JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Aquilaria subIntegra herbal tea leaves: 28-day repeated-dose oral sub-acute toxicity in Wistar rats

ACHARAPORN ISSURIYA[1], UTAI THONGBANG[2], CHONLATID SONTIMUANG[3], SINEENART SANPINIT[4], SASITORN CHUSRI[5], THAMMARAT KAEWMANEE[6], SURASAK LIMSUWAN[7,8]*

[1]*Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

[2]*Southern Laboratory Animal Facility, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

[3]*Office of the President, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

[4]*Department of Applied Thai Traditional Medicine, School of Medicine, Walailak University, Thasala, Nakhon Si Thammarat, 80160*

[5]*School of Health Science, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand*

[6]*Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Pattani 94000, Thailand*

[7]*Center of Antimicrobial Biomaterial Innovation-Southeast Asia and Natural Product Research Center of Excellence, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

[8]*Traditional Thai Medical Research and Innovation Center, Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand, Email: surasak.l@psu.ac.th*

**Corresponding Author*

ABSTRACT

Aquilaria subintegra Ding Hou (agarwood) tea has a high market value. Previous research found that agarwood leaf extract displayed potent anti-diabetic properties. However, the assessment of its safety is limited. In this work, the safety information at the no-observed-effect-level (NOEL) and the human equivalent dose (HED) of A. subintegra tea leaf extract for repeated-dose 28-day consumption was investigated. The tea leaves were extracted with hot water according to the usual method of consumption. The extract at 5, 50, and 300 g/kg body weight was orally administered daily up to 28 days in both genders of Wistar rat. The clinical signs and symptoms of toxicity were inspected including mortality, morbidity, and behavioral and functional integrity. Animal blood samples were checked for hematological and biochemical parameters, and internal organs (liver, spleen, kidney, lung, heart, ovary, and testis) were examined for any gross lesions and maintained for histopathological studies. Results showed that repeated oral dose (5, 50, and 300 mg/kg) for 28 days did not affect any of the measured toxicity parameters. The NOAEL of the extract was noted as more than 300 mg/kg body weight. The estimated HED was found to be approximately 40 mg/kg body weight. The results proved that A. subintegra tea is considered safe as there was no adverse effect related to its use.

KEYWORDS:
Agarwood; Aquilaria subintegra; Human equivalent dose (HED); No-observed-effect-level (NOEL); Safety; Toxicological study

ARTICLE HISTORY:
Received July 10, 2021
Accepted Nov 15, 2021
Published Dec 11, 2021

DOI:
10.5455/jcmr.2021.12.04.41

VOLUME: 12
ISSUE: 4
ISSN: 2146-8397

INTRODUCTION

Agarwood tea is produced from the leaves of Aquilaria spp., a tree in the family of Thymelaeaceae. There are approximately 25 species of Aquilaria spp., with Aquilaria agallocha, Aquilaria crassna, Aquilaria malaccensis, and Aquilaria sinensis being among the most often mentioned species in various published works [1, 2]. The most common plantation species for producing a tea drink are A. crassna, A. malaccensis, and A. sinensis [3]. The biological activities of Aquilaria spp. related to diabetes mellitus remedy have been reported. Hypoglycemic, antioxidant, and α-glucosidase inhibitory potentials of A. crassna leaf water extract in type II diabetic mice have been discovered [4]. Low doses of methanol and water extracts of A. malaccensis showed antihyperglycemic

activity by lowering glucose levels to within the normal range in type II diabetic mice [5]. Both ethanol and water extracts of A. sinensis leaves demonstrated antidiabetic activity in streptozotocin-induced diabetic rats and enhanced glucose uptake by rat adipocytes [6]. By suppressing hyperlipidemia, hepatopathy, nephropathy, and obesity, the water extracts of green tea fermented with Aquilariae Lignum (stem of A. agallocha) help to alleviate diabetes and associated consequences in db/db and high-fat diet animal models [7, 8]. One diabetic patient who drank agarwood leaf water infusion instead of water more than six months was reported, the blood glucose fell from 184 mg/dL to normal (117 mg/dL) [9]. However, no further clinical trial has been done to confirm this hypoglycemic action of agarwood tea and its scientific proof for the safe in humans is still lacking.

Aquilaria subintegra Ding Hou is cultivated widely in Southern Thailand. Due to the long period of time for growth and fragrant resinous wood induction, many local agriculturists capitalized on their planted tree leaves by manufacturing a tea drink [3, 6, 10]. The tea from A. subintegra leaves has a different and unique flavor from other Aquilaria species. Hypoglycemic property of A. subintegra tea was claimed by some diabetic patients in Thailand, while its assessment to deliver safety and clinical evidences has not been available yet. According to various scholarly articles, Aquilaria tea leaves have been administered orally as extract or infusion water for diabetic treatment [3, 6]. Therefore, to develop this plant as a herbal tea for diabetes the in vivo toxicity testing is required by controlling agencies prior to the start of clinical study. In this study, we aimed to evaluate the 28-day repeated-dose oral sub-acute toxicity of A. subintegra tea leaf extract in rats to ensure its safety for human consumption.

