation and finished preparation as soluble gel-base, ASA was firstly prepared as solid-in-oil encapsulation within Beeswax and mixed with Dimethyl Isosorbide (2%) to disinhibit the hydrolysis process before incorporation of the rest of herbal active ingredients and adjuvants. The battery of tests including physico-chemical stability and microbiological tests were performed for short-term clinical investigation, which confirmed HMC gel-based preparations were stabled. Later, 35 grams of each HMC gel were repacked and stored in the aluminum tubes (size of 45-gram tube) prepared for both safety and clinical efficacy investigation. In addition, identical placebo ASA gel-base in color and odor but free of any of the two active ingredients were prepared.

The safety study protocol

The efficacy and safety investigation in CVD patients and healthy volunteers were pre-approved by Chulalongkorn University and hospital ethical approval for patient accessibility was granted to investigate at Somdet Phra Yanna Sangworn (SDPY) Hospital Wiang Chai, Chiang Rai, Thailand. The HMC for CVD trial was registered with ISRCTN No.54360155 (http://www.isrctn.com/ISRCTN54360155) and conformed with the investigation in human which followed the guidelines of the Declaration of Helsinki

and Tokyo for humans which institutionally approved and informed consent obtained. The detail investigation of the HMC gel preparation will be reported in other relevance publication.

Study design

This is a prospective single-blinded (blinding to patient) two-week active interventions, the first and third week as active treatment, and placebo-controlled during the second week as cross-over design, providing with a three-day washout period. This is a pilot study cross-over design, as such should minimize subject variation among 20 healthy volunteers.

Inclusion criteria

Any 20 healthy volunteers both genders aged over 18 years old completed participation informed-consent were recruited from the SPDY Hospital Wiang Chai and confirmed to follow-up as per regular self-assessment criteria in the weekly diary of ADEs.

Exclusion criteria

Healthy volunteers with known allergy or suspected allergy to ASA, aloe vera and asiaticoside were excluded.

Assessment of safety and reporting of ADEs

During inclusion, physical examinations and blood sample were collected for liver enzyme assessment. Drug administration were detailed to participants by one of two research assistants. Participants were instructed to squeeze out 1 tablespoon of gel in the aluminum tube and gently apply externally on lower part of both legs for twice daily in the morning and before bedtime. They were allowed to stop the administration of the gel immediately and washout with water, should they experience any intolerable side effects especially any serious systemic symptoms including hypersensitivity reactions such as skin eruption as well as suspecting bleeding events. ADEs assessment were summary collections of self-reports by participants from weekly diary ADEs for one week. After each week follow-up, all aluminum tubes provided together with ADEs weekly diary report were returned to investigator. At each follow-up visits, regular physical examinations including hemodynamics assessment of arterial systolic/diastolic blood pressure and a palpable heart rate were assessed using standard sphygmomanometer. The last blood sample was collected after the final visit at the end of 3-week. This study was intended for investigation of ADEs and therefore the Naranjo algorithm scoring by interviewing approach for individual were performed after each week, through direct enquiry as in a challenge phase during the first week, then a de-challenge phase during the second week and again as a re-challenge phase during the third week meanwhile all patients remain in blinding during a three-day washout period for each week assessment. There was one volunteer reported with serious burning sensation and decided to drop out after the first week, details of which were given in Figure 1. We decided to choose only outstanding symptoms ADEs (Intolerable itching sensation and burning sensation) as criteria for Naranjo's algorithm assessment. Overall, ADEs reports were of three types as (i) the participant self-report weekly diary adverse event report (ii) the Safety Monitoring Protocol (SMP) as summary report prescribed by Thai FDA guidelines (http://drug.fda.moph.go.th/zone_service/files/ser016_07.pdf) and (iii) the summary report as per the Naranjo algorithm score Naranjo algorithm (Thai) (http://part.sopmoei.com/pharm/files/Naranjo.pdf). The analysis of results employed descriptive statistics using Statistical Package for the Social Sciences version 17.0 SPSS (PASW) 17.0 and were presenting as descriptive statistics as frequency in percentages, the relative risk (RR), attributable risk (AR) incidence were computed and the proportion of incidence with the likelihood ratio

Chi-square statistics test for predicting proportion of ADEs comparing active intervention with placebo intervention were described. The overall outstanding ADEs reports from participants' summary in the patients' weekly diary were summarized from all participants with active intervention as compared with placebo employing the likelihood ratio Chi-square statistics test (G) assuming the null hypothesis where the ADEs is not different.

