Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

ANTI-DIARRHEAL ACTIVITY AND TOXICITY OF LEARNG PID SAMUD RECIPE

Seewaboon Sireeratawong,[1]* Parirat Khonsung,[2] Urarat Nanna,[1] Supaporn Vannasiri,[3] Nirush Lertprasertsuke,[4] Tipaya Singhalak,[5] Noppamas Soonthornchareonnon,[6] Kanjana Jaijoy,[2]

[1]Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand. [2]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
[3]Division of Physiology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand. [4]Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. [5]Department of Animal Science, Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Chiang Mai, 50300 Thailand. [6]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.
* E-mail: seewaboon@gmail.com

Abstract

Learng Pid Samud (LPS) recipe is a traditional remedy in Thai folk medicine to ease the common diarrhea. The anti-diarrheal potential of LPS recipe was herein examined *in vitro* using a guinea-pig ileum model. The LPS exerted an inhibitory effect on acetylcholine-induced smooth muscle contraction in the guinea pig ileum. Significantly, not only did the LPS reduce the total amount of feces in the induced diarrhea rats, but also the intestinal transit in the charcoal meal test. A single oral administration with the recipe at 5,000 mg/kg did not cause acute toxicity and the daily oral administration (1,000, 2,000 and 4,000 mg/kg) for 90 days in rats did not produce any toxic signs and symptoms. In conclusion, the Learng Pid Samud recipe remedy is evidently safe and effective for the anti-diarrheal treatment which supports its therapeutic uses in the alternative medicine.

Keywords: Learng Pid Samud, Anti-diarrheal activity, Acute toxicity, Chronic toxicity

Introduction

Diarrhea is one of the most common illnesses in all age groups. It is an uncomfortable condition that can have many causes, and vital gastroenteritis (stomach flu) is one of the most common causes of diarrhea. Diarrhea will normally heal itself within a few days. However, in some cases, diarrhea can lead to dehydration or be a sign of a more serious problem (Fontaine, 1988; Snyder and Merson, 1982).

Learng Pid Samud (LPS) recipe is a household remedy in the National List of Essential Medicines of Thailand, for the treatment of non-infectious diarrhea. According to the applied Thai traditional medicine, this recipe is composed of fourteen kinds of herbal plants as shown in Table 1 (National Drug Committee, Ministry of Public Health, 2006). All plants in LPS recipe except resin from plants in Dipterocarpaceae family and resin of *L. chinensis* show gastrointestinal effects and anti-diarrheal activity (Rajkumar et al., 2011; Chengaiah et al., 2010; Meena et al., 2010; Rabbani et al., 2010; Sawangjaroen and Sawangjaroen, 2005; Taufiq-Ur-Rahman et al., 2005; Rabbani et al., 2004; Sharma et al., 2001; Das et al., 1999; Joy et al., 1998). However, the scientific data to support the reputed anti-diarrheal activity and toxicity of the LPS recipe have not yet been substantiated. Therefore, the objective of this study was to evaluate the anti-diarrheal activity and safety of this recipe.

Materials and methods

Preparation of Learng Pid Samud (LPS) extract

The constituents of LPS are listed in Table 1. The 14 kinds of plant materials were kindly provided by the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University. The plant was identified and kept at the herbarium library collection at Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Each plant material was 4.80 kg, but *C. longa* was 28.80 kg. Whole plant material (91.20 kg) was continuously extracted with three solvents, *n*-hexane, 95% ethanol and hot water, and spray dried.

Standardization of LPS extract

The quality control of raw materials and the extract was followed by Thai Herbal Pharmacopoeia including organoleptic examination, % loss on drying, extractive values, total ash and acid insoluble ash (Department of Medical Sciences, 2000). The percent amount of volatile oil, type of chemical constituents in oils (detected by GC/MS), chemical constituents in raw materials and the extract were also studied using thin layer chromatography (TLC) following the method of Farnsworth (Farnsworth, 1966).

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Experimental animals

Male Sprague-Dawley rats (140-180 and 180-200 g), female Sprague-Dawley rats (180-200 g) and male or female Guinea pig (500-700 g) were provided by the National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. The animals were housed under standard environmental conditions of temperature at 24 ± 1 °C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. The experimental protocols were authorized by the Animal Ethics Committee of Faculty of Medicine, Thammasat University, Pathumthani, Thailand (No. 0003/2007).

Table 1 Composition of LPS

Scientific name	Family	Part of used
Cyperus rotundus Linn	GRAMINEAE	Root
Curcuma zedoaria (Berg) Roscoe	ZINGIBERACEAE	Rhizome
Oroxylum indicum (L.) Kurz	BIGNONIACEAE	Bark
Musa (ABB group)	MUSACEAE	Root
Allium sativum L.	ALLIACEAE	Bulb
Uncaria gambir (Hunter) Roxb	RUBIACEAE	Branch
Acacia catechu (L.f.) Willd	LEGUMINOSAE	Branch
Lawsonia inermis L.	LYTHRACEAE	Leaf
Punica granatum Linn. vas *granatum*	PUNICACEAE	Leaf
Piper chaba Hunt	PIPERACEAE	Fruit
Curcuma longa Linn.	ZINGIBERACEAE	Rhizome
Laccifera chinensis Mahdihassan	LACCIFERIDAE	Resin
Hopea odorata Roxb., *Dipterocarpus alatus* Roxb., *Dipterocarpus intricatus* Dyer, *Shorea henryana* Pierre, *Shorea btuse* Wall., *Shorea siamensis* Miq.	DIPTEROCARPACEAE	Resin
Quercus infectoria Oliver	FAGACEAE	Gall

