Toxicology International, *Vol 28(4), 411-419, 2021, DOI: 10.18311/ti/2021/v28i4/28112*

Original Research
ISSN (Print): 0971-6580
ISSN (Online): 0976-5131

Evaluation of Haematological and Biochemical Activity of Thai Traditional Antinausea Remedy in Normal Albino rats

Watchara Damjuti[1*], Juthaporn Kwansang[1] and Jurairat Boonruab[2]

[1]Department of Integrative Medicine, Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi, Pathum Thani - 12130, Thailand; Watchara_d@rmutt.ac.th

[2]Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani - 12121, Thailand

History: Received 2021-07-08; Accepted 2021-08-23; Published 2021-12-22

Abstract

The present study was aimed to evaluate the effect of Thai Traditional anti-nausea remedy against haematological and biochemical effects in animals in the acute toxicity and sub-acute toxicity phase. Twenty-two male and female rats were divided into four groups with equal numbers of sex, each group were respectively received distilled water, 500, 1000 and 2000 mg/kg body weight as a single dose by gavage. While the experiment in the sub-acute phase, eighteen male and female rats were divided into three groups with equal numbers of sex. Each group were respectively given distilled water, 500 and 1000 mg/kg body weight once a day for the duration of the experiment until collecting blood samples. The blood samples were collected for haematological: Red Blood Cell (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), White Blood Cell (WBC), Neutrophil (NEU), Lymphocyte (LYMPH), Monocyte (MONO), Eosinophil (EO), Basophil (BASO) and biochemical analysis; Glucose (GLU), Blood Urea Nitrogen (BUN), Creatinine (CREA), Cholesterol (CHOL) and Triglyceride (TG). The results showed that the remedy caused behavioural adverse effects, but no mortality observed. In the acute toxicity phase, single oral administration of anti-nausea remedy increased mean difference compared to the control group on Creatinine level and decreased in AST level and ALP level ($P<0.05$). In the subacute study, daily oral administration of the remedy did not result in death or changes in the body weight and food consumption but also showed comparable results on renal function marker including increasing of BUN levels and decreasing of AST level and ALP level ($P<0.05$). In haematological and some biochemical analyses showed few disadvantageous effects. Based on the results obtained, although there were some changes in blood chemistry values, these were small changes not higher than reference values, indicating that the remedy was safe to take for a short period of time.

Keywords: Biochemical Activity, Haematological Activity, Kaelomvingvian Remedy, Thai Traditional Antinausea Remedy

1. Introduction

Traditional Thai medicine is a system of methods and practices, such as herbal medicine, bodywork practices and spiritual healing that is indigenous to the region currently known as Thailand. Thai Traditional Pharmacy practice in Thailand comprises of the use of Materia Medica or recipe derived from plants, animals or minerals, which are used as traditional medicines. Furthermore, it involves the art of compounding those ingredients into various

*Author for correspondence

dosage forms of the recipes. Thai herbal medicines can be divided into groups according to the abnormalities found in the diagnosis of practitioners. One of the most common disorders is nausea, vomiting, dizziness and headaches. These disorders are particularly important because they can increase the risk compounded in elderly persons with other neurologic deficits and chronic medical problems. The total incidence of dizziness, vertigo and imbalance is 5 to 10% and it rises to 40% in individuals over the age of 40[1]. In terms of treatment, most Thai traditional practitioners prefer to treat with herbal medicines. Especially, cordial which is invigorating and stimulating preparation that is intended for a medicinal purpose.

According to Herbal Products Act of Thailand (B.E. 2560), herb means natural produce obtained from plants, animals, microorganisms or minerals mixed with cooked or transformed into herbal products. Currently, the Ministry of Public Health has established a list of drugs from herbs under the National Drug List (B.E. 2556) in two categories including Thai traditional medicineand drugs developed from herbs. Thai law prescribes Thai traditional medicine to mean drugs that are directly derived from herbs or obtained from mixing, cooking or converting herbs intended for use in accordance with the knowledge of Thai traditional medicine[2]. Interestingly, Thai traditional medicine is a drug that has been used for a long time since past century has been divided according to the announcement of the Ministry of Health into 8 groups according to the symptoms of treatment such as circulatory system syndrome drugs, gastrointestinal syndrome drugs, obstetrics and gynecology syndrome drugs, antipyretics drugs, respiratory syndrome drugs, blood tonic, musculoskeletal syndrome drugs and restorative tonic. Circulatory system syndrome drugs are a group of drugs that are widely used such as Thiposot remedy, Theppajit remedy, Nawakot remedy and Intachak remedy[3]. Kaelomvingvian remedy or Thai traditional anti-nausea remedy (Pharmacopoeia of Wang Nam Yen Hospital, Sa Kaeo Province)is also one of circulatory system syndrome drugs that have been used for relieve dizziness, fatigue and insomnia symptom. With precautions, just being careful of eating together with drugs in the anticoagulant group or anti-platelet drugs and should be careful to use with patients who are allergic to pollen. Thai traditional anti-nausea remedy consists of various proportions of twenty-three ingredients including *Glycyrrhiza glabra* L., *Myristica fragrans* Houtt., *Syzygium aromaticum* (L.) Merrill and Perry, *Angelica sinensis* (Oliv.) Diels, *Ligusticum chuanxiong* Franch, *Chrysopogon zizanioides* (L.) Roberty, *Nelumbo nucifera* Gaertn., *Cinnamomum bejolghota* (Buch. -Ham.) Sweet, *Cinnamomum loureirii* Nees., *Cinnamomum verum* J. Presl, *Aquilaria crassna* Pierre, *Euphorbia antiquorum* L., *Artemisia annua* L., *Terminalia chebula* Retz., *Alyxiarein wardtii* Blume, *Dryobalanops aromatica* Gaertn., *Lagerstroemia floribunda* Jack, *Mesua ferrea* L., *Mimusops elengi* L., *Mammea siamensis* T. Anders, *Urceola rosea* (Hook.&Arn.) D.J. Middleton, *Dracaena loureirin* Gagnep, and sodium tetraborate decahydrate. From the fact that the medicinal formula contains various herbs, the composition of the drug resulting in a drowsiness effect by showing a successive GABAergic agonistic effect[4].