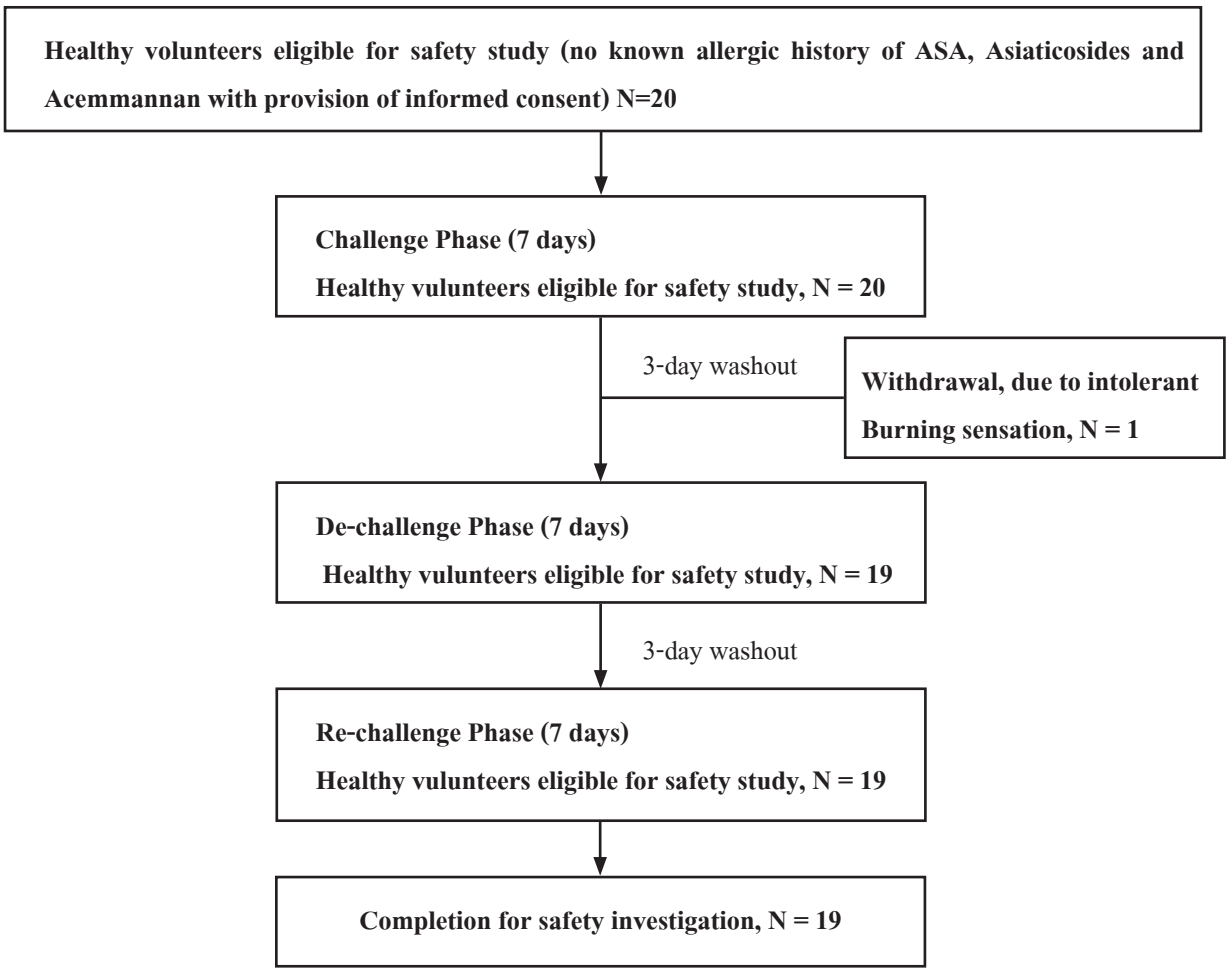

Figure 1 Flow diagrams for patient recruitment

Note. Burning sensation denoted by pain dissipated at the surrounding area with reddenig of skin.

