Original Article

The Clinical Safety of Sahastara Remedy Ethanolic Extract Capsules in Healthy Volunteers

Puritat Kanokkangsadal MSc[1], Preecha Wanichsetakul MD[2], Arunporn Itharat PhD[1,3]

[1] Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[2] Department of Obstetrics and Gynecology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[3] Center of Excellence in Applied Thai Traditional Medicine Research, Thammasat University, Pathum Thani, Thailand

Background: The Sahastara [SHT] remedy is a Thai traditional medicine which has long been used for muscle and joint pain treatment. The SHT remedy has potential to develop as modern drug in extract form. The clinical safety of SHT remedy extract need to be study as a part of phytomedicine development.

Objective: To investigate the clinical safety of capsules of SHT remedy ethanolic extracts in doses of 300 and 600 mg/day, in healthy volunteers.

Materials and Methods: Twenty-four eligible healthy volunteers were recruited into the study. They were divided into 2 groups (6 males and 6 females each group). They were required to take 1 or 2 capsules of 100 mg of SHT extract orally 3 times a day before meals for 28 days. The follow-up including history taking, complete physical examination, liver function test, and renal function test were performed every 14 days and continued for 14 days after the volunteers stopped taking SHT extract capsules.

Results: The SHT remedy extract capsules were quite safe to use in humans for 28 days. The results showed the most common adverse events of 600 and 300 dose groups were abdominal discomfort as 7 of 12 and 8 of 12, respectively. The severity was mild to moderate grade. The adverse events were relieved within 14 days after take medicine. Laboratory results showed no clinical significant different changing within group. There was also no significant difference between dose groups especially liver and renal functions including AST, ALT, ALP, and creatinine (p>0.05). Although BUN show statistically significant difference between dose group, it was not significantly changed in clinical consideration.

Conclusion: The SHT remedy extract capsules were safe to use in humans. There was no toxicity on liver or renal function and only mild systemic side effects. The 300 mg/day is the suggested dose to use in humans.

Keywords: Sahastara remedy, Safety, Anti-inflammatory, Quality control

J Med Assoc Thai 2018; 101 (10): 1429-36
Website: http://www.jmatonline.com

As a result of lower fertility rates and longer life expectancy, the world is an aging society. The world health organization [WHO] estimated people aged 60 and older are projected to be 1.5 billion in 2050 from 524 million in 2010(1). The problem of degenerative disease is also increasing. One of the most common degenerative diseases of the musculoskeletal system is osteoarthritis [OA]. OA leads patients to disability of joint function and poor quality of life. Inflammation is the main cause of joint pain, so anti-inflammatory drugs especially non-steroidal anti-inflammatory drugs [NSAIDs] are often used to treat OA. However, there are side effects and toxicity to liver and renal functions from NSAIDs in long term use. Alternative medicine such as herbal medicine has become interesting including Thai traditional medicine [TTM].

There are several ways to treat OA in TTM including massage, hot herbal ball compression, topical medicine and oral medicine. The Sahastara [SHT] remedy is most commonly used as oral medicine to treat muscle and joint pain and has been used for more than 50 years. The SHT remedy is published in the National List of Essential Medicine [NLEM] of Thailand which assumes it is quite safe to use in humans. It consists of 21 medicinal plants. The main ingredients are pepper (*Piper nigrum* Linn.) and long pepper (*Piper retrofractum* Vahl.). Previous studies demonstrated that SHT remedy extract showed high anti-inflammatory activity by inhibition of nitric oxide [NO] and prostaglandin E_2 [PGE_2] with IC_{50} 2.81µg/ml and 16.97 µg/ml, respectively(2), which are similar

Correspondence to:
Itharat A. Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand.
Phone & **Fax**: +66-2-9269749
Email: iarunporn@yahoo.com

How to cite this article: Kanokkangsadal P, Wanichsetakul P, Itharat A. The clinical safety of Sahastara remedy ethanolic extract capsules in healthy volunteers. J Med Assoc Thai 2018;101:1429-36.

to mechanisms of NSAIDs. SHT remedy ethanolic extract is reported to show no toxicity either acute or chronic in rat[3]. A clinical study of SHT remedy powdered drug in primary knee OA patients supported previous results that SHT remedy can reduce pain in knee OA and improve quality of life with equal efficacy to diclofenac[4]. However, the powdered drug leads patients to poor compliance because they must to take too many capsules per day. According to the reason above, SHT remedy has potential to be developed as a modern drug and could solve the inflammatory disorder problem. Thus, the clinical safety of capsules of SHT remedy ethanolic extract needs to be studied.