Although the mechanism of action of the drug has been studied; however, information regarding their hematological and biochemical activity is yet to be reported. Hematological and biochemical parameters are tools for toxicity screening. Hematological and biochemical parameters evaluation is a critical and sensitive measure that is used in toxicity research to extrapolate experimental findings to clinical studies[5]. Therefore, this study aims to determine the hematological and biochemical activity of Thai Traditional anti-nausea remedy in normal albino rats.

2. Material and Methods

2.1 Plant Material and Extraction

This research was supported by herbal raw materials from Academic and Production Services Centre for Aesthetic and Health, Rajamangala University of Technology Thanyaburi. The specimens were then re-authenticated by botanist and compared with the standard herbal materials and reference voucher specimen.Thai Traditional anti-nausea remedy were then prepared by grinding and mixing of G. *glabra* root (32g), *M. fragrans* fruit (24 g), *S. aromaticum* bud (12g), *A. sinensis* root (12g), *L. chuanxiong* rhizome (12 g), *C. zizanioide* soot(12g), *N. nucifera* stamen (12g), *C. bejolghota* bark (10g), *C. loureirii* bark (8g), *C. verum* bark (8g), *A. crassna* bark(8g), *E. antiquorum* pith (8g), *A. annua* aerial part (8g), *T. chebula* tubercle (8g), *A. reinwardtii* root (8g), *D. aromatic* leaf (6g), *L. floribunda* wood (6g), *M. ferrea* flower (6g), *M. elengi* flower (6g), *M. siamensis* flower (6g), *U. rosea* vine (5g), *D. loureiri* pith (4g) and 4g of cooked sodium tetraborate decahydrate by National Drug List (B.E. 2556) proportion[3]. The mixed powder was ground and weighed, then simmered in deionized water (60°C) according to the Thai traditional medicine pharmacy until exhaustion. The aqueous extracts were then filtered through Whatman number 1 filter paper and allowed to dry by freeze dryer (Labconco Corporation, USA) to obtain the extract yields (yield = 19.89% w/w). The crude extract was then stored in -20 °C for further use throughout studies.

2.2 Experimental Animals and Ethical Clearance

Adult albino Sprague Dawley rats (7–8 weeks old with a body weight range of 180-220g) from the National Laboratory Animal Centre, Mahidol University, Thailand, were used to evaluate toxicity testing. Rats were housed in the Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Thailand, under standard environmental conditions (24°C ± 1°C, 60%–70% humidity, 12-h light: 12-h dark cycle). Food and water were given ad libitum. All experimental procedures were conducted in accordance with guidelines of the Animal Care and Use Committee, Rajamangala University of Technology Thanyaburi, Thailand (Animal License No. RMUTT.TMC.2020.R001).

2.3 Acute Toxicity Testing

In accordance to OECD Test Guidelines 420, acute toxicity testing was done by dividing rats into 4 groups of 6 mice per group (3 females and 3 males)[6]. Group 1 was received distilled water p.o. as vehicle control by gavage. Groups 2-4 were experimental groups. Each group respectively received 500, 1000 and 2000 mg/kg p.o. as single dose and mice were kept without food for 3–4 h prior to dosing. Each was then observed every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for behavioural signs of toxicity (changes in skin, hair, eyes, mucous membranes and respiratory, circulatory, autonomic and central nervous systems, motor activity, convulsion, tremors, salivation, diarrhoea, lethargy or sleep). Daily feed and water intake were recorded for each rat. The animals were monitored for a total of 14 days for the long-term possible lethal outcome. The body weights of the animals were daily measured.

2.4 Sub-acute Toxicity Testing

In accordance to OECD Test Guidelines 425, sub-acute toxicity testing was done by dividing rats into 3 groups of 6 mice per group (3 females and 3 males)[7]. Group 1 received distilled water as vehicle control by gavage. Groups 2 and 3 were experimental groups. Each group respectively received 500 and 1000 mg/kg for 28 days and mice were kept without food for 3–4 h prior to dosing. On day 28, the rats were weighed and sacrificed. Blood samples were collected via cardiac puncture for biochemical and haematological analyses

2.5 Blood Collection and Biochemical Analysis

Before the blood collection, the rats will stop feeding for 18 hours. Each rat was intraperitoneally injected with thiopental sodium (150 mg/kg). After that, the blood (2 ml) will be collected by heart puncture technique and immediately stored in EDTA blood collection tubes. The samples were then centrifuged for 15 min at 3,000 rpm (1500 × g) at 4°C. Then, the supernatant (plasma) was filtered through 0.22 µM to a 0.5 ml-Eppendorf tube.