Isolated guinea pig ileum experiment

Briefly, Guinea pigs of both sexes were fasted 48 hour before the experiment. After the animals were sacrificed, the midline incision of the abdomen was made and the ileum was isolated. The ileum was cut into strips of 2 cm long. A piece of ileum was mounted in a bath of 20 ml Tyrode's solution (NaCl = 8.0, KCl = 0.2, $MgCl_2$ = 0.1, $CaCl_2$ = 0.2, NaH_2PO_4 = 0.05, $NaHCO_3$ = 1.0, and glucose = 1 g/L) with a controlled temperature of 37 °C and aerated with 95% O_2 and 5% CO_2. Isometric concentrations were recorded under a resting tension of 1 g via a force displacement transducer (FTO3 Grass Instrument Co., Quincy, MA) and displayed on a polygraph (PD7, grass Instrument Co.). After an equilibration period of 30 min, standard contractions produced by acethylcholine (ACh, 0.02 ug/mL) were recorded. The tissue was then washed out with Tyrode's solution. To test the inhibitory effect, the test substance was added into the organ-bath 3 minutes before the addition of ACh. The tissue was further washed 3-4 times after measuring the contractions at each dose of test substances. Results are expressed as percent inhibition of contraction (Department of Pharmacology, University of Edinburg, 1970).

Castor oil-induced diarrhea in rats

Mice were fasted 48 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. The experiment was performed according to the method of Venkatesan et al. (2005). Mice were given 0.5% carboxymethy cellulose (CMC) (orally) or LPS (orally) or atropine sulfate (intraperitoneally, i.p.). One hour later, they were given one milliliter castor oil orally and then weighed and placed individually in a transparent plastic box with absorbent paper underneath. Total amount of feces (in grams) after the castor oil administration was collected and weighed.

Small intestinal transit in rats

The experiment was performed according to the method of Venkatesan et al. (2005). Rats were fasted 48 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. The rats were given 0.5% CMC or LPS (orally) or atropine sulfate (i.p). Thirty minutes later, they were orally fed with 1 ml of 3% deactivated charcoal (in 0.5% CMC). Thirty minutes after the deactivated charcoal feeding, the rats were sacrificed with intraperitoneal injection of pentobarbital sodium (50 mg/kg) and the gastrointestinal tract was removed. Total length of the small intestine (pylorus to caecum), and the distance of the deactivated charcoal movement were measured. The small intestine transit was calculated and expressed as percentage of the deactivated charcoal movement.

Acute oral toxicity

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Acute oral toxicity test was performed according to the World Health Organization (WHO) guideline (WHO, 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals TG420 (OECD, 2001). Healthy fasted rats (five rats per group) were administrated a single oral dose of 5,000 mg/kg body weight while the control group received water vehicle. Body weight, signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for next 14 days. On the 15th day, all rats were kept fasted for 16-18 hours, and then sacrificed with pentobarbital sodium (50 mg/kg, i.p.) for necropsy examination. The internal organs were excised and weighed. The gross pathological observations of the tissues were performed.

Subchronic oral toxicity

According to WHO guideline (WHO, 2000) and the OECD TG408 (OECD, 1981), rats were divided into 6 groups of 20 animals (10 male and 10 female). The extract was administered orally at doses of 1,000, 2,000 and 4,000 mg/kg body weight to the three subsequent treatment groups for consecutive 90 days, while the control group received distilled water. Signs of toxicity, mortality and body weight changes were monitored daily.
At the end of the experiment, all animals were kept fasted for 16-18 h and then anesthetized with intraperitoneal injection of pentobarbital sodium at a dose of 50 mg/kg on day 91st and 118th (satellite groups). Blood samples for hematological and blood chemical analyses were taken from common carotid artery. The internal organs and some tissues were weighed to determine relative organs' weights and observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

Statistical analysis

Data were reported as mean ± standard error of mean (S.E.M.) and were compared using one-way analysis of variance (ANOVA), followed by post hoc least-significant difference test. The data obtained from acute toxicity studies were analyzed using Student's paired *t*-test. *P* values less than 0.05 were considered to be significant.

Results

LPS extrac*t*

The raw materials of LPS contained 14 varieties of plants. The chemical constituents in the raw materials were mainly fluorescence compounds (green, blue or red color), terpenes and tannins. The amount of tannin is present in six plants of this recipe including *Q. infectoria* (69.40%), *U. gambir* (45.10%), *P. granatum* var. *granatum* (24.94%), *L. inermis* (8.56%), *A. catechu* (7.98%), and *Musa* (ABB group) (2.04%). Four plants contained volatile oil: *C. longa* (6.92%), *C. zedoaria* (2.58%), *P. chaba* (0.95%), and *C. rotundus* (0.75%). Continuous extraction of crude extracts by three solvents: hexane, 95% ethanol and water gave two separate fractions in ratio of 4:1 w/w. The texture of the first extract was brown and sticky resin (16.48 kg) and the latter was yellow and fine powder (4.12 kg). Both fractions were combined and used for testing in further experiments. The percent yield of raw material was 22.59 %w/w.

Anti-diarrheal activity

As shown in Figure 1, the positive control atropine sulfate (30 ng/ml) effectively inhibited the ACh-induced ileum contraction by 81.11%. Similarly, LPS at the concentrations of 0.25, 0.50 and 1.00 mg/ml significantly inhibited the ACh-induced contractions (20-80%) in a concentration-dependent manner. The anti-diarrheal effect of LPS was further examined *in vivo* using experimental rats. The diarrhea in rats was induced by castor oil, and the total amount of feces in 4 h was recorded as shown in Figure 2. Atropine as well as LPS significantly showed > 50% decrease in the total amount of feces. Finally, the gastrointestinal motility was measured in rats, and the result clearly demonstrated that LPS, similar to atropine sulfate, effectively decreased the movement of deactivated charcoal from the stomach to the small intestine (Figure 3).

Acute oral toxicity

LPS at a dose of 5,000 mg/kg did not show any toxicity during the experimentation period. The internal organs of treatment rats such as brain, lung, heart, liver, spleen, adrenal gland, kidney, and sex organ showed no pathological abnormality relative to these organs of the control (data not shown).