Results

20 healthy volunteers were recruited through the individual on voluntary basis from hospital officers, SDPY hospital during September 1st to October 30th, 2015. A prospective recruitment was an observational basis. All volunteers contacted hospital surgery department for inquiry and learn about the ongoing research before interviewing by research assistants to screen for eligibility. All relevance data of participants' baseline demographic characteristics were provided in Table 1.

Table 1

Baseline demographic characteristics of healthy volunteers

Clinical Characteristics	Mean ± SD, (Range)
Gender (Men/Women)	Number of subject, N = 20, 11/9)
Age (years old)	29.2 ± 4.1, (13)
BMI (kg/m2)	23.1 ± 1.9, (6.9)
ALT (u/l)	28.80 ± 5.49, (16.6)
DSB (mmHg)	84.00 ± 5.62, (20.0)
SBP (mm Hg)	135.95 ± 2.76, (9.0)
HR (bpm)	70.55 ± 4.09, (14.0)

Note. DSB = Diastolic Blood Pressure, SBP = Systolic Blood Pressure, in mmHg; HR = Heart rate, bpm = beat per minute; BMI = Body Mass Index in kilogram per square metre; ALT =Alanine Aminotransferase in international unit per litre. The ALT determined whether skin absorption is significant thereby rendering liver enzyme response.

Outstanding Summary of ADEs

The ADEs summary as per SMP were provided in Table 2. These outstanding ADEs incidences were mild localized skin reactions and confined to skin area where medication was externally applied with pruritus and itching sensation (ICD-10 L29) of 50 (40.3%), with rash and other nonspecific skin eruption (ICD-10 R21) of 45 (36.2%), the non-specified contact dermatitis and uncomfortable skin sensations (ICD-10 L25) of 15 (12.1%). There were no systemic ADEs and negligible hemodynamic changes during physical examination with mean ± SD for SBP at recruitment of 135.95 ± 2.76 (mmHg) and after the final visit of 136.75 ± 2.14 (mmHg) (p = 0.130), DBP at recruitment of 84.00, ± 5.62 (mmHg) and 85.60 ± 3.08 (mmHg) (p = 0.099), and mean palpable heart rate of 70.55 ± 4.09 (beat per minutes) and 71.42 ± 4.0 (beat per minutes) (p = 0.401), and the alanine aminotransferase during the follow-up of 28.80 ± 5.49 (u/l) and 28.61 ± 5.50 (u/l) (p = 0.096) respectively. These parameters at final assessment reflected no significant different from baseline as given in Table 3.

Table 2

A summary report of Safety Monitoring Protocol (SMP) of Thai FDA regulation

Outstanding ADE	Number (Percentage) of ADE after 3 Weeks
Incidence of pruritus and itching sensation (ICD-10 L29)	50 (40.3%)
Incidence of rash and other nonspecific skin eruption (ICD-10 R21)	45 (36.2%)

Table 2

A summary report of Safety Monitoring Protocol (SMP) of Thai FDA regulation (continue)

Outstanding ADE	Number (Percentage) of ADE after 3 Weeks
Incidence of non-specified contact dermatitis and uncomfortable skin sensations (ICD-10 L25)	15 (12.1%)
Overall ADE reported	**124 (100.0%)**

Note. (1) ADE = Adverse Drug Event (2) ADE reported were localized mild reactions during the 3 weeks trial. (3) All ADEs were classed as torelated ADE, except one participant was withdrawn after 1 week due to intorelated burning sensation. (4) ICD-10 = International Classification of Disease Code of WHO