MATERIALS AND METHODS

Plant materials and preparation of agarwood tea leaf extract

Agarwood tea leaves were obtained from Thai Rung Agricultural Limited Partnership, a community enterprise manufacturer and distributor of agarwood leaf tea from A. subintegra species. The agarwood trees were cultivated in Songkhla and Pattani Provinces, Thailand. Plant samples were collected for botanical identification and voucher specimens were deposited at Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand. For extraction, the processed tea leaves were randomly collected and soaked with boiling water at 98 ± 2 °C for 30 min. The infusion water was then filtered and freeze dried. The extract power was kept in airtight container at -4 °C.

Experimental animals

Adult Wistar rats (200-300 g) were purchased from the National Animal Laboratory Center, Mahidol University (Nakhon Pathom, Thailand). Twenty rats of each gender were separately housed in steel mesh cages in a ventilated area with a 12 h light/dark artificial photoperiod (150-300 lux) and 10-20 air exchange levels per h at 23-25 °C and relative humidity of 50-55%. The animals were given free access to irradiation-sterilized pellet feed (Charoen Pokphand Foods Public Company Limited Bangkok, Thailand) and distilled water. The experiment was carried out in conformity with Good Laboratory Practice (GLP) and Organization for Economic Cooperation and Development (OECD) test guideline 407, Repeated Dose 28-day Oral Toxicity Study in Rodents guidelines [11] at the Southern Laboratory Animal Facility. Every attempt was made to ameliorate animal suffering and to diminish the number of animals utilized. This study procedure was authorized by Animal Ethical Committee of Prince of Songkla University (MOE 0521.11/1304).

Design of the animal experiments

Following a 2-week quarantine and acclimation period, 40 Wistar rats were randomly separated into 4 groups. Groups 1 to 3 were assigned as experimental groups which oral gavaged with 5, 50 or 300 mg/kg body weight/day of water extract for 28 days. Group 4 was the control group that administered with vehicle (distilled water) daily throughout the study period. The A. subintegra leaf extract power was dissolved with distilled water before the oral administration.

Cage-side observation and examination

Cage-side observation was made immediately, particularly for the first 1 h after the administration of the extract, every 2 h for 24 h, and then daily for 28 days. The mortality, morbidity, and clinical symptoms of toxicity were inspected as mentioned in the OECD guideline. The observations, such as the determination of survival numbers, skin and fur appearances, respiratory effects, autonomic effects, and central nervous system effects were monitored during the experimental period. Body weight and food consumption were recorded before extract administration and then documented daily during the test period.

Hematology and biochemistry analysis

On the 29th day, all rats were anesthetized and blood samples from each rat were collected for hematological and biochemical analysis. Hematological parameters including white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, and platelet count were checked (ADVA 2120i Hematology system). Blood biochemical parameters, including creatinine, blood urea nitrogen, alkaline phosphatase, and alanine aminotransferase were examined (Cobas® 6000 analyzer, Roche Diagnostics, Penzburg, Germany).

Organs collection and histological examination

Animal organs including liver, spleen, kidney, lung, heart, ovary, and testis were carefully collected after euthanasia. All organs were weighed, and the relative organ weights were calculated. The organs were further evaluated for any gross lesions and kept in a 10% buffered formaldehyde solution for

histological examinations. After that, the organs were immersed in a 10% formalin solution for overnight. The organ samples were then dried using a Leica ASP300 automated tissue processor (Wetzlar, Germany), cleared with xylol, and infiltrated by molten paraffin wax at 50 °C. After infiltration, the specimens were embedded into molds and carefully sectioned using a microtome. Tissue sections (3-4 µm) were then stained with hematoxylin-eosin and inspected at ×20 and ×40 magnifications under a light microscope. For the microscopic findings, an initial unblinded evaluation of both the control and treated groups was performed. When the preliminary assessment revealed any changes, blind and/or semiquantitative ratings were performed. Two well-trained researchers carried out the histopathological examination.

Statistical analysis

The results are presented as the mean ± standard deviation. The data were assumed to be normally distributed, and one-way ANOVA was used to compare them, followed by the Bonferroni post hoc comparisons test. The value of $p<0.05$ were considered statistically significant.

RESULTS

Cage-side observation for symptoms of toxicity

Cage-side observation for 28-day repeated oral dose toxicity testing revealed that the extract administered rats at all dosages displayed normality. All animal groups demonstrated no mortality, morbidity, and clinical symptoms of toxicity. The changes of rat behaviors and appearances such as skin and fur alteration, respiratory effects, autonomic effects, and central nervous system effects were unnoticed throughout the treatment period when compared with the control group (Table 1).