Subchronic oral toxicity

The subchronic oral administration of LPS (1,000, 2,000 and 4,000 mg/kg) resulted in no significant change in neither general behavior nor in health condition during the experimentation period. The body weights and weight gain of both female and male rats at various concentrations are listed in Table 2. The female rats (2,000 and 4,000 mg/kg) showed a significant decrease of the body weight gain from those of the control. Moreover, the male rats showed a significant decrease in the body weights (day 90th) and body weight gain in comparison with the control groups. Some internal organ weights of the treatment female and male rats significantly lower than that of the control (Table 3 and 4). For 1,000 and 2,000 mg/kg/day dose, a significant increase in mean corpuscular volume (MCV) in the female treatment rats was observed (Table 5). The female rats in the group treated with 4,000 mg/kg body weight showed the significantly increased mean corpuscular hemoglobin concentration (MCHC) more than that in the control group. In the male groups, MCV and mean corpuscular

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8
hemoglobin (MCH) in treated group with 4,000 mg/kg body weight was significantly higher than those in the control group (Table 6). The differential white blood cell count values of the female and male rats treated groups are shown in Table 7 and 8, respectively. In the female treatment rats, a significant decrease in monocyte was observed in the treated groups with 1,000, 4,000 mg/kg and satellite group. The eosinophil of female treatment rats (1,000 and 2,000 mg/kg) were significantly increased from those of the female control. Furthermore, a significant decrease in neutrophil was observed in the male satellite group. Blood chemical value of the female and male rats treated groups are summarized in Table 9 and 10, respectively. In the female and male treatment rats, some parameters of these values were significantly difference than that of the control group.

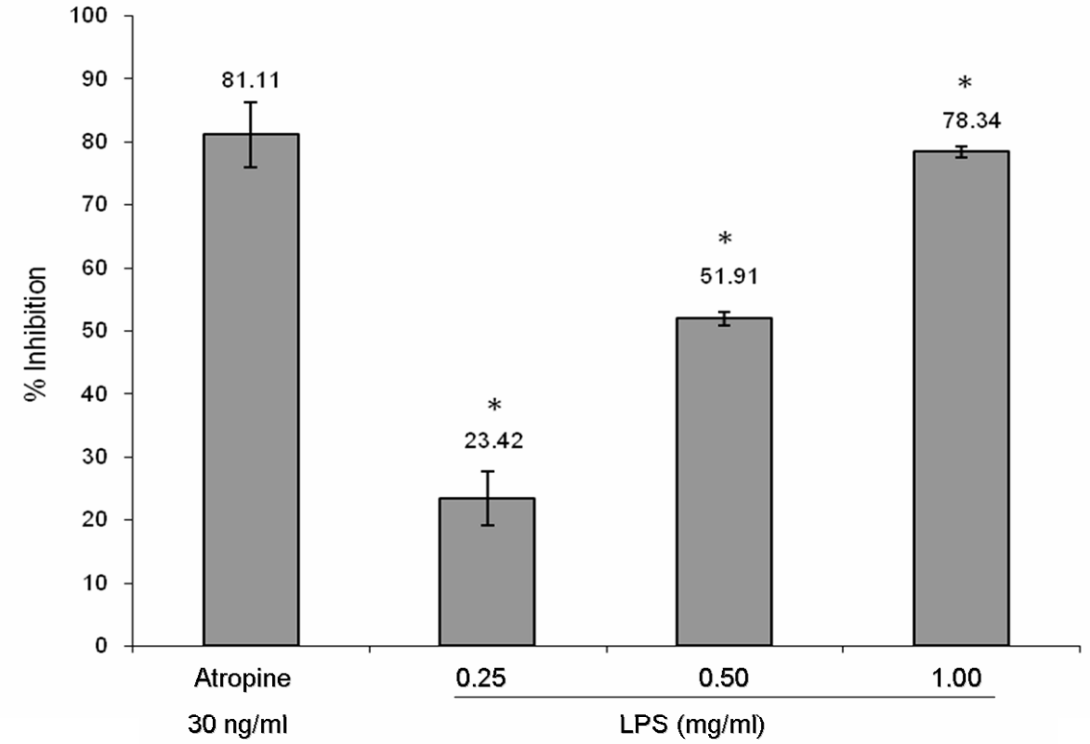

Figure 1 The inhibitory effect of atropine sulfate and LPS extract on acetylcholine-induced contraction of isolated guinea pig ileum. * Significantly different from control, $p<0.05$

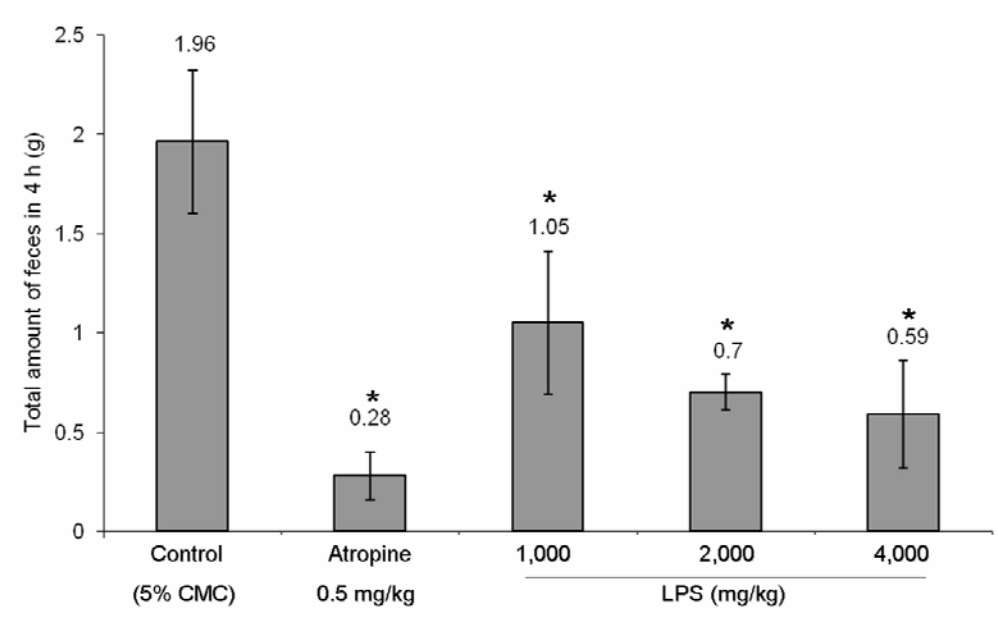

Figure 2 Effect of atropine sulfate and LPS extract on total feces amounts (g) after 4 h castor oil-induced diarrhea in rats. * Significantly different from control, $p<0.05$