Table 3

Hemodynamic and laboratory assessments at baseline and after three weeks

Clinical Characteristics	Mean ± SD, (Range)		p-value
	Baseline - Day 0	Final - After 3-Week	
Diastolic Blood Pressure (mm Hg)	84.00 ± 5.62, (20.0)	85.60 ± 3.08, (12.0)	p = 0.099
Systolic Blood Pressure (mm Hg)	135.95 ± 2.76, (9.0)	136.75 ± 2.14, (9.0)	p = 0.130
Heart Rate (bpm)	70.55 ± 4.09, (14.0)	71.42 ± 4.0, (12.0)	p = 0.401
ALT (u/l)	28.80 ± 5.49, (16.6)	28.61 ± 5.50, (16.6)	p = 0.096

Note. DSB = Diastolic Blood Pressure, SBP= Systolic Bollod Pressure, HR = Heart rate , bpm = beat per minutes, BMI = Body Mass Index in kilogram per square metre, ALT = Alanine Aminotransferase in international unit per litre *p-value by paired t-test with significnat at p-value < 0.05

Proportional risk incidence for the Active and Placebo during 3 weeks

During the three-week trials, all ADEs were computed as the proportion difference reported by each individual participant from each week and thereby analysis performed using the likelihood ratio Chi-square statistics test (G) with the hypothesis that the proportion was not difference at 80% power to detect with statistical significant at p-value < 0.05. Though higher incidence was noted for active treatment as compared with placebo (for Week 1 vs Week 2) yielding the RR and AR of active as compared with placebo of 1.113, 95% CI, 0.955 to 1.296) and of 0.057, 95% CI, -0.025 to 0.136 respectively. The same, with adjusted likelihood ratio Chi-square statistics test with p < 0.001 leading us to reject the null hypothesis based on our hypothesis. There was no plausible sufficient evidence to conclude that the risk proportion was not difference. Furthermore, we found that lower incidence was noted for active treatment as compared with placebo (for Week 3 vs Week 2) yielding the RR and AR for active as compared with placebo of 1.164, 95% CI, 0.924 to 1.467) and attributable risk of 0.080, 95% CI, -0.042 to 1.999 respectively during extended duration. For the same,

with adjusted likelihood ratio Chi-square statistics test suggesting us to retain the null hypothesis (p = 0.283) leading to our assumption to accept the null hypothesis to conclude that the proportion was in fact no statistical significant difference. In this respect the RR and AR from active treatment with extended duration was in the contrary less than that of placebo. The overall analysis results were given in Table 4 and Figure 2. Moreover, final assessment at the end of trial, there was no significant changes of hemodynamics parameters before treatment with mean ± SD of diastolic/ systolic blood pressure/ heart rate of 84.00 ± 5.62, 135.95 ± 2.76 mmHg, 70.55 ± 4.09 bpm and after treatment of 85.60 ± 3.08, 136.75 ± 2.14 mmHg, 71.42 ± 4.0 bpm, (p = 0.099, p = 0.130, p = 0.401) respectively. The same for alanine aminotransferase level before treatment with mean ± SD of 28.80 ± 5.49 and after treatment of 28.61 ± 5.50 (p = 0.096). Overall, physical function assessments and blood tests results were given in Table 3. This could confirm that there were no remarkably outstanding effects of the medication and the adjuvants on the short-term application both on the hemodynamics and liver enzyme, in this respect we test for alanine aminotransferase. This may possibly due to minimal skin absorption should be noted as intended topical application of this HMC. However, further in-vitro investigation on the release of the active Asiaticoside, Acemannan and acetyl salicylic acid may be performed.