Feed intake, body weight, and relative organ weight

During the experimental period, the food consumption patterns of animals in all groups were regular and constant. There was no significant difference in body weight between the four groups at each day during the entire treatment period (Fig. 1). Relative organ weight to body weight of 28-day repeated-dose oral sub-acute toxicity of water extract from A. subintegra tea leaves in rats are shown in Table 1. The consumption of the extract at all doses did not cause any significant changes in the relative weights or morphology of the liver, spleen, kidney, lung, heart, ovary, and testis among the test groups and when compared to the control group.

Hematology and clinical chemistry analysis

The 28-day repeated-dose oral sub-acute toxicity of 5, 50, and 300 mg/kg body weight A. subintegra tea leaf extract on the hematological parameters of rats are displayed in Table 3. All analyzed parameters revealed that there were no statistically significant changes among group receiving the extract and when compared to the respective control group for each parameter. There were no significant changes in blood biochemical parameters, including creatinine, blood urea nitrogen, alkaline phosphatase, and alanine aminotransferase, for both between the test group and compared to the control group during the study period (Table 4).

Histopathological examination

Histopathological examination of the vital organs including liver, spleen, kidney, lung, heart, ovary, and testis disclosed no significant histoarchitecture changes in all group of animals treated with 5, 50, and 300 mg/kg body weight A. subintegra tea leaf extract (Fig. 2). There were no evidence of necrosis, lesions, or pathological damage in the hepatocytes dispersed around the central veins, and they had a normal architecture. The spleen revealed normal histology in the white pulp, red pulp, and central arterials, with no histological abnormalities. Adequate glomeruli and renal tubules were intact without any signs of glomerular injury or lumen cast histopathological segments. Representative lung sections from treated and control groups presented normal alveolar and bronchiolar structures which were maintained and showed no inflammatory as evidenced by infiltration of immune cells. Inspection of ovarian tissues revealed a regular histological architecture with an outer simple cubical epithelium. At all doses of extract and control, there were no marked changes in testicular histology, seminiferous tubules were preserved, no diffuse or focal necrosis, and any inflammatory cell infiltration.

No-observed-adverse-effect-level (NOAEL) and human equivalent dose (HED)

The results from the 28-day repeated-dose oral sub-acute toxicity suggested that the no-observed-adverse-effect-level (NOAEL) of A. subintegra tea leaf extract was higher than 300 mg/kg body weight in rats (both genders), which no toxicity signs and symptoms or organ toxicity were detected. The estimated human equivalent dose (HED) calculated according to HED (mg/kg) = Animal does (mg/kg) × Km ratio [12] was 40 mg/kg body weight.

DISCUSSION

Medicinal plants and herbal teas play an important role in preventing and control diabetes mellitus [13, 14]. Many previous studies found that Aquilaria spp. showed various pharmacological properties that can potentially be used in the treatment of diabetes [3, 15]. For instance, antioxidant by DPPH radical scavenging and α-glucosidase inhibitory activities of A. crassna leaf water extract have been studied, and its IC50 values were found to be 34.6 and 36.3 µg/ml, respectively. A daily oral administration of 500-1000 mg/kg A. crassna leaf water extract for 4 weeks in streptozotocin-nicotinamide-induced type II diabetic mice significantly lowered the blood glucose by 66-86% [4]. Methanol and water extracts (50 mg/kg body weight) of A. malaccensis showed antihyperglycemic activity by lowering glucose levels to within the normal range in type II diabetic mice [5]. The methanol and water extracts of A. sinensis leaves at the dose of 1 g/kg body weight on

streptozotocin-induced diabetic rats demonstrated hypoglycemic activity which was equivalent to insulin at 4 U/kg. The glucose uptake enhancement activity of 10 µg/ml of methanol and water extracts was comparable to 1.5 nM insulin [6]. Due to the numerous potential biological activities related to hyperglycemic treatment, Aquilaria tea is becoming more popular [3]. Even with all these health benefits, Aquilaria tea, like any other herbal tea product, should be drank in moderation because negative effects may occur depending on the plant used, especially if taken in excessive doses. An anaphylactic or allergic reaction to chamomile tea [16, 17] and a generalized systemic allergic dermatitis triggered by cinnamon in a herbal tea [18] for examples are not unusual. Thus, a detailed examination of the toxicity effects of Aquilaria tea in animals will be enforced to have a better knowledge of its toxicity in humans.