Materials and Methods

Research design

The present study was a clinical trial phase 1 which investigated the clinical safety of SHT remedy ethanolic extract in healthy volunteers. The present study included two doses of 300 and 600 mg/day. The dose was calculated back from percentage of yield in extraction and effective dose of powdered drug from the study on OA knee patients. The study was approved by the Medical Ethics Committee of the Faculty of Medicine, Thammasat University which is accredited by the Thai FDA (Registry #MTU-EC-TM-1-179/57) and at the clinicaltrial.gov (Registry #NCT02568059).

Subjects

Twenty-four Thai healthy volunteers of both sex age between 20 and 45 years were screened by history taking, complete physical examination, and laboratory results included complete blood count [CBC], lipid profile, renal functions, liver functions, blood electrolyte, and urine analysis. Volunteers with severe peptic ulcer, high blood pressure (systolic >140 mmHg and diastolic >90 mmHg), impaired renal and liver functions were excluded from the study.

Drug preparation and quality control

The SHT remedy was prepared according to 2011 NLEM. Medicinal plants for SHT remedy were collected from several parts of Thailand or imported from other countries. The medicinal plants were identified, and specimen voucher numbers were obtained (Table 1). The medicinal plants were the same as the previous study of SHT powder in OA knee patients[4]. These ingredients were thin sliced, washed and dried by hot air oven. After that, all medicinal plants were coarse ground to prepare for extraction and then macerated with 95% ethanol for 3 days. Then, the solvent was filtered and evaporated by rotary evaporator. The residue of SHT remedy was repeat macerated 2 more times. All filtrates were combined and percentage of yield was calculated.

Table 1. Medicinal plants in Sahastara remedy formulation (1,000 g)

Thai name	Scientific name	Voucher specimen	Part used	Weight (g)
Prik-Thai	*Piper nigrum* Linn.	SKP146161401	Fruit	240
Jet-Ta-Mul-Plerng-Dang	*Plumbago indica* Linn.	SKP148160901	Root	224
Sa-mhor-thai	*Terminalia chebula* Retz.	SKP049200301	Fruit	104
Dee-plee	*Piper retrofractum* Vahl.	SKP146160301	Fruit	96
Tong-Tank	*Baliospermum montanum* Muell.A.	SKP121021301	Root	80
Wan-Nam	*Acorus calamus* Linn.	SKP015010301	Rhizome	88
Has-sa-khun-tade	*Kleinhovia hospita* Linn.	SKP183110801	Root	48
Ka-ra-boon	*Cinnamomum camphora* Linn.	SKP096030301	-	14
Dok-Chan	*Myristica fragrans* Houtt.	SKP121130601	Aril of seed	13
Luk-Chan	*Myristica fragrans* Houtt.	SKP121130601	Seed	12
Tien-Dang	*Lepidium sativum* Linn.	SKP057121901	Seed	11
Tien-Ta-Tuk-Ka-Tan	*Anethum graveolens* Linn.	SKP199010701	Fruit	10
Ma-Ha-Hing	*Ferula assafoetida* Linn.	SKP199060101	Resin	10
Tien-Sut-Ta-But	*Pimpinella anisum* Linn.	SKP199160101	Fruit	9
Tien-Khao	*Cuminum cyminum* Linn.	SKP199030301	Fruit	8
Jing-Jor	*Merremia vitifolia* (Burm.f.) Hallier f.	SKP054132201	Root	8
Tien-Dum	*Nigella sativa* Linn.	SKP160141901	Seed	7
Kote-Kag-Kra	*Anacyclus pyrethrum* (L.) DC.	SKP051011601	Root	6
Kote-Ka-Mao	*Atractylodes lancea* (Thunb) DC.	SKP051011201	Rhizome	5
Kote-Kan-Prao	*Picrorhiza kurroa* Benth.	SKP177161101	Root	4
Kote-Pung-Pla	*Terminalia chebula* Retz. (gall)	SKP019200301	Gall	3

The ethanolic extract was tested for quality including anti-inflammatory activity by NO inhibition (IC_{50} less than 30 µg/ml)[5] and piperine content not less than 190 mg/g.extract measured by HPLC. The 100 mg of the SHT remedy ethanolic extract was mixed with drug excipients for encapsulation in capsule No.0 (500 mg). These capsules passed quality standards including contaminant testing, weight variation and disintegration time. The SHT remedy ethanolic extract capsules were sealed in aluminum foil.