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

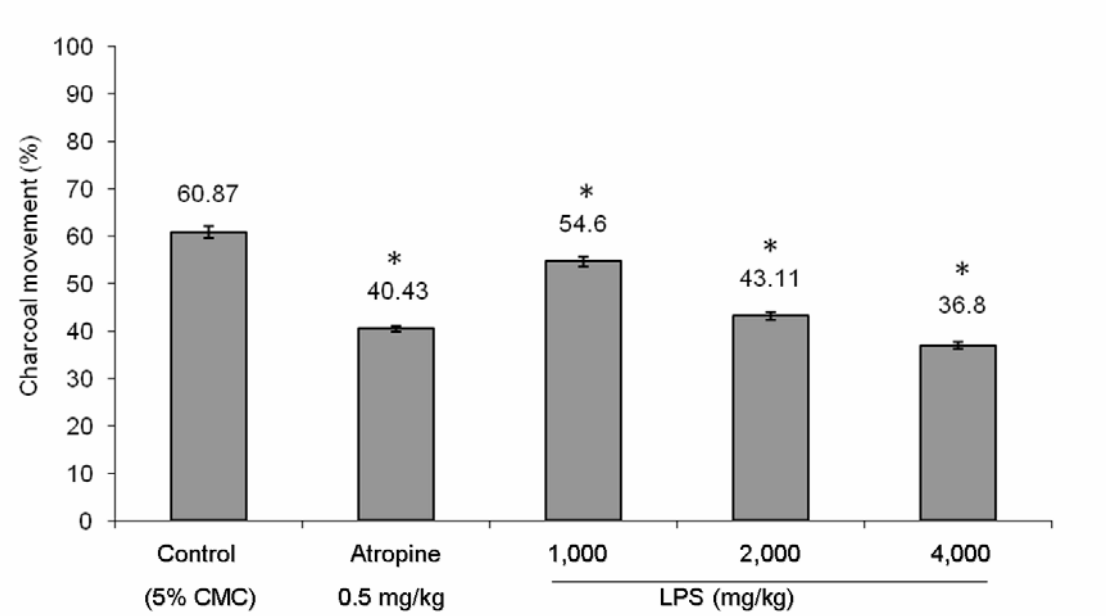

Figure 3 The inhibitory effect of atropine sulfate and LPS extract on charcoal movement in gastrointestinal tract of rats. * Significantly different from control, $p<0.05$

Table 2: Body weights of rats under subchronic doses of LPS

	Body weight (g)			
	Day 0	Day 90	Day 118	Weight gain on day 90
Female				
Control	235.40 ± 4.89	280.40 ± 9.96	-	47.00 ± 6.27
Control [a]	251.20 ± 2.33	282.80 ± 7.08	286.00 ± 6.06	31.60 ± 7.82
LPS				
1,000 mg/kg	245.20 ± 3.97	282.40 ± 3.65	-	37.20 ± 4.77
2,000 mg/kg	246.00 ± 3.07	271.80 ± 3.85	-	25.80 ± 3.57*
4,000 mg/kg	249.40 ± 5.19*	264.00 ± 4.11*	-	16.40 ± 4.99*
4,000 mg/kg [a]	242.40 ± 6.07	273.10 ± 3.40	275.60 ± 2.28	34.90 ± 5.02
Male				
Control	243.60 ± 4.77	380.00 ± 11.15	-	138.20 ± 13.53
Control [a]	238.20 ± 7.09	358.80 ± 13.00	357.60 ± 14.51	120.60 ± 15.34
LPS				
1,000 mg/kg	235.40 ± 6.16	327.80 ± 11.22*	-	92.40 ± 9.85*
2,000 mg/kg	234.80 ± 3.74	319.60 ± 13.69*	-	84.80 ± 11.93*
4,000 mg/kg	245.20 ± 6.06	347.00 ± 6.67*	-	101.80 ± 8.14*
4,000 mg/kg [a]	235.80 ± 6.46	336.60 ± 7.89*	323.20 ± 7.07	100.80 ± 6.71*

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.
* Significantly different from control, $p<0.05$.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Table 3 Organ weights of female rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Lung	1.98 ± 0.31	2.00 ± 0.20	1.72 ± 0.30	2.22 ± 0.31	1.59 ± 0.12	1.45 ± 0.10
Heart	1.32 ± 0.04	1.24 ± 0.04	1.15 ± 0.05*	1.13 ± 0.05*	1.21 ± 0.04	1.16 ± 0.03
Liver	8.95 ± 0.48	8.24 ± 0.40	8.30 ± 0.40	8.92 ± 0.32	9.26 ± 0.20	7.00 ± 0.02
Spleen	0.84 ± 0.03	0.78 ± 0.04	0.69 ± 0.03*	0.87 ± 0.11	0.77 ± 0.02	0.70 ± 0.03
Adrenal gland	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Kidney	1.38 ± 0.16	1.26 ± 0.04	1.38 ± 0.05	1.31 ± 0.03	1.32 ± 0.05	1.24 ± 0.05
Testis	1.69 ± 0.18	1.63 ± 0.08	1.60 ± 0.04	1.54 ± 0.01	1.77 ± 0.03	1.71 ± 0.04

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.* Significantly different from control, p<0.05.