2.6 Hematological Analysis

The blood samples from animals (both treated and vehicle control groups) were tested for hematological study. CBC parameters, Hemoglobin (Hb), total RBC, Packed Cell Volume (PVC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), platelet count, White Blood Cells (WBC) count, Neutrophils (N), Lymphocytes (L), Monocytes (M) and Eosinophils (E) were determined with homolyze photometers. Detection of blood chemistry Hitachi 902 (Boehringer Mannheim, Germany) automatic analysis with the Roche Diagnostics product Albumin detection kit (ALB), Alkaline Phosphatase (ALP), Total Bilirubin (Bili-T), Blood Urea Nitrogen (BUN), Cholesterol (CHOL), Creatinine (CREA), Glucose (GLU), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Total Protein (TP), Triglyceride (TG), Uric Acid (UA), Globulin (GLOB).

2.7 Statistical Analysis

Results are presented as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance, followed by Tukey's HSD *post hoc* test using SPSS software, SPSS INC., USA

3. Results and Discussion

In the acute toxicity phase, non-mortality was observed in all groups after received the extract. Furthermore, no death was occurred during 14 days the animals that received 500, 1000 and 2000 mg/kg and no significant differences in body weights and food consumption was observed between the control group and the experimental groups (Figure 1). Furthermore, the results of blood chemistry profiles and hematological analysis were showed in Figure 2 and Table 1 respectively. In the sub-acute toxicity phase, no death was occurred during 28 days the animals that received 500 and 1000 mg/kg and no significant differences in body weights and food consumption was observed between the control group and the experimental groups (Figure 1). Furthermore, the results of blood chemistry profiles and hematological analysis were showed in Figure 3 and Table 1 respectively. Even though, there is no shown the lethality effect, but the animal obviously showed a still, calm, reduced movement, when receiving extracts of all dosages and clearly showed more about 1-2 hours after receiving the extract in the acute toxicity study. In accordance with the previous study, the aqueous extract of Thai traditional

anti-nausea remedy was found to be a hypnotic and sedative agent by successive GABAergic agonistic effect resulting in locomotor deactivation and anti-anxiety[4]. In term of blood chemistry profiles of rats in acute toxicity testing, the blood was drawled and then examined Blood Urea Nitrogen level (BUN), Creatinine level, Aspartate Transaminase or Aspartate Aminotransferase level (AST) level, Alanine Transaminase level (ALT), Alkaline Phosphatase level (ALP), Total Protein Level, albumin level and globulin level (Figure 2). The results were found that the high dose (2000 mg/kg) was significantly showed to increase the mean different compared to the control group on Creatinine level and decreased in AST level and ALP level.

Creatinine is a chemical waste product that's produced by muscle metabolism, the creatinine level indicates glomerular filtrate function. In the female rats, the highest dose (2000 mg/kg) showed to be increased when compared to others ($P<0.05$). Similarly, to male groups, the Creatinine level was also tended to be marginally increased in all groups. The results of Creatinine level, combined with marginally increased BUN levels, show that high doses of extracts can affect kidney function by increasing the filtration rate of the kidney.

In addition, the evaluation in sub-acute phase revealed, the liver function which is an important organ in changing chemical structure in order to make a drug reaction. The extract impacted on AST level and ALP level by decreasing in high dose (2000 mg/kg). AST is an enzyme that helps metabolize amino acids. In contrast, ALP is an enzyme found in the liver and bone and is important for breaking down proteins. The low AST levels are remarkable in the normal liver function. However, low AST may signal vitamin B6 deficiency, vitamin B6 deficiency is uncommon, but it's more likely to occur in underlying health conditions when kidney disorders occur since vitamin B6 required for AST enzyme to function[8,9]. In blood chemistry profiles, the experimental results were similar to the acute toxicity study. When the extract was continuously given for 14 days, it was found that BUN levels were significantly increased at 500 and 1,000 mg/kg in both sexes (Figure 3). Moreover, the decrease of ALP levels indicated the abnormal of the liver or bones. However, they may also indicate malnutrition, kidney cancer tumors, intestinal issues, a pancreas problem or a serious infection[10].