Procedure

The healthy volunteers received information about the study and were advised to report to the investigator and signed the consent form. The screening was performed including history taking, complete physical examination, blood and urine collection for laboratory test (CBC, fasting blood sugar, lipid profile, liver function test, renal function test, blood electrolyte, and urine analysis). The eligible volunteers were recruited into the study. They were divided into 2 groups based on dose with 6 males and 6 females in each group for 300 mg/day and 600 mg/day of SHT remedy extract capsules. In dose 300 mg/day, each volunteer received 100 mg SHT remedy extract 1 capsule before meals 3 times per day, while dose 600 mg/day received 100 mg SHT remedy extract 2 capsules before meals 3 times per day. They received SHT capsules for 28 days. The follow-up was performed every 14 days (day 14, day 28) and also after stopped taking SHT capsules for 14 days (day 42).

The first step of the present study, the dose 300 mg/day was done first. After finished of this dose and there was no problem in healthy volunteer, the study on dose 600 mg/day was followed.

Outcome assessment

The history taking, complete physical examination, daily notes, and laboratory results were used to evaluate clinical safety.

Statistical analysis

The results were represented as median (P_{25} to P_{75}). The Wilcoxon sign rank test was used to analyze differences between days within a group. Mann-Whitney U test was used to analyze differences between 2 dose groups. A *p*-value less than 0.05 was taken to indicated statistical significance (SPSS version16.0, USA).

Results

The SHT remedy extract pass acceptable standard of biological activity (NO inhibition) and chemical content (piperine content). The capsule of SHT remedy extract also pass the requirement of quality standard including contaminant test, weight variation, and disintegration time (Table 2).

From a total of twenty-four eligible volunteers, no volunteer was excluded and all volunteers completed the study (Figure 1). There was no significant difference between 2 dose groups in baseline characteristics (Table 3). All volunteers had good compliance average of more than 90% that was assessed from daily notes combined with counting remaining capsules in each follow-up.

The most common adverse event [AE] found in both groups was abdominal discomfort; it was found in both SHT remedy dose 300 and 600 mg/day at 58.33% and 66.67%, respectively. The severity of AE was mild to moderate grade. There were further AEs that arose in the study including belching and peripheral hot sensation (especially hands and feet). However, AEs in the present study relieved within 2 weeks after starting the medicine. There were many reasons for AEs relieved such as changing of volunteer's behavior, increasing drug tolerance, and the complying with the suggestion of previous study to take SHT remedy directly before meals or drink more water. The AEs occurred in the 600 mg/day dose more than 300 mg/day (Table 4).

Blood pressure is an important issue with SHT

Table 2. The quality control of SHT remedy extract and capsule of SHT remedy extract

Sample	Testing	Requirement	Results	Conclusion
SHT remedy extract	Anti-inflammatory activity by NO inhibition	IC_{50} <30 µg/ml	6.274±0.796 µg/ml*	Pass
	Piperine content	Piperine ≥190 mg/g.extract	224.94 mg/g.extract	Pass
Capsule of SHT remedy extract	Bacterial contamination	<$5x10^5$ CFU/g	<10 CFU/g	Pass
	Yeast & Mold contamination	<$5x10^3$ CFU/g	10 CFU/g	Pass
	Disintegration time	<30 minutes	14.01 minutes	Pass
	Weight variation: average weight**	>462.5 mg	534 mg	Pass

SHT = Sahastara; NO = nitric oxide

* Data represented as mean of IC_{50} ± SEM (n = 3), ** No more 2 capsule had weight different from 5% of average weight

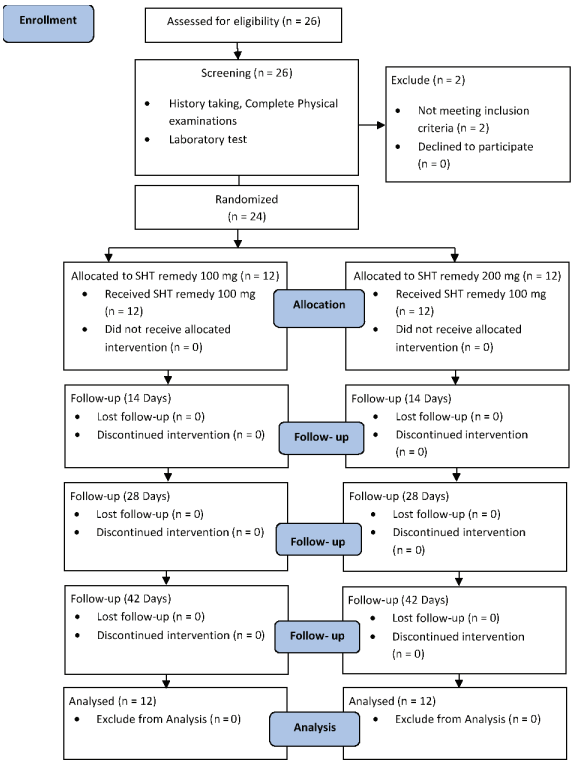

Figure 1. Flow of volunteers.

remedy because SHT remedy is to be used with caution in hypertensive patients. However, the present study shows a statistically significant decrease of systolic blood pressure [SBP] and diastolic blood pressure [DBP] when compared to day 0 in both groups but all results were in normal range. According to the present study, SHT remedy had no hypertensive effect (Table 5).