Table 4 Organ weights of male rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Lung	1.76 ± 0.26	1.80 ± 0.15	1.53 ± 0.13	1.49 ± 0.09	1.70 ± 0.12	1.35 ± 0.14
Heart	0.96 ± 0.03	1.00 ± 0.02	0.97 ± 0.03	0.92 ± 0.02	1.05 ± 0.03*	0.96 ± 0.10
Liver	5.95 ± 0.26	6.44 ± 0.19	6.56 ± 0.16*	6.35 ± 0.13	7.37 ± 0.24*	6.48 ± 0.68
Spleen	0.70 ± 0.03	0.69 ± 0.04	0.70 ± 0.02	0.68 ± 0.02	0.70 ± 0.02	0.74 ± 0.08
Adrenal gland	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Kidney	1.01 ± 0.04	0.94 ± 0.02	0.98 ± 0.02	0.94 ± 0.02*	0.99 ± 0.11	0.96 ± 0.09
Ovary	0.05 ± 0.00	0.0 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.01

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.
* Significantly different from control, p<0.05.

Table 5 Hematological examinations of female rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Red blood cell ($x10^6$/μl)	7.81 ± 0.11	7.95 ± 0.29	7.74 ± 0.09	7.88 ± 0.10	7.59 ± 0.09	7.70 ± 0.17
Hemoglobin (g/dl)	14.71 ± 0.22	14.86 ± 0.48	14.76 ± 0.21	14.97 ± 0.20	14.39 ± 0.18	14.67 ± 0.30
Hematocrit (%)	42.52 ± 0.56	42.58 ± 1.49	42.56 ± 0.57	43.33 ± 0.51	40.99 ± 0.44	42.01 ± 0.87
MCV(fl)	54.47 ± 0.18	53.54 ± 0.26	55.00 ± 0.13*	55.03 ± 0.20*	54.00 ± 0.13	54.64 ± 0.17
MCH (pg)	18.84 ± 0.14	18.66 ± 0.14	19.08 ± 0.07	19.02 ± 0.08	18.94 ± 0.06	19.04 ± 0.08
MCHC (g/dl)	34.57 ± 0.21	34.90 ± 0.17	34.69 ± 0.06	34.57 ± 0.10	35.10 ± 0.11*	34.85 ± 0.09
Platelet ($x10^5$/μl)	5.53 ± 0.12	5.68 ± 0.09	5.66 ± 0.15	5.89 ± 0.11	5.85 ± 0.15	5.65 ± 0.21

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.* Significantly different from control, p<0.05.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Table 6: Hematological examinations of male rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Red blood cell ($x10^6/\mu l$)	8.70 ± 0.07	8.84 ± 0.10	8.18 ± 0.17	8.40 ± 0.20	13.33 ± 4.96	8.84 ± 0.10
Hemoglobin (g/dl)	15.90 ± 0.11	15.92 ± 0.20	15.05 ± 0.29	15.51 ± 0.39	15.52 ± 0.35	15.43 ± 0.50
Hematocrit (%)	43.08 ± 3.31	46.66 ± 0.59	43.87 ± 0.91	44.94 ± 1.08	45.22 ± 0.97	44.93 ± 1.40
MCV(fl)	53.34 ± 0.20	52.78 ± 0.22	53.66 ± 0.30	53.52 ± 0.25	54.27 ± 0.24*	52.74 ± 0.23
MCH (pg)	18.29 ± 0.08	18.02 ± 0.10	18.40 ± 0.39	18.46 ± 0.09	18.62 ± 0.09*	18.11 ± 0.06
MCHC (g/dl)	34.42 ± 0.13	34.12 ± 0.12	34.31 ± 0.19	34.46 ± 0.10	34.33 ± 0.12	33.34 ± 0.14
Platelet ($x10^5/\mu l$)	5.79 ± 0.21	5.55 ± 0.15	5.78 ± 0.17	5.61 ± 0.32	5.59 ± 0.23	5.82 ± 0.12

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.
* Significantly different from control, p<0.05.

Table 7: Differential white blood cell counts of female rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
White blood cell ($x10^3/\mu l$)	2.33 ± 0.13	2.40 ± 0.17	2.46 ± 0.09	2.33 ± 0.08	2.51 ± 0.15	2.51 ± 0.13
Neutrophil (%)	15.99 ± 1.90	20.52 ± 3.99	15.51 ± 2.05	15.35 ± 1.25	13.26 ± 1.32	12.94 ± 0.85
Lymphocyte (%)	79.34 ± 2.18	76.32 ± 4.92	80.67 ± 2.22	79.23 ± 1.14	84.61 ± 1.71	84.23 ± 1.03
Monocyte (%)	1.66 ± 0.55	0.24 ± 0.09*	0.55 ± 0.21*	1.61 ± 0.46	0.20 ± 0.11*	0.40 ± 0.15*
Eosinophil (%)	0.83 ± 0.27	1.50 ± 0.53	1.96 ± 0.32*	2.05 ± 0.34*	1.02 ± 0.43	0.84 ± 0.41
Basophil (%)	2.00 ± 0.33	1.46 ± 0.62	1.32 ± 0.48	1.75 ± 0.35	0.91 ± 0.21	1.59 ± 0.64

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.
* Significantly different from control, p<0.05.