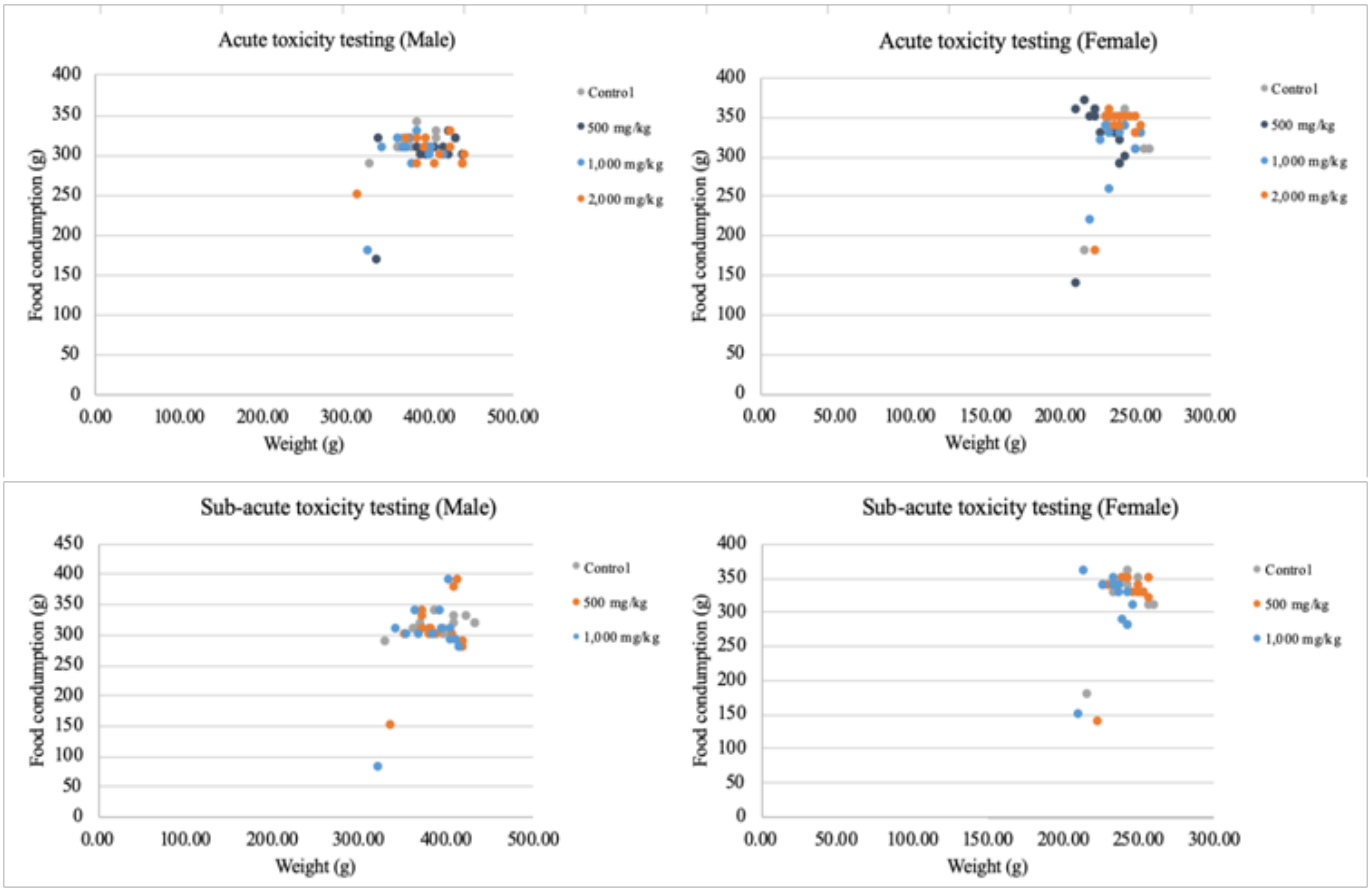
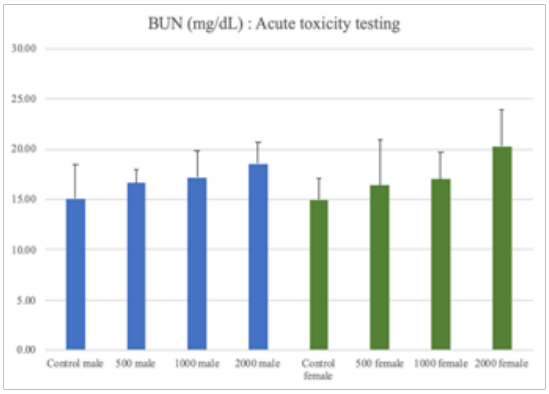
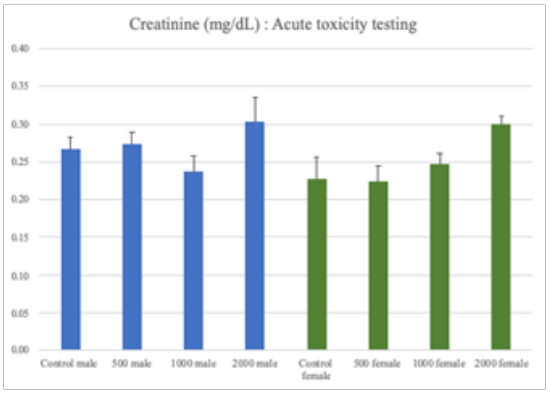


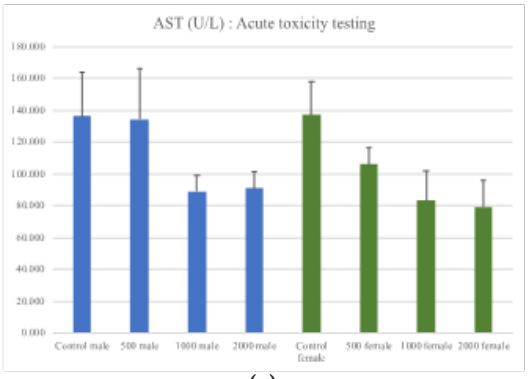
Figure 1. Acute and sub-acute toxicity testing – nutritional consumption and weight for Thai Traditional Anti-nausea Remedy.