Table 4

Adverse Drug Events Reports of Participant Weekly Diary, Proportion Difference and Comparison During Three Weeks Exposure (Active vs Placebo)

Treatment Type(Period) [No.of participants with events / no events]	**With ADE Incidence**	**No ADE Incidence**	**Likelihood - Chi-square Statistics**
Active (W1) (HMC) [16 / 4]	52	5	*p < 0.001
Placebo (W2) [12 / 7]	41	9	RR of 11.13% (1.113, 95% CI, 0.955 - 1.296) AR of 5.7% (0.057, 95% CI, -0.025 - 0.136)
Active (W3) (HMC) [13 / 6]	31	13	*p = 0.283
Placebo (W2) [12 / 7]	41	9	RR of-6.59% (-0.659,95% CI, -0.682 - 1.083) AR of-2.71% (-0.271,95% CI, -0.175 - 0.037)

Note. Subject (N) = reported from numbers of subjects in each week; RR = Relative Risk as defined by ratio of [probability of risk between HMC/probability of risk Placebo]; AR = Attributable Risk as defined by ratio of [incidence for HMC- incidence Placebo] / incidence HMC; One subject dropouted at the end of week 1

*p-value adjusted standardized likelihood ratio Chi-sqaure test, significant at p < 0.05, W1 = Week1, W2 = Week2, W3 = Week3

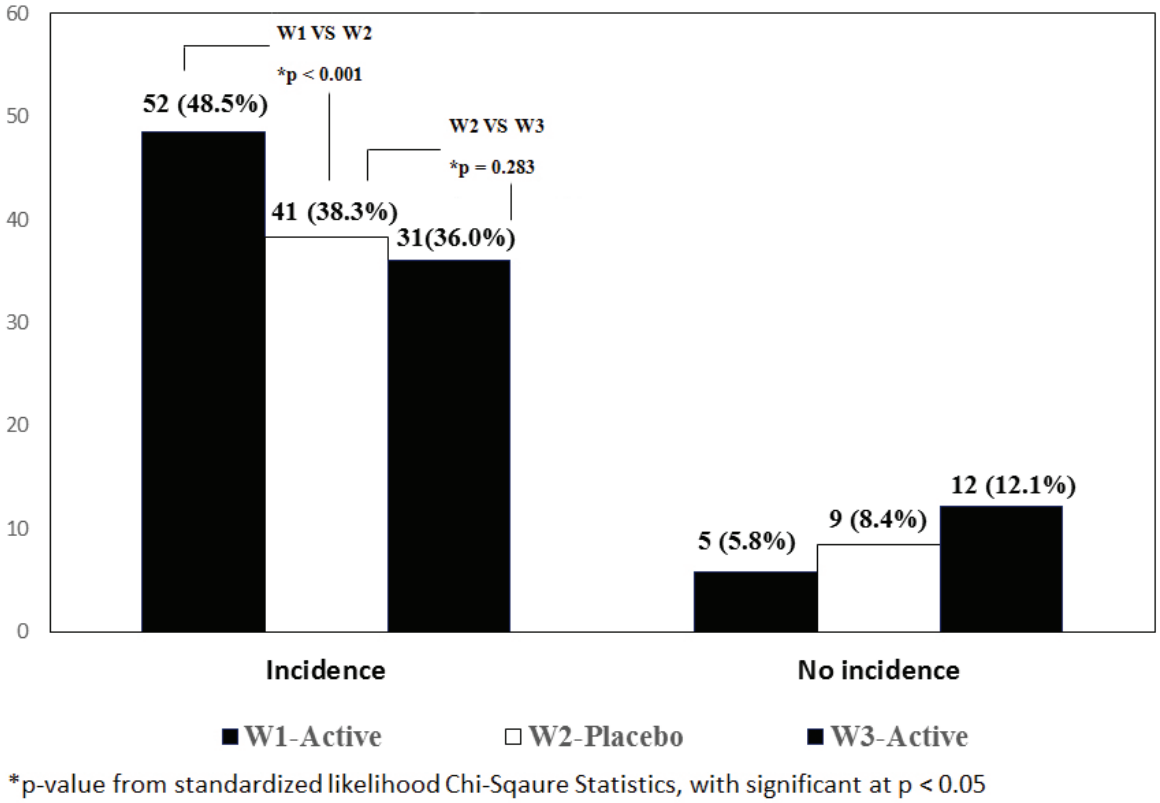

Figure 2 Three week adverse events reported during exposure comparing active vs placebo in a 3-week cross-over setting for all events reported combined