Aquilaria subintegra is cultivated widely by local agriculturists in Southern Thailand for tea leaves. However, this tea product has never been registered for considering as food. For registration as a novel food according to the Thai Food and Drug Administration (FDA), the scientific evidence for the safe consumption of this tea is required. To anticipate the safe dosage for human intake, in vivo toxicological study of this plant product is helpful. In this present study, we evaluated the 28-day repeated-dose toxicity effects of A. subintegra tea leaf extract on both gender of rats. As expected, no clinical evidences of toxicity were observed up to the dosage level of 300 mg/kg body weight of the animals which was equivalent to 40 mg/kg body weight for humans, referred to NOAEL and HED values, respectively. The NOAEL of the tea leaf extract from this study was close to or higher than green tea extract evaluated in male and female F344/NTac rats and B6C3F1 mice for 14 weeks at 62.5, 125, 250, 500, and 1000 mg/kg body weight [19]. The NOAEL of green tea for the liver in both rodent species was 500 mg/kg body weight. However, in the nose of rats, the NOAEL was 62.5 mg/kg body weight in males, and no NOAEL was found in females. In both genders of mice, there was no NOAEL of green tea extract was found in the nose. These results indicate that the consumption of A. subintegra leaf tea is probably as safe as or safer than drinking green tea. However, the experimental period of our study was shorter, and the highest test concentration of this present study was lower than that of the green tea toxicity test. In addition, a previous study demonstrated that the kaempferol isolated from A. subintegra leaves exhibited no significant cytotoxicity activities in several human cell lines [20]. The safety evaluations of other Aquilaria spp. have been reported. The leaves of A. sinensis had no acute oral toxicity and genetic toxicity effects [21]. A single dose at 2000 mg/kg body weight and 21-day repeated doses up to 1000 mg/kg body weight of water extract from A. malaccensis in male ICR mice showed no mortality in both acute and sub-acute toxicity studies except for one animal from 1000 mg/kg body weight group [22]. Acute and sub-chronic toxicity studies of A. malaccensis methanol leaf extract in Sprague-Dawley rats revealed that oral lethal dose (LD50) was higher than 2000 mg/kg body weight for acute toxicity, and 250-500 mg/kg body weight of extract produced no signs of 28-day repeated does toxicity [2]. The acute toxicity in mice of A. crassna leaf water and ethanol extracts revealed that, even at high doses (2 and 15 g/kg body weight), no irregular signs and symptoms of toxicity or mortality were observed in any of animals [23, 24]. In the hepatoprotective evaluation of A. agallocha leaf ethanol extract in Sprague-Dawley rats, the extract exhibited non-toxic up to the dose of 2000 mg/kg body weight [25]. From these previous studies, non-toxic reported concentrations were higher than that of our experiment. However, the highest test concentration of this present study was lower than the previous works, which was 300 mg/kg body weight and displayed harmlessness. In addition to that, extracts from different solvent and plant species may have different toxicity effects. Taken together, our results and prior findings demonstrated that, depending on the doses and plant species examined, Aquilaria tea leaves, particularly from A. subintegra, are relatively safe to consume without causing significant toxicity.

The results suggest that A. subintegra tea leaves is safe for consumption as 28-day repeated-dose oral sub-acute toxicity in both genders of Wistar rats showed no mortality and clinical signs of toxicity. The changes of rat behaviors, physical appearances, and food consumption patterns were unnoticed. Hematology, blood biochemical, and histopathological examinations of the vital organs revealed no significant changes. The NOAEL of A. subintegra tea leaf extract was noted as more than 300 mg/kg body weight. The estimated HED was found to be approximately 40 mg/kg body weight. It is obvious that this tea is safe and suitable for further clinical evaluation in diabetic patients.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ACKNOWLEDGEMENTS

This research was funded by Government budget grant (Integrating research and innovation; TTM620126S, Fiscal year 2019), Prince of Songkla University, and Co Research Program (PsuCor-2/61), Southern Thailand Science Park, Prince of Songkla University, Songkhla, Thailand. We thank Mr. Somporn Kongpakdee, Thai Rung Agricultural Limited Partnership, for providing A. subintegra tea leaves.

REFERENCES

1. Mohamed R, Jong PL, Zali MS. Fungal diversity in wounded stems of Aquilaria malaccensis. Fungal Diversity. 2010; 43(1):67-74.
2. Zulkifle NL, Sabri NA, Omar NAM, Shaari MR, Tajuddin SN. Acute and sub-chronic toxicity study of Aquilaria malaccensis leaves extract in Sprague-Dawley rats. Chemistry of Advanced Materials. 2018; 3(1):8-15.
3. Adam AZ, Lee SY, Mohamed R. Pharmacological properties of agarwood tea derived from Aquilaria (Thymelaeaceae) leaves: An emerging contemporary herbal drink. Journal of Herbal Medicine. 2017; 10:37-44.
4. Manok S, Sungthong B, Sato H, Sugiyama E, Sato VH. Hypoglycemic

and antioxidant activities of the water extract of Aquilaria crassna leaves in streptozotocin-nicotinamide-induced type-2 diabetic mice. Natural Product Communications. 2016; 11(6):757-761.