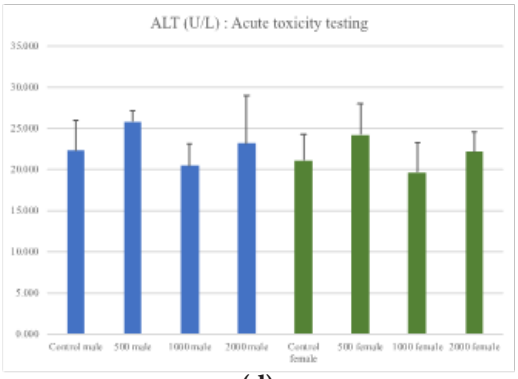
(a)



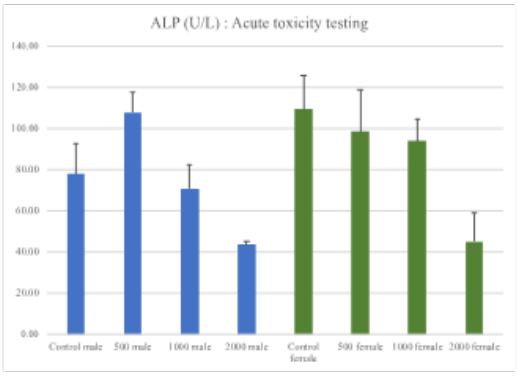
(b)



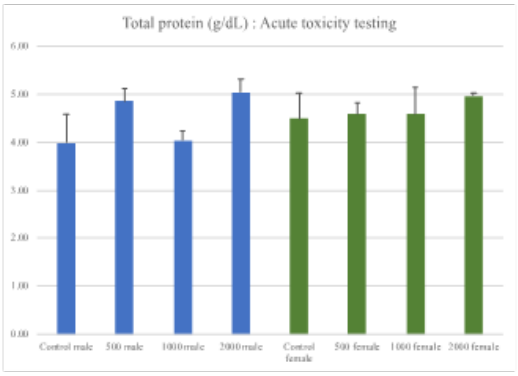
(c)



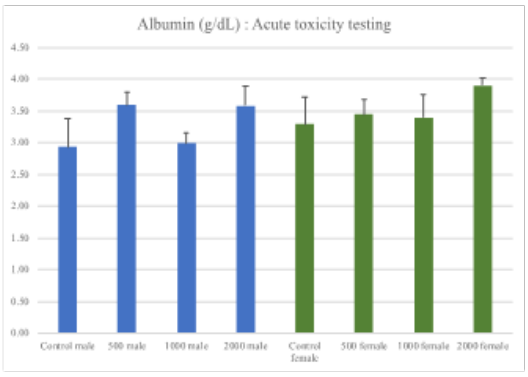
(d)



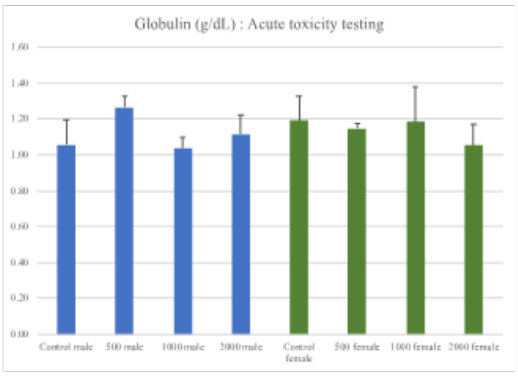
(e)



(f)

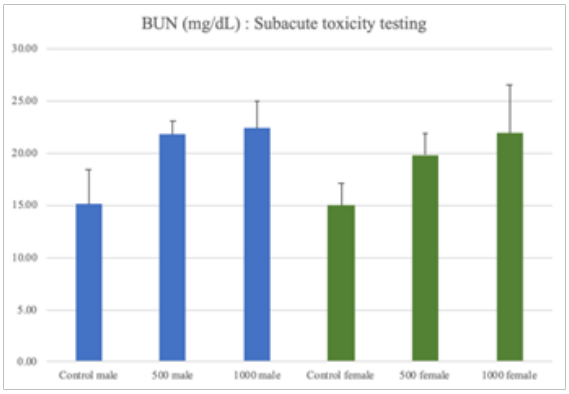


(g)

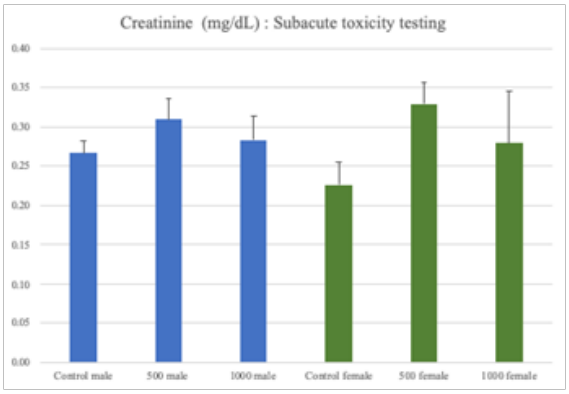


(h)

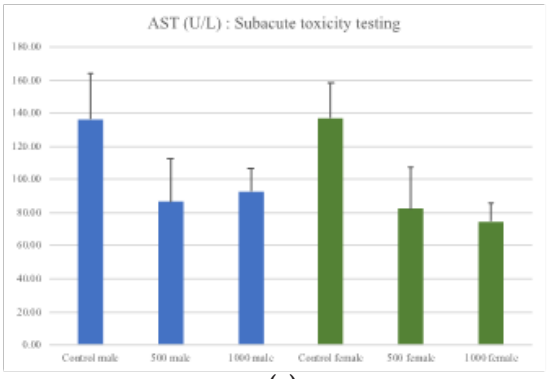
Figure 2. Blood chemistry profiles of rats in acute toxicity testing: **(a)** BUN level; **(b)** Creatinine level; **(c)** AST level; **(d)** ALT level; **(e)** ALP level; **(f)** Total protein level; **(g)** Albumin level; and **(h)** Globulin level.