Note. Percentage of reported ADE incidence calculated as proportion of all ADEs reported per week. Estimate Difference of Risk indicated the proportion of ADE difference based on numbers of participants [with events / no events] and the p-value was estimated by adjusted standardized likelihood ratio Chi-square test with a significant level at $p < 0.05$ (given in table 3) where events combined during W1 + W2 (107) and W2 + W3 (93); One subject droped out at the end of W1, W1 = Week 1, W2 = Week 2 and W3 = Week 3, X-Axis is Type of Report and Y-Axis = Percentage of Report.

Naranjo's Scores Assessment

The assessments of individual participants using the Naranjo algorithms by direct inquiry from data gathering during each follow-up visit from 19 participants were performed. However, among 19 participants, the ADEs outstanding should be interpreted as possible with the average Naranjo's score between 1-4. These results were unable to rule out should the effects could be due to the adjuvant of the same formulation thus worth further investigation. Should the formulation intended for further investigation in patients, further formulation improvement may be anticipated. The overall analysis of naranjo's score for individual assessment criteria with the average score were given in the Table 5.

Table 5

Naranjo Algorithm assessment of each individual after 3 weeks (N=19)

Assessment item	Naranjo Score, Mean ± SD (Range, Min-Max)
1. Are there previous conclusive reports on this reaction?	0.0 ± 0, (0, 0 to 0)
2. Did the adverse event occur after the suspected drugs was administered?	1.37 ± 0.95, (0, 0 to 0)
3. Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	0.0 ± 0, (0, 0 to 0)
4. Did the adverse reaction reappear when the drug was re-administered?	0.42 ± 1.53, (3, -1 to 2)
5. Are there alternative causes (other than the drug) that could have on their own caused the reaction?	0.0 ± 0, (0, 0 to 0)
6. Did the reaction reappear when a placebo was given?	0.26 ± 0.45, (1, -1 to 0)
7. Was the drug detected in the blood (or fluids) in concentration known to be toxic?	0.0 ± 0, (0, 0 to 0)
8. Was the reaction severe when the dose was increased or less severe when the dose was decreased?	0.0 ± 0, (0, 0 to 0)
9. Did the patients have a similar reaction to the same or similar drug in any previous exposure?	0.0 ± 0, (0, 0 to 0)
10. Was the adverse events confirmed by any objective evidence?	0.0 ± 0, (0, 0 to 0)
Total average of Naranjo's score	**1.42 ± 2.00, (5, -1 to 4)**

Note. The assessed events selected were outstanding events according to participants (Itching with uncomfortable skin stickiness; Skin reddening with mild burning sensation, Sweating and swelling at area applied); Naranjo's score was a combined score in average of 19 participants.The assessment began after the ends of trial as all of the adverse reactions was mild and well tolerated. Interpretation of score: 9 or greater is definitive, 5-8 is probable, 1-4 is possible

Conclusion

Our findings concluded that this HMC containing 2% Asiaticosides and 1% Acemannan in a 2% ASA-base external preparation could produce burning sensation. The incidence of adverse events as high as 48.5% among 19 patients treated for 3 weeks. These effects could be similarly spotted in the placebo preparation at 38.3% and appear less in extended period of active drug application of 36.0%. The ADEs were localized skin reactions, no significant hemodynamics changes and no abnormal liver enzyme detected during short-term investigation. This confirmed safety of HMC topical preparation. However, formulation further development is needed should the preparation intended for external use for therapeutic investigation.