5. Fayyadh AA, Ibrahim H, Zain HHM, Al-Qubaisi MS. The effect of agarwood leaf extracts on blood glucose level of type II diabetes mellitus in ICR male mice. Research Journal of Pharmacy and Technology. 2020; 13(1):237-242.
6. Pranakhon R, Pannangpetch P, Aromdee C. Antihyperglycemic activity of agarwood leaf extracts in STZ-induced diabetic rats and glucose uptake enhancement activity in rat adipocytes. Songklanakarin Journal of Science and Technology. 2011; 33(4):405-410.
7. Kang SJ, Lee JE, Lee EK, Jung DH, Song CH, Park SJ, Choi SH, Han CH, Ku SK, Lee YJ. Fermentation with Aquilariae Lignum enhances the anti-diabetic activity of green tea in type II diabetic db/db mouse. Nutrients. 2014; 6(9):3536-3571.
8. Lee JE, Kang SJ, Choi SH, Song CH, Lee YJ, Ku SK. Fermentation of green tea with 2% Aquilariae Lignum increases the anti-diabetic activity of green tea aqueous extracts in the high fat-fed mouse. Nutrients. 2015; 7(11):9046-9078.
9. Akrarapholchote S. Krisna leaf tea. First News and Media Group, Bangkok, pp 66-67; 2008.
10. Zhou M, Wang H, Suolangjiba, Kou J, Yu B. Antinociceptive and anti-inflammatory activities of Aquilaria sinensis (Lour.) Gilg. Leaves extract. Journal of Ethnopharmacology. 2008; 117(2):345-350.
11. OECD. The Organization for Economic Cooperation and Development. Repeated dose 28-day oral toxicity study in rodents. In: OECD Guideline for the Testing of Chemicals. Section 4: Health Effects, No. 407, Paris, pp 1-13; 2008.
12. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy. 2016; 7(2):27-31.
13. Zhang B, Yue R, Huang X, Wang Y, Jiang Y, Chin J. Effect of herbal tea on glycemic control in patients with type 2 diabetes: Protocol for a systematic review and meta-analysis. Medicine (Baltimore). 2019; 98(50):e18346.
14. Sun J, Ren J, Hu X, Hou Y, Yang Y. Therapeutic effects of Chinese herbal medicines and their extracts on diabetes. Biomedicine and Pharmacotherapy. 2021; 142:111977.
15. Hashim YZ, Kerr PG, Abbas P, Mohd Salleh H. Aquilaria spp. (agarwood) as source of health beneficial compounds: A review of traditional use, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2016; 189:331-360.
16. Nakagawa M, Hanada M, Amano H. A case of anaphylactic reaction to chamomile tea in a patient with mugwort pollinosis. Allergology International. 2019; 68(3):396-398.
17. Anzai A, Vazquez Herrera NE, Tosti A. Airborne allergic contact dermatitis caused by chamomile tea. Contact Dermatitis. 2015; 72(4):254-255.
18. Mertens M, Gilissen L, Goossens A, Lambert J, Vermander E, Aerts O. Generalized systemic allergic dermatitis caused by Cinnamomum zeylanicum in a herbal tea. Contact Dermatitis. 2017; 77(4):259-261.
19. Chan PC, Ramot Y, Malarkey DE, Blackshear P, Kissling GE, Travlos G, Nyska A. Fourteen-week toxicity study of green tea extract in rats and mice. Toxicologic Pathology. 2010; 38(7):1070-1084.
20. Bahrani H, Mohamad J, Paydar M, Rothan HA. Isolation and characterisation of acetylcholinesterase inhibitors from Aquilaria subintegra for the treatment of Alzheimer's disease (AD). Current Alzheimer Research. 2014; 11(2):206-214.
21. Li Q, Huang J, Yang Y, Chen M, Liang Y, Chen X. Research on the acute toxicity and genetic toxicity of Aquilaria sinensis (Lour.) Gilg: leaf. Chinese Journal of Health Laboratory Technology. 2015; 25:1518-1521.
22. Musa NHC, Zain HHM, Ibrahim H, Jamil NNM. Evaluation of acute and sub-acute oral toxicity effect of Aquilaria malaccensis leaves aqueous extract in male ICR mice. Natural Product Sciences. 2019; 25(2):157-164.
23. Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S, Chudapongse N. Antibacterial activity of Aquilaria crassna leaf extract against Staphylococcus epidermidis by disruption of cell wall. Annals of Clinical Microbiology and Antimicrobials. 2013; 12:20.
24. Ghan SY, Chin JH, Thoo YY, Yim HS, Ho CW. Acute oral toxicity study of Aquilaria crassna and α-tocopherol in mice. International Journal of Pharmaceutical Sciences and Research. 2016; 7(4):1456-1461.
25. Alam J, Mujahid M, Badruddeen, Jahan Y, Bagga P, Rahman MA. Hepatoprotective potential of ethanolic extract of Aquilaria agallocha leaves against paracetamol induced hepatotoxicity in SD rats. Journal of Traditional and Complementary Medicine. 2017; 7(1):9-13.

Table 1: Cage-side observation of effects of 28-day repeated-dose oral sub-acute toxicity of water extract from Aquilaria subintegra tea leaves in rats.