Table 8: Differential white blood cell counts of male rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
White blood cell ($x10^3/\mu l$)	2.53 ± 0.10	2.58 ± 0.14	2.62 ± 0.13	2.88 ± 0.17	2.83 ± 0.13	2.51 ± 0.10
Neutrophil (%)	19.62 ± 1.63	14.91 ± 3.04	15.54 ± 2.07	15.46 ± 2.67	16.33 ± 1.88	11.78 ± 1.62*
Lymphocyte (%)	77.88 ± 1.98	82.52 ± 3.17	75.39 ± 3.07	81.91 ± 3.04	78.72 ± 1.57	85.17 ± 1.99
Monocyte (%)	0.70 ± 0.14	0.72 ± 0.37	2.63 ± 1.96	0.46 ± 0.12	1.36 ± 0.81	1.20 ± 0.82
Eosinophil (%)	0.81 ± 0.41	0.22 ± 0.11	0.82 ± 0.29	0.74 ± 0.33	1.12 ± 0.36	0.51 ± 0.29
Basophil (%)	1.12 ± 0.17	1.61 ± 0.51	0.53 ± 0.15	1.26 ± 0.38	1.51 ± 0.31	1.35 ± 0.40

Values expressed as mean ± S.E.M (n = 10)
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.
* Significantly different from control, p<0.05.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Table 9: Clinical blood chemistry examinations of female rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Glucose (mg/dl)	86.89 ± 3.15	90.80 ± 4.19	88.30 ± 2.23	88.10 ± 2.73	99.67 ± 3.42*	97.44 ± 1.03*
BUN (mg/dl)	23.67 ± 0.80	26.00 ± 0.65	24.50 ± 0.87	25.00 ± 0.92	23.67 ± 0.73	26.89 ± 0.68*
Creatinine (mg/dl)	0.60 ± 0.02	0.68 ± 0.04	0.66 ± 0.03	0.60 ± 0.02	0.51 ± 0.02*	0.58 ± 0.02
Total protein (g/dl)	5.93 ± 0.06	5.86 ± 0.10	6.21 ± 0.05*	6.03 ± 0.10	6.02 ± 0.12	5.76 ± 0.13
Albumin (g/dl)	1.50 ± 0.05	1.58 ± 0.02	1.64 ± 0.03*	1.51 ± 0.02	1.64 ± 0.05*	1.61 ± 0.05
Total bilirubin (mg/dl)	0.47 ± 0.03	0.40 ± 0.05	0.45 ± 0.03	0.43 ± 0.02	0.47 ± 0.02	0.36 ± 0.04*
Direct bilirubin (mg/dl)	0.16 ± 0.02	0.12 ± 0.04	0.12 ± 0.02	0.10 ± 0.02	0.16 ± 0.02	0.08 ± 0.02*
SGOT (U/l)	106.00 ± 1.85	85.20 ± 6.08*	107.20 ± 2.11	106.00 ± 1.83	95.78 ± 2.56*	94.44 ± 2.52*
SGPT (U/l)	36.67 ± 2.02	31.40 ± 1.81	35.80 ± 1.62	31.50 ± 1.49*	30.00 ± 1.87*	34.11 ± 1.09
ALP (U/l)	51.89 ± 4.42	47.20 ± 4.99	43.70 ± 1.97*	44.30 ± 1.44*	40.56 ± 1.84*	49.89 ± 1.74

Values expressed as mean ± S.E.M (n = 10) * Significantly different from control, $p<0.05$.
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days.

Table 10: Clinical blood chemistry examinations of male rats under subchronic doses of LPS

			LPS (mg/kg)			
	Control	Control [a]	1,000	2,000	4,000	4,000 [a]
Glucose (mg/dl)	78.11 ± 3.91	94.50 ± 3.01*	70.30 ± 2.84	77.22 ± 2.94	91.90 ± 3.02*	86.10 ± 3.35
BUN (mg/dl)	19.11 ± 1.06	23.40 ± 0.87*	22.40 ± 1.30*	23.78 ± 1.13*	26.10 ± 1.40*	21.80 ± 0.83
Creatinine (mg/dl)	0.58 ± 0.03	0.58 ± 0.02	0.49 ± 0.02	0.51 ± 0.03	0.49 ± 0.06	0.53 ± 0.01
Total protein (g/dl)	5.96 ± 0.11	5.74 ± 0.13	5.89 ± 0.12	5.98 ± 0.24	6.09 ± 0.09	5.62 ± 0.06
Albumin (g/dl)	1.50 ± 0.08	1.33 ± 0.03*	1.43 ± 0.05	1.43 ± 0.09	1.47 ± 0.03	1.39 ± 0.04
Total bilirubin (mg/dl)	0.41 ± 0.03	0.45 ± 0.02	0.40 ± 0.01	0.47 ± 0.03	0.43 ± 0.03	0.41 ± 0.02
Direct bilirubin (mg/dl)	0.12 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	0.13 ± 0.04	0.10 ± 0.03	0.11 ± 0.01
SGOT (U/l)	135.00 ± 4.97	95.40 ± 2.90*	130.90 ± 3.48	126.56 ± 5.34	113.70 ± 7.51*	104.50 ± 1.09*
SGPT (U/l)	38.33 ± 1.62	36.50 ± 1.79	37.40 ± 1.59	39.89 ± 0.93	35.60 ± 153	31.60 ± 1.65*
ALP (U/l)	66.22 ± 2.96	51.90 ± 3.47*	69.50 ± 2.06	72.22 ± 1.49	72.70 ± 1.84	53.50 ± 3.06*

Values expressed as mean ± S.E.M (n = 10) * Significantly different from control, $p<0.05$.
a: a satellite group was treated with oral dose of test substance for 90 days following by no treatment for 28 days

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8

Discussion

Learng Pid Samud is used as anti-diarrheal remedy in Thai folk medicine. In the present study, the anti-diarrhea activity of this recipe was verified *in vitro* (isolate guinea-pig ileum) and *in vivo* (small intestinal transit and castor oil-induced diarrhea in rats). Diarrhea is a condition in which the frequency of bowel movements is increased or the form of stool decreased. The effects such as inhibition of ileum contractions, slowing motility and propulsion of intraluminal contents are generally known to take part in anti-transit effect (Shook et al., 1989). Gastrointestinal (GI) transit may be coordinated by relaxation of the circular muscle and constriction of the longitudinal muscle (Prins et al., 2000; Shibata et al., 1999). Nerve supply of GI tract has two types, enteric system (myenteric and submucosal plexus) and extrinsic nerves (parasympathetic and sympathetic nerves). The enteric system controls the movement and secretion within the gut. The stimulation of parasympathetic nerves lead to the increase of motility and secretion within the tract and relaxation of the gut sphincters. In contrast, the sympathetic nerves reduce blood flow to the gut, and decrease secretions, motility and contractions (Ganong, 2001).