The blood chemistry including glucose blood sugar [GBS] and lipid profile (i.e., total cholesterol, HDL, LDL, and triglyceride) were also performed to assess health status of volunteers.There was no statistically significant difference between the 2 dose groups in blood chemistries. However, the HDL of 600 mg/day group was higher than 300 mg/day group while LDL of 300 mg/day group was higher than 600 mg/day. In addition, the results showed HDL significantly increase and LDL significantly decrease when compared to Day 0 within groups in both group of doses. It similarly to total-cholesterol which was also significantly decrease in 300 mg/day group and has tend to decrease in 600 mg/day group. The renal and liver functions are the most important safety issue for herbal medicine especially in long term use. The renal functions in the present study showed BUN was no significantly changed in 300 mg/day group, while it was significantly changed in 600 mg/day group when compared from day 0 within dose group. The BUN was also statistically significant difference between dose group in day 14 and day 28 but it was not significant in clinical consideration. Meanwhile, creatinine had no significant difference between dose groups and within group. Liver function including AST, ALT, ALP, albumin, globulin, total protein, total bilirubin, and direct bilirubin showed elevation of ALT in 600 mg/day dose group and decrease of globulin in 300 mg/day dose group from baseline. However, all laboratory results were not changed more than reference range (Table 6).

Table 3. Baseline characteristics

Baseline characteristics	Results[a]		*p*-value
	SHT 300 mg/day	SHT 600 mg/day	
Sex, n (%)	12 (100)	12 (100)	1.000[b]
Male	6 (50)	6 (50)	
Female	6 (50)	6 (50)	
Age (years)	27.50 (24.00 to 34.75)	27.00 (25.25 to 28.75)	0.954[c]
Body mass index [BMI] (kg/m^2)	21.85 (20.20 to 24.57)	20.15 (19.15 to 21.90)	0.143[c]
Systolic blood pressure [SBP] (mmHg)	110.00 (92.50 to 117.50)	110.00 (100.00 to 120.00)	0.339[c]
Diastolic blood pressure [DBP] (mmHg)	70.00 (70.00 to 80.00)	75.00 (70.00 to 80.00)	0.598[c]
Blood urea nitrogen [BUN] (mg/dL)	11.05 (9.60 to 15.15)	10.90 (7.38 to 12.43)	0.378[c]
Creatinine (mg/dL)	0.76 (0.65 to 1.00)	0.78 (0.65 to 0.94)	0.751[c]
AST (U/L)	19.50 (16.50 to 25.00)	18.00 (16.25 to 21.25)	0.309[c]
ALT (U/L)	26.50 (24.00 to 45.00)	26.00 (16.75 to 31.25)	0.271[c]
ALP (U/L)	68.50 (62.25 to 71.75)	72.50 (47.75 to 83.00)	0.514[c]

SHT = Sahastara; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

[a] Data represented as median (P_{25} to P_{75}), [b] Chi-square-test, [c] Mann-Whitney U test

Table 4. Adverse events

Adverse events	SHT 300 mg/day (n = 12)				SHT 600 mg/day (n = 12)			
	n (%)	Day 1-14	Day 15-28	Day 29-42	n (%)	Day 1-14	Day 15-28	Day 29-42
Body hot sensation	3 (25.00)	$3^{e,g,h}$	$2^{g,h}$	0	7 (58.33)	$7^{m,o,p,r,t,u,x}$	$3^{t,u,x}$	0
Abdominal discomfort	7 (58.33)	$6^{a,c\text{-}d,e,g,i\text{-}j}$	$3^{c,g,j}$	$2^{g,j}$	8 (66.67)	$8^{m\text{-}o,t\text{-}v,w,x}$	$3^{m,u,x}$	0
Dry lip and throat	4 (33.33)	$4^{d,g,i}$	$3^{c,g,i}$	0	1 (8.33)	1^{q}	1^{q}	1^{q}
Dizziness	2 (16.67)	1^{g}	$2^{e,g}$	0	1 (8.33)	1^{o}	0	0
Nausea	1 (8.33)	0	1^{e}	0	0 (0.00)	0	0	0
Sleepy	4 (33.33)	$3^{g,h,j}$	$3^{e,g,j}$	$2^{e,j}$	4 (33.33)	$4^{n,t,v,w}$	0	0
Diarrhea	1 (8.33)	1^{g}	1^{g}	0	3 (25.00)	$3^{n,v,x}$	$2^{n,x}$	0
Belch	6 (50.00)	$6^{a,c\text{-}e,g,i}$	$3^{a,c,g}$	0	6 (50.00)	$6^{m,o,p,s,t,s}$	$3^{p,s,x}$	0