Cage-side observation	Control	Tea extract (mg/kg body weight)		
		5	50	300
Skin and fur appearances	Normal	Normal	Normal	Normal
Abdominal distension	Nil	Nil	Nil	Nil
Color and consistency of feces (bolus)	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil
Urination	Normal	Normal	Normal	Normal
Breathing abnormalities	Nil	Nil	Nil	Nil
Vocalization	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Discharge from the eyes	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil
Twitches or convulsions	Nil	Nil	Nil	Nil
Gait	Normal	Normal	Normal	Normal
Body posture	Normal	Normal	Normal	Normal
Behavior pattern	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil

Cage-side observation was made immediately after the administration of the extract and every 2 h for 24 h, and then daily for 28 days. Ten rats/group (5 males and 5 females). Water was used as vehicle control.

Table 2: Relative organ weight to body weight (%) of 28-day repeated-dose oral sub-acute toxicity of water extract from Aquilaria subintegra tea leaves in rats.

Organs	Control	Tea extract (mg/kg body weight)		
		5	50	300
Liver	2.980 ± 0.314	3.164 ± 0.276	3.069 ± 0.371	3.026 ± 0.157
Spleen	0.224 ± 0.047	0.214 ± 0.030	0.247 ± 0.127	0.208 ± 0.043
Kidney	0.533 ± 0.025	0.576 ± 0.024	0.551 ± 0.019	0.571 ± 0.034
Lung	0.400 ± 0.047	0.383 ± 0.049	0.380 ± 0.074	0.402 ± 0.060
Heart	0.589 ± 0.136	0.570 ± 0.133	0.525 ± 0.118	0.522 ± 0.120
Ovary	0.246 ± 0.098	0.275 ± 0.023	0.246 ± 0.013	0.237 ± 0.033
Testis	0.895 ± 0.056	0.875 ± 0.072	0.879 ± 0.069	0.882 ± 0.063

Ten rats/group (5 males and 5 females) treated with different concentrations (5, 50, and 300 mg/kg body weight) of water extract. Water was used as vehicle control. Values are mean ± SD. There was no significant difference ($p>0.05$) between the four groups of each organ by one-way ANOVA followed by Bonferroni's post hoc comparisons test.

Table 3: Hematological parameters of 28-day repeated-dose oral sub-acute toxicity of water extract from Aquilaria subintegra tea leaves of rats.

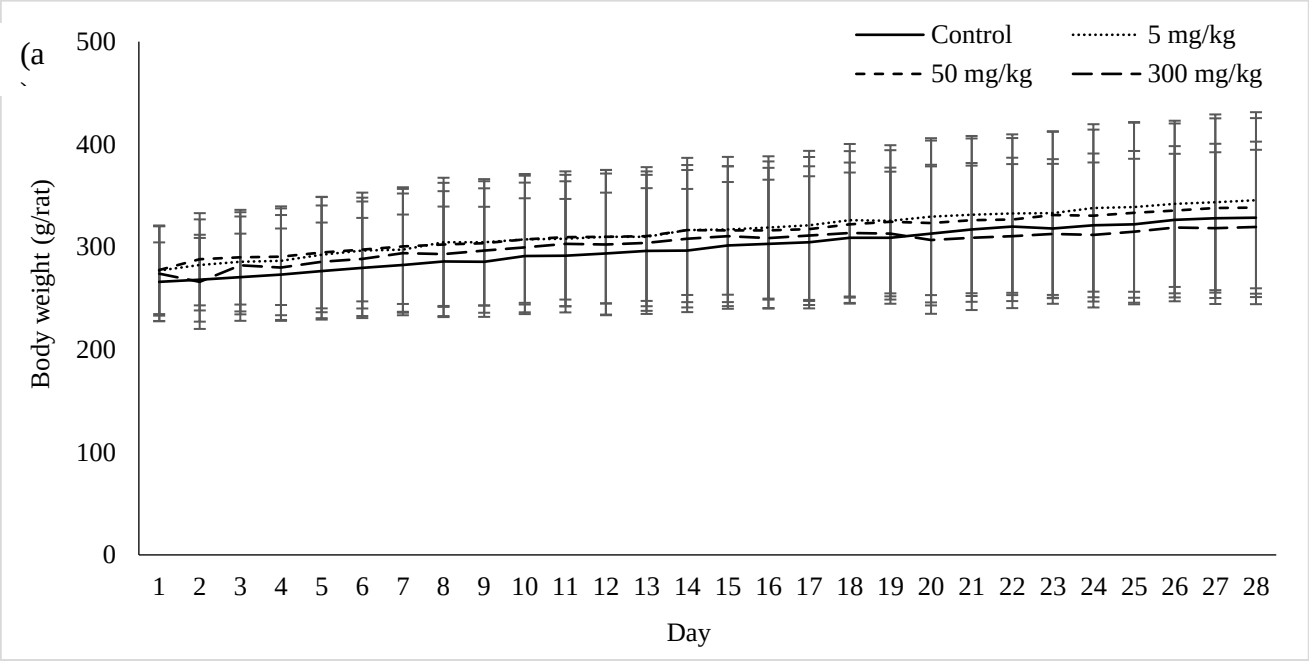
Hematological parameters	Control	Tea extract (mg/kg body weight)		
		5	50	30
WBC (103/µL)	3.17 ± 1.40	3.39 ± 1.31	2.96 ± 1.70	2.52 ± 1.29
RBC (106/µL)	8.24 ± 0.35	8.31 ± 0.36	8.29 ± 0.36	8.23 ± 0.32
Hb (g/dL)	14.91 ± 0.54	15.09 ± 0.56	14.98 ± 0.49	14.93 ± 0.47
Hct (%)	47.19 ± 2.36	47.88 ± 2.09	46.90 ± 1.65	46.58 ± 1.74
MCV (fL)	57.30 ± 1.79	57.63 ± 1.01	56.60 ± 1.68	56.62 ± 1.28
MCH (pg)	18.08 ± 0.52	18.17 ± 0.27	18.09 ± 0.42	18.16 ± 0.55
MCHC (g/dL)	31.61 ± 0.62	31.52 ± 0.39	31.94 ± 0.78	32.07 ± 1.02
RDW (%)	12.55 ± 0.43	12.27 ± 0.29	12.24 ± 0.22	12.32 ± 0.39
Plt count (103/µL)	793.50 ± 93.71	815.89 ± 60.13	739.13 ± 103.16	723.63 ± 127.37