ACh is a neurotransmitter from parasympathetic nervous system. It acts on muscarinic and nicotinic cholinergic receptors. This neurotransmitter produces a contractile response in the ileum. Atropine is a competitive antagonist for the muscarinic ACh receptor (Pappano and Kutzung, 2004). In this study, LPS as well as atropine sulfate showed an inhibitory effect on ACh-induced contractions of isolated guinea pig ileum. Thus, the anti-spasmodic activity of LPS may involve a cholinergic mechanism.

The castor oil test in rats has been extensively used to screen and assess anti-diarrhea agents. This test is both secretion and motility diarrhea model (Yegnanaravan and Shrotri, 1982). Castor oil causes diarrhea due to the irritating properties of its active ricinoleic acid. Ricinoleic acid (hydroxyl fatty acid) increases the peristalsis activity, decreases the small intestinal transit time and produces a permeability change in the intestinal mucosal membrane to electrolytes and water resulting in a hypersecretory response (Kutchai, 2004; Gaginella et al., 1975; Ammon et al., 1974). Moreover, its irritating properties are a cause of the inflammation of intestinal mucosal membrane, leading to the release of prostaglandins, which stimulates the small intestinal movement and water and electrolytes secretion (Beubler and Juan, 1979; Pierce et al., 1971). The reduction in severity and frequency of defecation can be attributed to the anti-intraluminal fluid accumulation activity (Nwodo and Alumanah, 1991). The increase in intestinal transit time by atropine could also take place due to the reduction in gastric emptying (Izzo et al., 1999). Atropine produces the reduction in the number of stools and increases the intestinal transit time possibly due to its anti-cholinergic effect (Brown and Taylor, 2000). In this experimental model, LPS effectively decreases the total amount of feces in 4 hours after castor oil administration. These results suggest the LPS and atropine may share a similar mechanism of action.

The effect of LPS on small intestinal transit was examined using deactivated charcoal with atropine sulfate as the reference drug. Similar to atropine, the LPS recipe lessened the moving distance of deactivated charcoal through the ileum. These results suggest that the mechanism of LPS's anti-diarrheal activity may be due to the slowing propulsion of intraluminal contents and/or inhibition of the ileum contraction.

Acute oral toxicity and subchronic oral toxicity tests were further studied in rats to assess the safety of LPS. In acute toxicity, a single oral administration of LPS (5,000 mg/kg) was given to both sexes of rats. This recipe did not significantly change the body weight and the internal organ weight of treated female and male rats. There were no significant differences in general behaviors and toxic signs. Gross and pathological examinations of the internal organs revealed no pathological abnormality. The results suggest that LPS is not toxic after a single oral administration.

For the subchronic toxicity test, the observed changes such as toxic signs, animal behavior, health monitoring, final body and organ weights are attributable to usual change in food intake or metabolism. Neither morbidity nor sickness was observed during the entire experimentation period. The results show a significant increase in the body weight of the male rats when compared with the control groups. Moreover, significant changes in the hematological values and biochemical examination fall within the normal ranges (Angkhasirisap et al., 2002; Inala et al., 2002; Caisey and King, 1980). Necropsy and histopathological examinations further confirm no macroscopic or microscopic changes in these internal organs or tissues in any treated rats.

In conclusion, Learng Pid Samud recipe has an effective anti-diarrheal activity and no toxicity which supports its therapeutic uses in traditional medicine for treatment of diarrhea.

Acknowledgements

We are gratefully acknowledged and also financially supported by the National Research Council of Thailand.