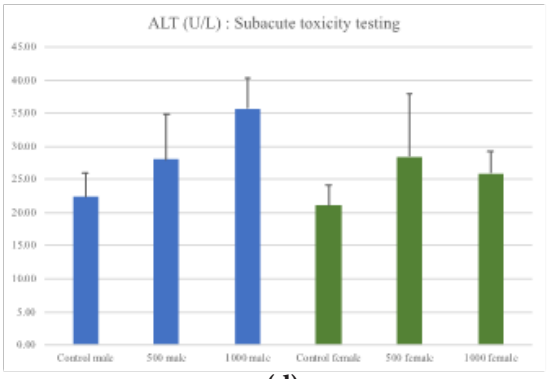
(a)



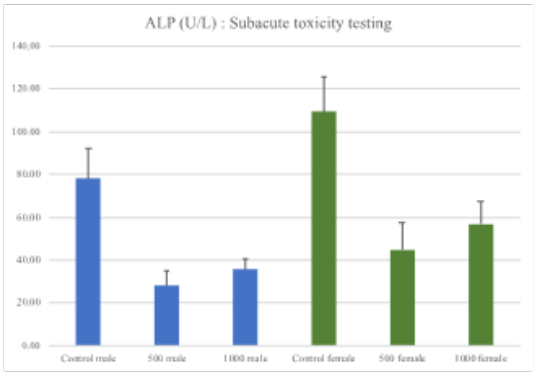
(b)



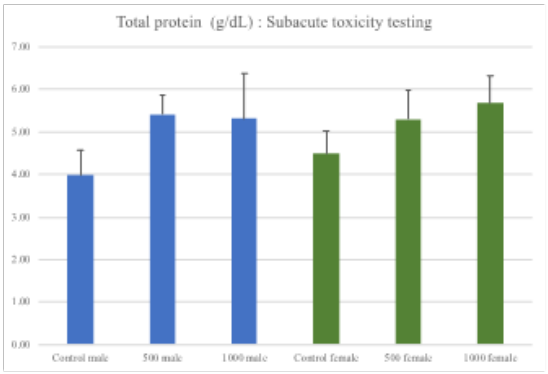
(c)



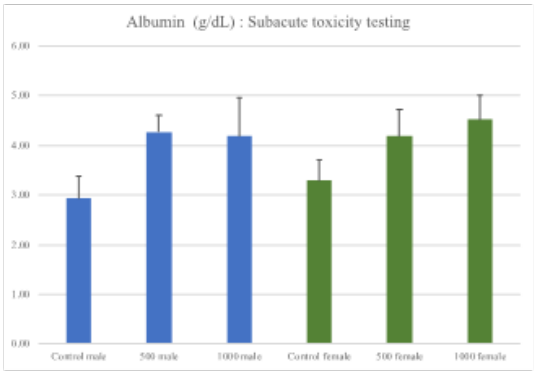
(d)



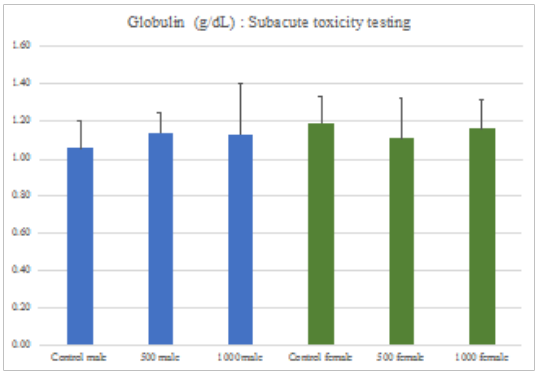
(e)



(f)



(g)



(h)

Figure 3. Blood chemistry profiles of rats in acute toxicity testing: **(a)** BUN level; **(b)** Creatinine level; **(c)** AST level; **(d)** ALT level; **(e)** ALP level; **(f)** Total protein level; **(g)** Albumin level; and **(h)** Globulin level.

Table 1. Showed the hematology values in mice of acute and subacute toxicity testing