Discussion

The incidence of ADEs and proportional difference among all active and placebo during three week interventions were compared as given in table 2 and table 3 with percentage of events reported illustrated in the figure 2. The first week events reported were higher than the second and the third week in a row. Most participants could continue trial up to 3 weeks. They reported that these reactions were only localized and acceptable which allow them to continue for 3 weeks. However, one participant dropped out early at the end of first week due to uncomfortable burning sensation. During the second week, placebo intervention also rendered some localized skin reaction though less frequent than the first week. The reported incidence seems to repeatedly occur in the same subject, may probably due to individual reaction or specific skin type per se. The RR and AR incidence among active and placebo intervention provided significant higher incidence in the active group leading to rejection of the null hypothesis whereby the likelihood Chi-square statistic test, $p < 0.001$. As such it could be likely that the reported incidence was probably as results from the HMC. However, after comparing the placebo and the active intervention during the second and the third week, it was founded that placebo intervention though had higher incidence as compared with the active intervention reporting with the likelihood Chi-square test, $p = 0.283$ suggested us to retain the null hypothesis, surprisingly the overall RR and AR of active HMC was less than that of placebo. There was no sufficient evidence to confirm that ADEs in placebo group was more or less similar to the HMC after extended period application. The skin sensitization may lead to higher threshold or lower skin sensitivity such that adaptation of the skin to treatment should have been observed. The medications induced adverse effects may possibly temporary and could disappear during prolonged treatment which have been observed with tretinoin on long term skin exposure previously reported (Goh, 1990). In addition, whether participants' self-reporting recall bias or possibly patients tended to violate the trial through application of less amount of active as compared with placebo during the next following second and third week. These could be limitations of the trial, and the overall ADEs founded maybe caused by added adjuvant. Whether the ADEs incidence may cause directly by the base formulation or whether specific skin hypersensitivity and skin threshold adaptability in a later application remains to be answered. Nevertheless, adjuvants base should be critically selected in the clinical trial of drug aims for external use. Although asiaticoside was well tolerated in experimental animals by oral route with no sign of toxicity dose up to 1 mg per kilogram body weight (Izu, et al., 1992), asiaticoside has no known toxicity in recommended oral doses, certain ADEs though rare e.g. headache, stomach discomfort, nausea, dizziness and extreme drowsiness depending on the doses may be spotted. Moreover, some degree of skin allergy with burning sensation and contact dermatitis for topical asiaticoside had been reported caused by oral ingestion (Izu, et al., 1992). In this attempts there were no observable ADEs likely to be similar to internal use. The topical application could be a safe topical route as there is no possible adverse events observed. Since an acute bullous allergic reaction and contact urticaria have been reported as a results of aloe vera gel (Morrow, Rapaport, & Strick, 1980), however, this topical preparation contains only the more purified fraction of Acemannan, there should be minimal risk of such acute allergic reaction and contact dermatitis observed. Nevertheless, subsequent to three week interventions, overall reporting incidence was less troublesome and well tolerated. As such, the base vehicles for the formulation may play role in rendering similar localized skin reaction, which could be particular nuisance leading to noncompliance if such medication shall be administered to patients due to patients' higher skin sensitivity. This could be expected differently

from the healthy skin. At the end of the 3 weeks, there were no statistically significant different for physical function especially hemodynamic parameters including systolic/diastolic blood pressure, heart rate and laboratory finding for the alanine aminotransferase. These results affirmed our opinion that HMC shall render minimal systemic adverse events. However, further investigation looking at efficacy assessment criteria in chronic venous insufficiency is needed despite its safety profile.

The Naranjo algorithms assessment demonstrated certain limitations of the study due mainly to (i) Some limitations in determination of the plasma level of active substance due to standard substance and laboratory facility not feasible. (ii) The short period of 3-day washout as intended prior to the next de-challenge and re-challenge period may be insufficient to allow proper detection of ADEs as well as possible recall bias. (iii) Only selected outstanding adverse.

Funding

This study was funded by the National Research University, Office of the Higher Education Commission (WCU-58-304-AS) for Chulalongkorn University Research Fund under the research cluster in Ageing.