SHT = Sahastara

Data represented as amount of person, $^{a\text{-}x}$ Volunteers number 1-24

Table 5. Blood pressure compared between 2 dose groups

Physical examination	Follow-up	Results[a]		*p*-value[b]
		SHT 300 mg/day (n = 12)	SHT 600 mg/day (n = 12)	
SBP (ref. ≤140 mmHg)	Day 0	110.00 (92.50 to 117.50)	110.00 (100.00 to 120.00)	0.339
	Day 14	110.00 (100.00 to 110.00)	100.00 (100.00 to 110.00)†	0.321
	Day 28	100.00 (90.00 to 110.00)	110.00 (100.00 to 110.00)††	0.089
	Day 32	100.00 (100.00 to 110.00)	100.00 (92.50 to 110.00)	0.662
DBP (ref. ≤90 mmHg)	Day 0	70.00 (70.00 to 80.00)	75.00 (70.00 to 80.00)	0.598
	Day 14	70.00 (70.00 to 80.00)	70.00 (70.00 to 77.50)	0.600
	Day 28	70.00 (70.00 to 70.00)†	70.00 (70.00 to 77.50)	0.166
	Day 32	70.00 (70.00 to 70.00)	70.00 (70.00 to 70.00)†	0.580

SHT = Sahastara; SBP = systolic blood pressure; DBP = diastolic blood pressure

[a] Data represented as median (P_{25} to P_{75}), [b] Mann-Whitney U test

† Significant difference from day 0 within group (p<0.05), †† Significant difference from day 0 within group (p<0.01)

Discussion

The quality control is the most common problem of herbal remedy because a remedy usually contains with many medicinal plants. In the current study showed the way to control the quality of SHT remedy for clinical research. The quality control related with previous study on efficacy of SHT remedy powdered drug in OA knee patients. Not only biological activity in anti-inflammatory but also chemical content in remedy were controlled. The present study might be referenced to suggest the standard of SHT remedy extract. The piperine was selected because *Piper* species were the main medicinal plants in the remedy and there are many reports which showed that piperine related with anti-inflammatory effects[6]. Although piperine was selected as marker in the present study, there are other chemical compounds in SHT remedy that related with anti-inflammatory and other pharmacological activity such as chebulagic acid[7], plumbagin[8], and beta-asarone[9]. However, these compounds are minor compound and have small amount in this extract, so the present study used major ingredient in SHT remedy to be ethanolic extract.

There are few reports on SHT remedy extract especially in human applications. Powdered SHT drug has been studied on OA knee patients. The current study is the first report on SHT remedy ethanolic extract study on humans. The results on the present study relate with the previous study on safety of powdered SHT drug where abdominal discomfort was found as the most common AEs. In the same study, the AEs cause from spicy medicinal plants in SHT remedy such as *Piper* species (pepper, long pepper) with piperine as a major constituent[4]. Piperine is a pungent alkaloid and might be the reason for AEs in the current study. The present study could be supported by the study of piperine which is an agonist of transient receptor potential vanilloid 1 [TRPV-1][10]. The receptor is believed to transduce the sensation of noxius heat and pain[11]. TRPV-1 expressing sensory nerve was found in the stomach and duodenum[12]. In addition, TRPV-1 activation also stimulates the thickening of protective layer in the stomach and duodenum[13]. It might explain the reduction of side effects after taking