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Hct: Hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, Plt: platelet. Ten rats/group (5 males and 5 females) treated with different concentrations (5, 50, and 300 mg/kg body weight) of water extract. Water was used as vehicle control. Values are mean ± SD. There was no significant difference ($p>0.05$) between the four groups of each parameter by one-way ANOVA followed by Bonferroni's post hoc comparisons test.

Table 4: Biochemical parameters of 28-day repeated-dose oral sub-acute toxicity of water extract from Aquilaria subintegra tea leaves of rats.

Biochemical parameters	Control	Tea extract (mg/kg body weight)		
		5	50	300
Creatinine (mg/dL)	0.30 ± 0.05	0.27 ± 0.02	0.30 ± 0.05	0.29 ± 0.02
Blood urea nitrogen (mg/dL)	23.50 ± 2.80	23.50 ± 2.51	22.50 ± 2.00	23.11 ± 1.27
Alkaline phosphatase (U/L)	54.17 ± 13.64	51.83 ± 11.92	57.83 ± 18.12	58.00 ± 13.32
Alanine aminotransferase (U/L)	37.20 ± 5.25	39.56 ± 4.07	33.88 ± 9.76	35.13 ± 5.08

Ten rats/group (5 males and 5 females) treated with different concentrations (5, 50, and 300 mg/kg body weight) of water extract. Water was used as vehicle control. Values are mean ± SD. There was no significant difference ($p>0.05$) between the four groups of each parameter by one-way ANOVA followed by Bonferroni's post hoc comparisons test.