References

1. Ammon, H.V., Thomas, P.J. and Phillips, S. (1974). Effect of the oleic acid and recinolic acid on net jejunal water and electrolyte movement. J. Clin. Invest. 53: 374 – 379.
2. Angkhasirisap, W., Inala, P., Sirimontaporn, A., Inpunkaew, R., Rungrojejinda, K., Kengkoom, K., Ratanasak, W. and Buripakdi Lawson, D. (2002). Blood chemistry profiles of outbred Sprague-Dawley rat in The Facility of National Laboratory Animal Centre. 28th Congress on Science and Technology of Thailand.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8
3. Beubler, E. and Juan, H. (1979). Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. J. Pharm. Pharmacol. 31: 681 – 685.
4. Brown, J.A. and Taylor, P. (2000). Muscarinic receptor agonists and antagonist. In: Hardman, J.G., Limbrid, L.E. (eds) Goodman and Gilman's The pharmacological Basis of therapeutics 10th ed. McGraw Hill, New York, pp 115 – 158.
5. Caisey, J.D. and King, D.J. (1980). Clinical chemical values for some common laboratory animals. Clin. Chem. 26: 1877 – 1879.
6. Chengaiah, B., Rao, K.M., Kumar, K.M., Alagusundaram, M. and Chetty, C.M. (2010). Medicinal importance of Natural dyes-a review. Int. J. PharmTech. Res. 2(1): 144 – 154.
7. Das, A.K., Mandal, S.C., Banerjee, S.K., Sinha, S., Das, J., Saha, B.P. and Pal, M. (1999). Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. J. Ethnopharmacol. 68(1-3): 205 – 208.
8. Department of Medical Sciences, Ministry of Public Health. (2000). Thai herbal pharmacopoeia, Volume II, Bangkok, Thailand: Prachachon Company.
9. Department of Pharmacology, University of Edinburg. (1970). Pharmacological experiments on isolated preparations.
10. Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. J. Pharm. Sci. 55(3): 225 – 265.
11. Fontaine, O. (1988). Bacterial diarrhoea and treatment. Lancet. 1(8596): 1234 – 1235.
12. Gaginella, T.S., Stewart, J.J., Olsen, W.A. and Bass, P. (1975). Action of recinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption *in vitro*. J. Pharmacol. Exp. Ther. 195: 355 – 356.
13. Ganong, W.F. (2001). Regulation of gastrointestinal function. In: Ganong, W.F. (ed) Review of Medicinal Physiology 12th ed. McGraw-Hill, USA, pp 464 – 497.
14. Inala, P., Sirimontaporn, A., Inpunkaew, R., Rungrojejinda, K., Kengkoom, K., Ratanasak, W. and Buripakdi Lawson, D. (2002). Hematological analysis of outbred Sprague-Dawley rat in The Facility of National Laboratory Animal Centre. 28th Congress on Science and Technology of Thailand.
15. Izzo, A.A., Mascolo, N., Capasso, R., Germano, M.P., De Pasquale, R. and Capasso, F. (1999). Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. Naunyn Schmiedeberg' s Arch. Pharmacol. 360(2): 221 – 223.
16. Joy, P.P., Thomas, J., Mathew, S. and Skaria, B.P. (1998). Medicinal Plants. Aromatic and Medicinal Plants Research Station, Kerala Agricultural University, India.
17. Kutchai, H.C. (2004). The gastrointestinal system: Gastrointestinal regulation and motility. In: Berne, R.M., Levy, M.N., Koeppen, B.M., Stanton, B.A. (eds) Physiology 5th ed. Mosby, Missouri, USA, pp 559.
18. Meena, A.K., Yadav, A.K., Niranjan, U.S., Singh, B., Nagariya, A.K. and Verma, M. (2010). Review on *Cyperus rotundus* – A potential herb. IJPCR. 2: 20 – 22.
19. National Drug Committee, Ministry of Public Health. (2006). National List of Essential Medicines of Thailand.
20. Nwodo, O.F. and Alumanah, E.O. (1991). Studies on *Abrus precatorius* seeds. II: Antidiarrhoeal activity. J. Ethnopharmacol. 31(3): 395 – 398.
21. Organization of Economic Co-operation and Development. (2001). The OECD guideline for testing of chemical: 420 Acute Oral Toxicity. France.
22. Organization of Economic Co-operation and Development. (1981). The OECD guideline for testing of chemical: 408 Subchronic Oral Toxicity-Rodent: 90-day Study. France.
23. Pappano, A.J. and Kutzung, B.G. (2004). Cholinoceptor-Blocking Drugs. In: Kutzung, B.G. (ed) Basic and Clinical Pharmacology 9th ed. McGraw-Hill, USA, pp 109 – 121.
24. Pierce, N.F., Carpenter, C.C.J., Elliot, H.I. and Greenough, W.B. (1971). Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology. 60: 22 – 32.
25. Prins, N.H., Akkermans, L.M., Lefebvre, R.A. and Schuurkes, J.A. (2000). 5-HT(4) receptors on cholinergic nerves involved in contractility of canine and human large intestine longitudinal muscle. Br. J. Pharmacol. 131(5): 927 – 932.
26. Rabbani, G.H., Larson, C.P., Islam, R., Saha, U.R. and Kabir, A. (2010). Green banana-supplemented diet in the home management of acute and prolonged diarrhea in children: a community-based trial in rural Bangladesh. Trop. Med. Int. Health. 15(10): 1132 – 1139.
27. Rabbani, G.H., Teka, T., Saha, S.K., Zaman, B., Majid, N., Khatun, M., Wahed, M.A. and Fuchs, G.J. (2004). Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. Dig. Dis. Sci. 49(3): 475 – 484.
28. Rajkumar, M.H., Sringeswara, A.N. and Rajanna, M.D. (2011). Ex-situ conservation of medicinal plants at University of Agriculture Sciences, Bangalore, Karnataka. Recent Research in Science and Technology 3(4): 21-27.
29. Sawangjaroen, N. and Sawangjaroen, K. (2005). The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: *Blastocystis hominis*. J. Ethnopharmacol. 98(1-2): 67 – 72.
30. Sharma, P.C., Yelne, M.B. and Dennis, T.J. (2001). Delhi: Central Council for Research in Ayurveda and Siddha. Database on medicinal plants used in Ayurveda.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.8
31. Shibata, C., Sasaki, I., Naito, H., Ueno, T. and Matsuno, S. (1999). The herbal medicine Dai-Kenchu-Tou stimulates upper gut motility through cholinergic and 5-hydroxytryptamine 3 receptors in conscious dogs. Surgery. 126(5): 918 – 924.
32. Shook, J.E., Lemcke, P.K., Gehrig, C.A., Hruby, V.J. and Burks, T.F. (1989). Antidiarrheal properties of supraspinal mu and delta and peripheral mu, delta and kappa opioid receptors: inhibition of diarrhea without constipation. J. Pharmacol. Exp. Ther. 249(1): 83 – 90.
33. Snyder, J.D. and Merson, M.H. (1982). The magnitude of the global problem of acute diarrhoea disease: A review of active surveillance of data. Bull. WHO. 60: 605 – 613.
34. Taufiq-Ur-Rahman, M., Shilpi, J.A., Ahmed, M. and Faiz Hossain, C. (2005). Preliminary pharmacological studies on *Piper chaba* stem bark. J. Ethnopharmacol. 99(2): 203 – 209.
35. Venkatesan, N., Thiyagarajan, V., Narayanan, S., Arul, A., Raja, S., Kumar, S.G.V., Rajarajan, T. and Perianayagam, J.B. (2005). Anti-diarrhoeal potential of *Asparagus racemosus* wild root extracts in laboratory animals. J. Pharm. Pharmaceut. Sci. 8(1): 39 – 46.
36. World Health Organization. (2000). General guidelines for methodologies on research and evaluation of traditional medicine. Switzerland.
37. Yegnanaravan, R. and Shrotri, D.S. (1982). Comparison of antidiarrhoeal activity of some drugs in experimental diarrhoea. Indian J. Pharmacol. 14(4): 293 – 299.