Table 6. Laboratory results compared between 2 dose groups

Laboratory	Follow-up	Results[a]		*p*-value[b]
		SHT 300 mg/day (n = 12)	SHT 600 mg/day (n = 12)	
Blood sugar				
Glucose (ref. 74 to 106 mg/dL)	Day 0	89.50 (82.25 to 92.00)	85.50 (80.00 to 89.25)	0.124
	Day 14	88.50 (81.75 to 92.00)	86.00 (77.75 to 88.75)	0.224
	Day 28	87.00 (80.25 to 90.75)	87.00 (79.75 to 92.25)†	0.931
	Day 42	85.00 (78.25 to 90.25)	85.00 (81.25 to 90.00)	0.771
Lipid profile				
HDL to cholesterol (ref. 40 to 60 mg/dL)	Day 0	54.00 (41.25 to 65.75)	63.00 (52.50 to 69.50)	0.214
	Day 14	58.00 (41.00 to 68.75)†	64.50 (53.50 to 74.00)†	0.204
	Day 28	60.50 (43.25 to 72.75)††	66.00 (58.50 to 76.00)††	0.285
	Day 42	52.00 (39.00 to 62.75)	62.50 (49.25 to 70.50)	0.225
Total cholesterol (ref. 0 to 200 mg/dL)	Day 0	240.50 (180.75 to 280.25)	198.00 (183.50 to 214.00)	0.242
	Day 14	219.50 (170.25 to 270.50)†	193.00 (169.50 to 219.50)	0.160
	Day 28	219.50 (184.25 to 257.25)	193.00 (183.00 to 224.25)	0.242
	Day 42	221.50 (184.50 to 265.00)	206.50 (185.75 to 228.25)	0.347
LDL to cholesterol (ref. 0 to 100 mg/dL)	Day 0	138.50 (105.75 to 174.50)	108.00 (101.25 to 138.75)	0.204
	Day 14	131.50 (99.25 to 178.50)†	103.50 (96.00 to 139.50)	0.133
	Day 28	128.00 (96.25 to 170.00)†	103.00 (94.75 to 140.50)	0.386
	Day 42	136.00 (108.75 to 163.75)	126.50 (105.25 to 145.75)	0.402
Triglycerides (ref. 0 to 150 mg/dL)	Day 0	96.00 (55.00 to 117.25)	64.50 (42.50 to 108.75)	0.248
	Day 14	66.00 (50.50 to 99.25)	75.00 (43.50 to 120.75)	0.931
	Day 28	84.50 (62.25 to 115.00)	65.50 (47.00 to 97.75)	0.402
	Day 42	80.50 (58.50 to 110.75)	69.50 (55.75 to 90.25)	0.402
Renal functions				
BUN (mg/dL) (ref. range 7.0 to 18.0)	Day 0	11.05 (9.60 to 15.15)	10.90 (7.38 to 12.43)	0.378
	Day 14	12.10 (10.83 to 14.90)†	8.65 (6.63 to 11.18)†	0.010
	Day 28	11.10 (10.55 to 11.75)	9.35 (8.08 to 10.03)	0.011
	Day 42	11.50 (10.25 to 13.45)	12.55 (10.53 to 14.48)†	0.453
Creatinine (mg/dL) (ref. range 0.7 to 1.3)	Day 0	0.76 (0.65 to 1.01)	0.78 (0.65 to 0.94)	0.751
	Day 14	0.81 (0.69 to 1.01)	0.81 (0.70 to 0.94)	0.954
	Day 28	0.80 (0.66 to 0.99)	0.79 (0.72 to 0.95)	0.862
	Day 42	0.84 (0.68 to 1.00)	0.77 (0.65 to 0.92)	0.603
Liver functions				
AST (U/L) (ref. range 15 to 37)	Day 0	19.50 (16.50 to 25.00)	18.00 (16.25 to 21.25)	0.309
	Day 14	18.50 (15.25 to 25.75)	20.00 (16.75 to 22.50)	0.931
	Day 28	21.00 (15.50 to 26.00)	18.50 (15.50 to 21.75)	0.400
	Day 42	17.50 (15.25 to 23.25)	18.00 (16.25 to 20.00)	0.862
ALT (U/L) (ref. range 30 to 65)	Day 0	26.50 (24.00 to 45.00)	26.00 (16.75 to 31.25)	0.271
	Day 14	27.50 (21.00 to 39.25)	27.00 (16.75 to 34.25)	0.370
	Day 28	28.50 (22.00 to 41.75)	30.00 (20.75 to 36.75)†	0.665
	Day 42	24.50 (21.75 to 27.75)†	26.50 (23.00 to 39.75)	0.602
ALP (U/L) (ref. range 50 to 136)	Day 0	68.50 (62.25 to 71.75)	72.50 (47.75 to 83.00)	0.488
	Day 14	65.00 (59.00 to 74.00)	73.00 (48.25 to 86.00)	0.525
	Day 28	68.50 (62.00 to 70.75)	69.00 (48.25 to 81.00)	0.707
	Day 42	69.00 (57.25 to 74.50)	76.50 (50.00 to 88.00)	0.470
Total protein (g/dL) (ref. range 6.4 to 8.2)	Day 0	7.95 (7.63 to 8.18)	7.85 (7.38 to 8.08)	0.543
	Day 14	7.85 (7.70 to 8.18)	7.85 (7.70 to 8.08)	0.816
	Day 28	7.90 (7.63 to 8.30)	7.75 (7.43 to 8.25)	0.750
	Day 42	7.80 (7.43 to 7.98)	7.85 (7.70 to 8.10)	0.466
Albumin (g/dL) (ref. range 3.4 to 5)	Day 0	4.15 (4.03 to 4.38)	4.20 (4.03 to 4.38)	0.705
	Day 14	4.20 (4.10 to 4.38)	4.35 (4.20 to 4.48)	0.348
	Day 28	4.25 (4.10 to 4.40)	4.30 (4.10 to 4.48)	0.838
	Day 42	4.20 (4.10 to 4.38)	4.30 (4.13 to 4.50)	0.483
Globurin (g/dL) (ref. range 1.5 to 3.5)	Day 0	3.70 (3.60 to 4.05)	3.60 (3.20 to 4.00)	0.201
	Day 14	3.65 (3.38 to 3.78)	3.50 (3.40 to 3.80)	0.663
	Day 28	3.65 (3.40 to 3.80)	3.50 (3.20 to 3.78)	0.416
	Day 42	3.45 (3.33 to 3.68)	3.45 (3.40 to 3.70)	0.618
Total bilirubin (mg/dL) (ref. range 0.2 to 1.0)	Day 0	0.65 (0.43 to 0.88)	0.60 (0.43 to 0.78)	0.662
	Day 14	0.60 (0.33 to 0.80)	0.50 (0.43 to 0.60)	0.481
	Day 28	0.90 (0.60 to 0.98)	0.40 (0.40 to 0.50)	0.006
	Day 42	0.70 (0.60 to 0.78)	0.40 (0.30 to 0.60)	0.005
Direct bilirubin (mg/dL) (ref. range 0.0 to 0.2)	Day 0	0.20 (0.10 to 0.20)	0.10 (0.10 to 0.10)	0.014
	Day 14	0.10 (0.10 to 0.18)	0.10 (0.10 to 0.10)	0.284
	Day 28	0.10 (0.10 to 0.18)	0.10 (0.10 to 0.20)	0.563
	Day 42	0.10 (0.10 to 0.18)	0.10 (0.10 to 0.10)	0.623