References

Bergan, J. J., Schmid-Schonbein, G. W., Smith, P. D., Nicolaides, A. N., Boisseau, M. R., & Eklof, B. (2006). Chronic venous disease. *The New England Journal of Medicine*, *355*(5), 488-498.

Bylka, W., Znajdek-Awizen, P., Studzinska-Sroka, E., & Brzezinska, M. (2013). Centella asiatica in cosmetology. *Postepy Dermatol Alergol*, *30*(1), 46-49.

Criqui, M. H., Jamosmos, M., Fronek, A., Denenberg, J. O., Langer, R. D., Bergan, J., & Golomb, B. A. (2003). Chronic venous disease in an ethnically diverse population: the San Diego population study. *American Journal of Epidemiology*, *158*(5), 448-456.

Del Río Solá, M. L., Antonio, J., Fajardo, G., & Vaquero Puerta, C. (2012). Influence of aspirin therapy in the ulcer associated with chronic venous insufficiency. *Annals of Vascular Surgery*, *26*(5), 620-629.

Eberhardt, R. T., & Raffetto, J. D. (2005). Chronic venous insufficiency. *Circulation*, *111*(18), 2398-2409.

Evans, C. J., Fowkes, F. G., Ruckley, C. V., & Lee, A. J. (1999). Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein study. *Journal of Epidemiology and Community Health*, *53*(3), 149-153.

Goh, S. H. (1990). The treatment of visible signs of senescence: The Asian experience. *British Journal of Dermatology*, *122*(Special), 105-109.

Gohil, K. J., Patel, J. A., & Gajjar, A. K. (2010). Pharmacological review on Centella asiatica: A potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences*, *72*(5), 546-556.

Graham, I. D., Harrison, M. B., Nelson, E. A., Lorimer, K., & Fisher, A. (2003). Prevalence of lower-limb ulceration: A systematic review of prevalence studies. *Advances in Skin & Wound Care*, *16*(6), 305-316.

Hausen, B. M. (1993). Centella asiatica (Indian pennywort), an effective therapeutic but a weak sensitizer. *Contact Dermatitis*, *29*(4), 175-179.

Izu, R., Aguirre, A., Gil, N., & Diaz-Perez, J. L. (1992). Allergic contact dermatitis from a cream containing Centella asiatica extract. *Contact Dermatitis*, *26*(3), 192-213.

Kanchanabat, B., Wongmahisorn, Y., Stapanavatr, W., Kanchanasuttirak, P., & Manomaiphiboon, A. (2010). Clinical presentation and patterns of venous reflux in Thai patients with chronic venous insufficiency (CVI). *European Journal of Vascular and Endovascular Surgery*, *40*(3), 399-402.

Kolluri, R. (2011). Compression therapy for treatment of venous disease and limb swelling. *Current Treatment Options in Cardiovascular Medicine*, *13*(2), 169-178.

Layton, A. M., Goodfield, M. J. D., Ibbotson, S., & Davies, J. A. (1994). Randomised trial of oral aspirin for chronic venous leg ulcers. *The Lancet*, *344*(8916), 164-165.

Morrow, D. M., Rapaport, M. J., & Strick, R. A. (1980). Hypersensitivity to aloe. *Archives of Dermatology*, *116*(9), 1064-1065.

Raju, S., Hollis, K., & Neglen, P. (2007). Use of compression stockings in chronic venous disease: patient compliance and efficacy. *Annals of Vascular Surgery*, *21*(6), 790-795.

Sierra-Garcia, G. D., Castro-Rios, R., Gonzalez-Horta, A., Lara-Arias, J., & Chavez-Montes, A. (2014). Acemannan, an extracted polysaccharide from Aloe vera: A literature review. *Natural Product Communications*, 9(8), 1217-1221.

Tilbrook, H., Forsythe, R. O., Rolfe, D., Clark, L., Bland, M., Buckley, H., . . . Hinchliffe, R. J. (2015). Aspirin for venous ulcers: Randomised trial (AVURT): study protocol for a randomised controlled trial. *Trials*, *16*(2015), 513.