Parameters	Acute toxicity (Mean ± SD)								Sub-acute toxicity (Mean ± SD)			
	Vehicle control		500 mg/kg		1,000 mg/kg		2,000 mg/kg		500 mg/kg		1,000 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
RBC (10^6/ul)	7.64±0.21	7.74±0.18	7.79±0.14	7.67±0.23	7.88±0.10	8.21±0.19	8.45±0.37	8.11±0.28	7.92±0.20	8.08±0.53	6.91±1.18	8.42±0.19
HGB (g/dl)	14.50±0.26	14.73±0.31	14.33±0.15	14.43±0.32	14.73±0.06	15.57±0.23	15.90±0.96	14.70±0.75	14.70±0.20	14.97±1.15	12.83±2.14	15.47±0.71
HCT (%)	42.67±1.15	43.20±1.22	42.60±0.79	43.53±0.38	43.73±0.70	47.10±0.46	48.60±2.91	44.20±3.08	44.27±0.12	44.67±4.29	37.50±7.07	46.60±1.99
MCV (fl)	55.83±1.27	55.77±0.40	54.67±2.01	56.80±1.65	55.50±1.57	57.40±1.54	57.50±1.01	54.4±2.00	55.93±1.27	55.17±1.72	54.13±1.89	55.33±1.16
MCH (pg)	18.97±0.46	19.03±0.12	18.43±0.49	18.83±0.23	18.70±0.35	18.93±0.15	18.83±0.29	18.10±0.36	18.60±0.46	18.53±0.38	18.57±0.35	18.37±0.45
MCHC (g/dl)	34.00±0.35	34.07±0.42	33.63±0.35	33.13±0.68	33.70±0.46	33.07±0.65	32.73±0.32	33.30±0.62	33.20±0.46	33.57±0.65	34.37±0.91	33.20±0.50
RDW (fl)	29.20±0.26	29.27±1.27	28.47±1.12	30.07±0.47	30.03±0.87	30.50±0.75	28.33±0.86	27.37±1.10	27.33±0.91	27.23±0.31	25.33±1.07	27.93±0.64
RDW (%)	18.60±0.69	18.87±0.98	18.93±0.85	19.03±0.85	20.07±0.15	19.73±0.91	18.83±1.33	18.80±0.66	17.97±1.77	18.03±0.40	15.70±1.51	19.40±0.79
RET (10^3/ul)	241.90±5.90	211.77±21.00	221.13±51.25	221.57±24.48	265.57±26.95	252.53±58.69	225.07±42.48	235.80±11.35	233.90±31.19	216.13±28.05	189.73±61.85	217.93±16.54
RET (%)	3.17±0.12	2.74±0.34	2.84±0.67	2.90±0.40	3.37±0.39	3.07±0.67	2.65±0.40	2.91±0.06	2.95±0.32	2.69±0.48	2.69±0.46	2.59±0.14
PLT (10^3/ul)	846.67±38.28	914.33±69.97	828.00±111.72	858.33±111.55	869.33±42.22	846.33±54.60	904.00±49.79	908.33±55.61	966.00±72.81	994.33±84.56	876.33±285.09	1053.00±49.67
PDW (fl)	8.47±0.31	9.03±0.50	8.80±1.04	9.13±0.23	8.90±0.26	8.73±0.40	9.23±0.29	8.83±0.55	8.60±0.36	8.53±0.40	8.03±0.31	8.23±0.49
MPV (fl)	7.17±0.25	7.57±0.35	7.47±0.47	7.63±0.06	7.47±0.21	7.47±0.23	7.77±0.15	7.43±0.31	7.30±0.26	7.23±0.23	6.93±0.31	7.07±0.40
P-LCR (%)	7.63±0.87	9.67±2.50	9.47±3.16	9.90±0.53	9.03±1.25	9.07±1.63	10.83±0.49	9.03±1.87	8.23±1.50	7.67±1.59	6.43±1.27	6.67±2.34
PCT (%)	0.61±0.04	0.69±0.07	0.62±0.06	0.65±0.09	0.65±0.04	0.64±0.05	0.71±0.05	0.67±0.07	0.71±0.04	0.720.09	0.60±0.20	0.74±0.05
WBC (10^3/ul)	7.31±0.58	5.95±0.98	7.58±1.20	9.57±2.06	7.67±0.63	8.26±1.63	7.53±1.65	7.69±1.18	6.82±0.40	5.55±1.85	6.48±0.95	8.66±1.98
NEU (10^3/ul)	1.08±0.09	1.02±0.29	0.74±0.56	1.33±0.35	1.28±0.19	0.55±0.78	0.72±0.18	0.55±0.13	0.51±0.07	0.50±0.04	0.25±0.21	0.42±0.31
LYMPH (10^3/ul)	5.57±0.55	4.46±0.72	6.16±1.29	7.51±1.65	5.78±0.53	6.92±1.03	6.12±1.39	6.56±1.01	5.66±0.53	4.61±1.70	5.71±0.91	7.38±1.48
MONO (10^3/ul)	0.57±0.08	0.37±0.09	0.56±0.35	0.58±0.09	0.49±0.04	0.69±0.09	0.59±0.30	0.47±0.17	0.55±0.10	0.35±0.10	0.38±0.08	0.67±0.11
EO (10^3/ul)	0.09±0.02	0.09±0.02	0.10±0.03	0.14±0.01	0.11±0.02	0.09±0.03	0.09±0.03	0.09±0.01	0.09±0.00	0.08±0.04	0.10±0.05	0.16±0.13
BASO (10^3/ul)	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.01	0.02±0.01	0.01±0.01	0.02±0.02	0.01±0.01	0.01±0.01	0.04±0.06	0.03±0.01

In the main structural analysis of the drug formula, *G. glabra* which is the largest proportion in the formulation, it is categorized as moderately toxic based on its lethal doses of 50% (LD50) value and its toxicity risk is lowest when administered orally. However, *G. glabra* should be avoided during pregnancy and in neonates because some clinical studies have shown a decrease in gestational age, preterm delivery, and changes in hypothalamic-pituitary-adrenocortical axis and cognitive functions in delivered children when used during pregnancy[11]. While the plants that are in the second proportion of the drug formula is *M. fragran*. *M. fragran* usually used in small amounts to flavor seasonal beverages and food. Myristicin, a sympathomimetic with hallucinogenic properties, is thought to be the compound responsible for *M. fragran* hallucinogenic effects[12]. Eugenol is a volatile phenolic constituent of essential oil obtained from *S. aromaticum*. Although eugenol is regarded to be acceptable as a food additive, there has been a lot of worry regarding its toxicity, some research revealed that oral intake of eugenol in different doses during a 15-days period may cause some changes in blood chemistry[13]. Ligustilide which is the major compound from *L. chuanxiong*. *L. chuanxiong* is one of the most safest ingredients in the recipe by exhibit oral and intra-peritoneal LD_{50} in mice were 7.23 g/kg and 2.25 g/kg respectively[14]. In addition, *N. nucifera* is another safe component. *N. nucifera* showed no-observed-adverse-effect level (NOAEL) in animal model by exhibited excess of 5000 mg/kg[15].