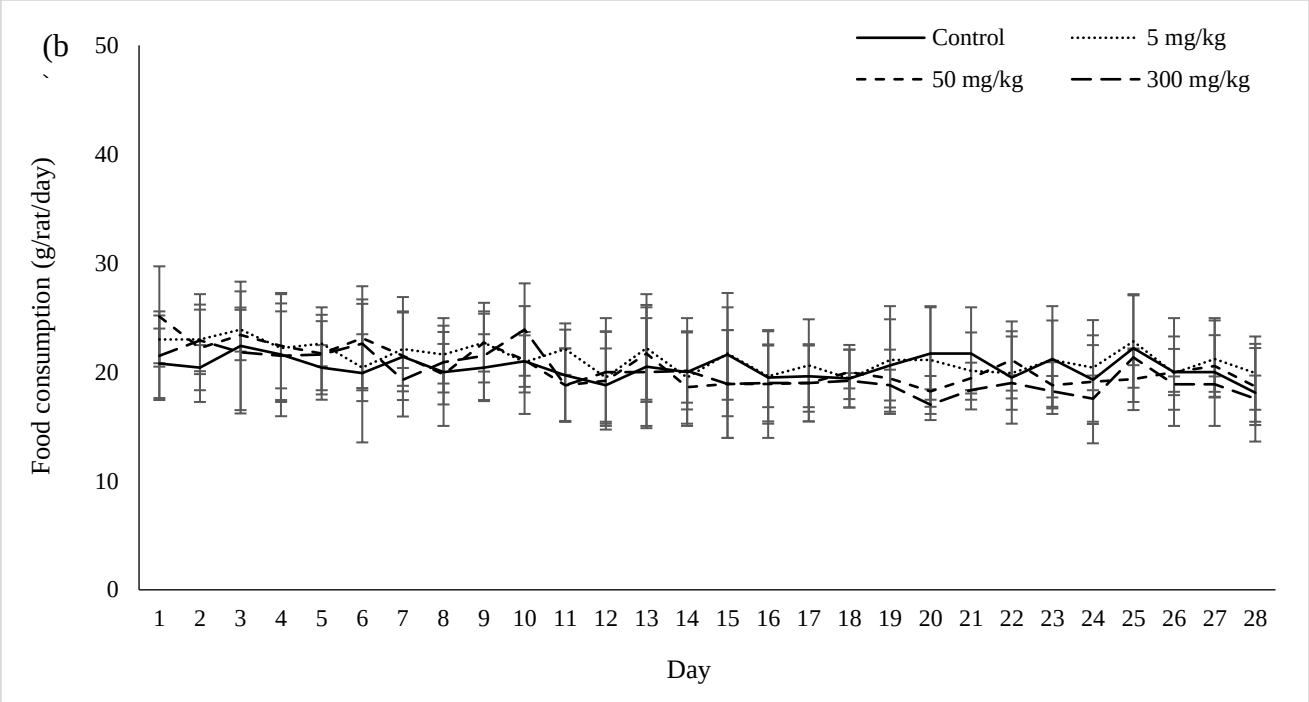


Fig. 1: Average food consummation (a) and body weight (b) of rats (10 rats/group; 5 males and 5 females). in 28-day repeated-dose oral sub-acute toxicity test of water extract from Aquilaria subintegra tea leaves (5, 50, and 300 mg/kg body weight). Water was used as vehicle control. Values are mean ± SD. There was no significant difference ($p>0.05$) between the four groups at each time point by one-way ANOVA followed by Bonferroni's post hoc comparisons test.

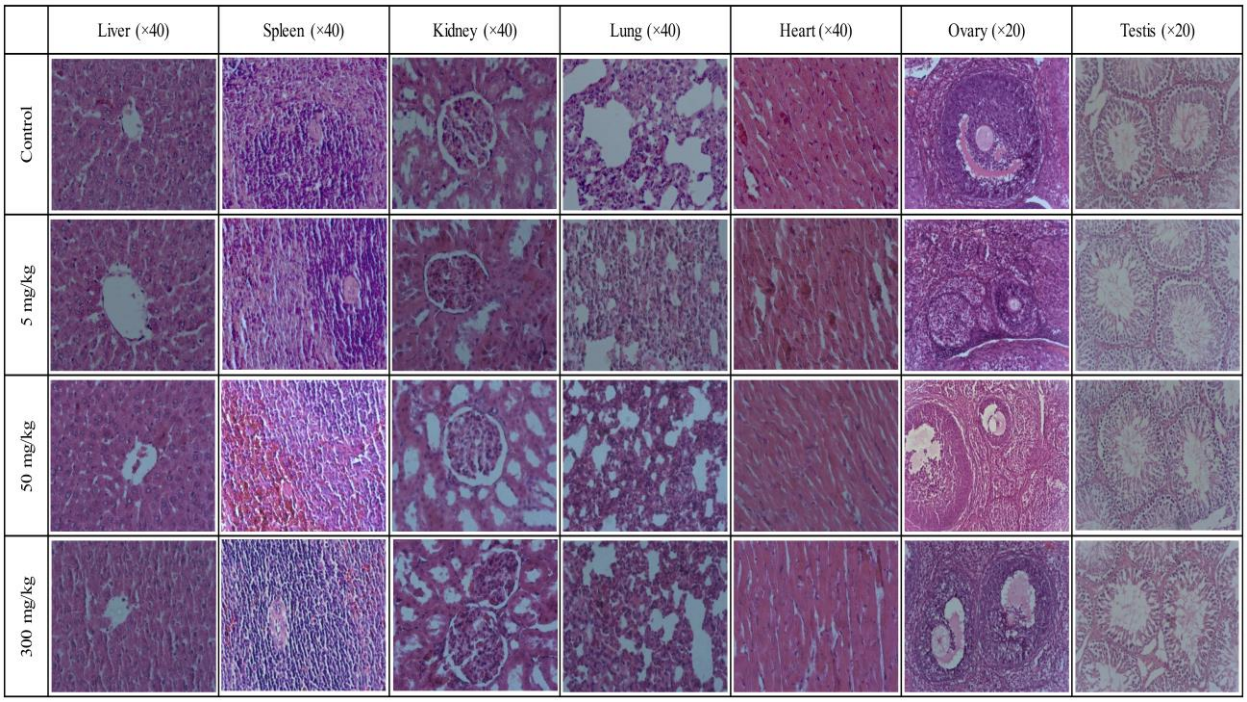


Fig. 2: Histopathology results of hematoxylin and eosin (H&E)-stained liver, spleen, kidney, lung, heart, ovary, and testis in rats treated with 28-day repeated-dose oral sub-acute toxicity of water extract from Aquilaria subintegra tea leaves (5, 50, and 300 mg/kg body weight). Water was used as vehicle control.