SHT = Sahastara; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

[a] Data represented as median (P_{25} to P_{75}), b Mann-Whitney U test

† Significant difference from day 0 within group (p<0.05), †† Significant difference from day 0 within group (p<0.01)

SHT remedy for 2 weeks. The SHT remedy extract was similarly studied of Benjakul [BJK] remedy ethanolic extracts which also contained with piperine as a main compound. The most common side effect of BJK in healthy volunteers was abdominal discomfort. The side effects was relieve after taking medicine for 2 weeks[14].

Although SBP and DBP had significantly reduced from baseline in both dose groups, it was not changed from the reference range. It can be assumed that SHT remedy extract does not affect blood pressure. The current study is related with the study of piperine and SHT remedy in hypertensive rats that demonstrated piperine and SHT remedy did not affect blood pressure in hypertensive and normal wistar rats. In addition, SHT remedy showed vasorelaxation effect by increased acetylcholine and vasculoprotective effect in nitric oxide impaired rats[15]. The present study also confirmed the previous work with OA knee patients that showed no significant change in blood pressure from baseline when powdered SHT remedy was taken for 28 days. From the above reason, SHT remedy extract not only appropriate to use in OA knee patients, but also might be considered to use in hypertensive patients that often present in elderly people as well as OA knee.

The SHT remedy extract capsules did not show toxicity on liver and renal function, which is similar to the report on toxicity in rats that demonstrated SHT extract single oral dose 5,000 mg/kg body weight in rats and the SHT extract at the dose 10, 100 and 1,000 mg/kg body weight oral feeding daily for 270 days did not show significant acute and chronic toxicity in rats[3]. It also relates with SHT powder drug in OA knee patients that showed powdered SHT remedy was not toxic to liver and renal functions and safer than diclofenac[4]. The present study confirms the safety of SHT remedy extracts for humans with no toxicity on liver and renal function. According to the study, renal and liver function tests tend to decrease from baseline. However, there is no laboratory test changed more than the reference range. It can be assumed that the SHT remedy did not affect renal and liver function when used for 28 days.

Moreover, the SHT remedy extract might be used in the dyslipidemia patient because the SHT remedy showed increase HDL and decrease LDL. This results was similarly to effect of piperine in high-fat diets rat which piperine could increase HDL and decrease LDL, VLDL, and total-cholesterol without changing food intake[16]. However, the changing of HDL and LDL need more study to confirmed because the diet control was not applied in the present study.