4. Conclusion

In conclusion, the toxicity studies of aqueous extract from Thai traditional anti-nausea remedy may affect kidney function by increasing the condition of the kidneys load. Based on the results obtained, although there were some changes in blood chemistry values, these were small changes not higher than reference values, indicating that the remedy was safe to take for a short period of time. Although the results of this study suggest that short-term use is safe, patients who need long-term use of this drug should be advised and cautious about the use of the drug under prescription by a professional practitioner.

5. Conflict of Interest

Authors declare that they have no conflict of interest.

6. Acknowledgement

The authors wish to thank the Applied Thai Traditional Medicine Clinic and Academic and Production Services Centre for Aesthetic and Health, Rajamangala University of Technology Thanyaburi, for providing the herbal material. The authors also wish to thank Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi, for supporting the facilities throughout research study.

7. References

1. Saber Tehrani AS, Coughlan D, Hsieh YH, Mantokoudis G, Korley FK, Kerber KA, Frick KD, Newman-Toker DE, Schneider S. Rising annual costs of dizziness presentations to U.S. Emergency Departments. Academic Emergency Medicine. 2013; 20(7):689–96. PMid:23859582. https://doi.org/10.1111/acem.12168
2. Thai Traditional and Alternative Health Profile: Thai Traditional Medicine, Indigenous Medicine and Alternative Medicine 2014-2016. Nonthaburi: Department of Thai Traditional and Alternative Medicine, Ministry of Public Health; 2017.
3. National Drug System Development Committee. Thailand National List of Essential Medicines (NLEM). Government gazette. 2018; 135(14):228–31.
4. Damjuti W, Kwansang J, Boonruab J. Effect of Thai traditional anti-nausea remedy on hypnotic and sedative activity in animal experimental models: Interaction with drugs acting at GABAA receptor. J Adv Pharm Technol Res. 2019; 10(2):85–9. PMid:31041188 PMCid:PMC6474159. https://doi.org/10.4103/japtr.JAPTR_361_18
5. Delwatta SL, Gunatilake M, Baumans V, Seneviratne MD, Dissanayaka MLB., Batagoda SS, Udagedara AH,Walpola PB. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Anim Models Exp Med. 2018; 1(4):250–4. PMid:30891574 PMCid:PMC6388088. https://doi.org/10.1002/ame2.12041
6. Organisation for Economic Cooperation and Development (OECD). Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure. 2002.
7. Organisation for Economic Cooperation and Development (OECD). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. 2008.
8. Chimata M, Masaoka H, Fujimaki M, Hamada N, Oda K, Sudo M, Nagase M. Low serum aminotransferase activity in patients undergoing regular hemodialysis. Nihon Jinzo Gakkai Shi. 1994; 36(4):389–95.

9. Ono K, Ono T, Matsumata T. The pathogenesis of decreased aspartate aminotransferase and alanine aminotransferase activity in the plasma of hemodialysis patients: The role of vitamin B6 deficiency. Clin Nephrol. 1995; 43(6):405–8.

10. Siddique A, Kowdley KV. Approach to a patient with elevated serum alkaline phosphatase. Clin Liver Dis. 2012; 16(2):199–229. PMid:22541695 PMCid:PMC3341633. https://doi.org/10.1016/j.cld.2012.03.012

11. Nazari S, Rameshrad M, Hosseinzadeh H. Toxicological effects of *Glycyrrhiza glabra* (Licorice): A review. Phytother Res. 2017; 31(11):1635–50. PMid:28833680. https://doi.org/10.1002/ptr.5893

12. Ehrenpreis JE, DesLauriers C, Lank P, Armstrong PK, Leikin JB. Nutmeg poisonings: A retrospective review of 10 years experience from the Illinois Poison Center, 2001-2011. J Med Toxicol. 2014; 10(2):148–51. PMid:24452991 PMCid:PMC4057546. https://doi.org/10.1007/s13181-013-0379-7

13. Li J, Yu Y, Yang Y, Liu X, Zhang J, Li B, Zhou X, Niu J, Wei X, Liu Z. A 15-day oral dose toxicity study of aspirin eugenol ester in Wistar rats. Food Chem Toxicol. 2012; 50(6):1980–5. PMid:22516304. https://doi.org/10.1016/j.fct.2012.03.080

14. Zhang H, Han T, Yu C-H, Jiang Y-P, Peng C, Ran X, Qin L-P. Analysis of the chemical composition, acute toxicity and skin sensitivity of essential oil from rhizomes of *Ligusticum chuanxiong*. J Ethnopharmacol. 2012; 144(3):791–6. PMid:23085396. https://doi.org/10.1016/j.jep.2012.10.010

15. Kunanusorn P, Panthong A, Pittayanurak P, Wanauppathamkul S, Nathasaen N, Reutrakul V. Acute and subchronic oral toxicity studies of Nelumbo nucifera stamens extract in rats. J Ethnopharmacol. 2011; 134(3):789–95. PMid:21291992. https://doi.org/10.1016/j.jep.2011.01.037