Comparing the dosage for safety, 300 mg/day demonstrated minor AEs and no toxicity to liver and renal function while 600 mg/day recorded some change in laboratory results including BUN and ALT with significant difference from baseline, even though it was in reference value range. The present study suggests that SHT remedy ethanolic extract capsules 300 mg/day is an appropriate dose to use in humans, having fewer side effects compared to 600 mg/day.

Conclusion

The SHT remedy ethanolic extract capsules at 300 and 600 mg/day show good safety to use in healthy volunteers for 28 days without renal and liver toxicity and mild systemic side effects. It is suggested that 300 mg/day of SHT remedy extract is preferable to 600 mg/day to use in humans.

What is already known on this topic?

The SHT remedy ethanolic extract has good anti-inflammatory activity. In the traditional use as powder drug, the SHT remedy has equal efficacy and more safety when compare to diclofenac in OA knee patients.

What this study adds?

This study shows the clinical safety of SHT remedy in ethanolic extract form. This study become the background knowledge for clinical trial phase 2 of SHT remedy ethanolic extract in patients.

Acknowledgement

The present study was supported by the National Research Fund and Center of Excellence in Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Thailand

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. World Health Organization. Global health and aging. Global health and aging. Bethesda, MD: NIH Publication; 2011.
2. Kakatum N, Jaiarree N, Makchucit S, Itharat A. Antioxidant and anti-inflammatory activities of Thai medicinal plants in Sahasthara remedy for muscle pain treatment. J Med Assoc Thai 2012; 95 Suppl 1:S120-6.
3. Sireeratawong S. Acute and chronic toxicity of Sahastara recipe extract. Pathum Thani, Thailand: Faculty of Medicine, Thammasat University; 2014.

4. Pinsornsak P, Kanokkangsadal P, Itharat A. The clinical efficacy and safety of the sahastara remedy versus diclofenac in the treatment of osteoarthritis of the knee: a double-blind, randomized, and controlled trial. Evid Based Complement Alternat Med 2015;2015:103046.
5. Kanokkangsadal P, Pinsornsak P, Makchuchit S, Itharat A. Correlation of extraction time with biological activities and stability test of crude Sahastara remedy. Thammasat Med J 2013;13: 496-503.
6. Meghwal M, Goswami TK. Piper nigrum and piperine: an update. Phytother Res 2013;27: 1121-30.
7. Reddy DB, Reddy TC, Jyotsna G, Sharan S, Priya N, Lakshmipathi V, et al. Chebulagic acid, a COX-LOX dual inhibitor isolated from the fruits of Terminalia chebula Retz., induces apoptosis in COLO-205 cell line. J Ethnopharmacol 2009;124: 506-12.
8. Wang T, Wu F, Jin Z, Zhai Z, Wang Y, Tu B, et al. Plumbagin inhibits LPS-induced inflammation through the inactivation of the nuclear factor-kappa B and mitogen activated protein kinase signaling pathways in RAW 264.7 cells. Food Chem Toxicol 2014;64:177-83.
9. Muthuraman A, Singh N. Attenuating effect of Acorus calamus extract in chronic constriction injury induced neuropathic pain in rats: an evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC Complement Altern Med 2011;11:24.
10. McNamara FN, Randall A, Gunthorpe MJ. Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). Br J Pharmacol 2005;144:781-90.
11. Caterina MJ. Vanilloid receptors take a TRP beyond the sensory afferent. Pain 2003;105: 5-9.
12. Holzer P. Sensory neurone responses to mucosal noxae in the upper gut: relevance to mucosal integrity and gastrointestinal pain. Neurogastroenterol Motil 2002;14:459-75.
13. Akiba Y, Furukawa O, Guth PH, Engel E, Nastaskin I, Kaunitz JD. Sensory pathways and cyclooxygenase regulate mucus gel thickness in rat duodenum. Am J Physiol Gastrointest Liver Physiol 2001;280:G470-4.
14. Amorndoljai P, Kietinun S, Somparn N. Study on safety of Benjakul recipies extract tablets in normal volunteers. Thammasat Med J 2011;11:195-202.
15. Booranasubkajorn S, Huabprasert S, Wattana-rangsan J, Chotitham P, Jutasompakorn P, Laohapand T, et al. Vasculoprotective and vaso-dilatation effects of herbal formula (Sahatsatara) and piperine in spontaneously hypertensive rats. Phytomedicine 2017;24:148-56.
16. Shah SS, Shah GB, Singh SD, Gohil PV, Chauhan K, Shah KA, et al. Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats. Indian J Pharmacol 2011;43: 296-9.