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ACUTE ORAL TOXICITY STUDY OF *AQUILARIA CRASSNA* AND α-TOCOPHEROL IN MICE

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Key words:

Acute oral toxicity; *Aquilaria crassna*; α-Tocopherol; LD_{50}; Mice

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ABSTRACT: The combination of agarwood (*Aquilaria crassna*) young leave with α-tocopherol (α-TOH) showed a synergistic effect which helps to protect the food from lipid oxidation. The present study was designed to elucidate the acute toxicity of the mixture of *A. crassna* young leaves crude ethanolic extract (CE) and α-TOH on Balb/c mice. This acute oral toxicity study was carried out based on OECD 423 guidelines by using female Balb/c mice weighing 15-25 g. First group was served as control group which received dimethyl sulfoxide (vehicle) while second, third and fourth group were orally administered with single dose of 2000 mg/kg of CE, α-TOH and a mixture of CE and α-TOH (1:2), respectively. Throughout 14 days of observation, no behavioural changes were seen in all the treated animals. Body weight for each mouse and the relative organ weights for liver, kidney, lung, heart and spleen showed insignificant different ($p>0.05$) when compared to the control group. These results indicate the safety of the acute exposure of the *A. crassna* CE, α-TOH and the mixture of *A. crassna* CE and α-TOH at dose of 2000 mg/kg in female mice, without causing any adverse effects. The oral lethal dose(LD50)of *A. crassna* CE, α-TOH and CE/α-TOH mixture were suggested to be greater than 2000 mg/kg body weight in female mice.

INTRODUCTION: Plants have been extensively used as medicines since a thousand years ago and the use of herbal products should be based on scientific origin in order to make sure the plants are safe to consume. Unfortunately, many people underestimate the toxicity of natural products and use them without further scientifically proven.

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Atropa belladonna and *Digitalis purpurea* are typical example for toxic herbal product which show severe systemic toxicity if taken orally [1]. Therefore, in order to prevent exposure to poisonous plants, the use of an appropriate animal model in toxicity test was conducted in present study. This is a key stage in ensuring the safety of the plant and acute toxicity studies are just one of the toxicity tests that are commonly used [2].

Agarwood (*Aquilaria crassna*) is a kind of dark resinous heartwood which belongs to the *Aquilaria* genus of the Thymelaeceae family [3]. The leaves of *A. crassna* are commonly consumed in the form of herbal tea for the treatment of high blood pressure,

constipation, headache and diabetes [4-6]. In the recent years, the pharmacological properties of *A. crassna* leaves such as antibacterial, antipyretic and antioxidant have been previously reported [5-6]. The exhibited antioxidant activity is likely attributed to the presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides, mangiferin and genkwanin in *A. crassna* leaves [7-8]. α-Tocopherol(α-TOH) is a lipid-soluble antioxidant which readily donate hydrogen from the hydroxyl (-OH) group on ring structure to free radicals and itself becomes unreactive. It has been reported that plant polyphenols can help to reduce α-tocopheroxyl radical by regenerating α-TOH back to its active form, producing synergistic antioxidant effect [9-10].

Antioxidant synergism has been observed when *A. crassna* young leaves crude extract (CE) combined with α-TOH at a ratio of 1:2 [11]. However, the safety for this plant is not well established. Up to date, no data have been documented on acute toxicity for the crude ethanolic leaves extract of *A. crassna*. The only data in this regard is the toxicity study for the aqueous leaves extract of *A. crassna* at 2000 and 15000 mg/kg body weight (bw) using mice [6]. Scientific evidence for the antioxidant efficacy of *A. Crassna* young leaves and α-TOH are widely studied but systemic safety studies are still lacking. Therefore, it is essential to determine the potential adverse effects that might be expected after introduction of this CE and α-TOH (CE/α-TOH) mixture into the food system.

The present study was aimed to investigate the possible acute oral toxic effect of CE/α-TOH mixture in female Balb/c mice at a dose of 2000 mg/kg bw and to determine the LD_{50} value of the mixture in experimental animals. The acute oral toxicity testing was carried out under the Organization for Economic Cooperation and Development (OECD) 423 guideline [12].

METHODS AND MATERIALS:

Chemicals:

Ethanol and dimethyl sulfoxide (DMSO) were supplied by Merck, Darmstadt from Germany. α-Tocopherol was purchased from Sigma Aldrich, St. Louis, USA.

Extraction of Plant Materials:

A. crassna young leaves (first five leaves from the leaf buds) were collected from a plantation in Klang, Selangor, Malaysia and oven dried for 22 h at 50^{o}C in a convention oven (Model UNB 400, Memmert, Germany). The dried young leaves were grounded into powder form and extracted with 61.01% ethanol at room temperature [13]. All the extracts were concentrated using multivapor (Multivapor P-6, Buchi, Switzerland) at 40^{o}C, followed by freeze drying (Alpha 1-4 LD Plus, Christ, Germany) at -50^{o}C for 24 h. The resulting crude extract (CE) was stored at -18^{o}C prior to further use.

Selection of Experimental Animals:

The present toxicity study was carried out in accordance to OECD 423 Test Guidelines with a single dose exposure [12]. A total of 20 healthy 8-12 weeks old female Balb/c mice, weighing about 15-25 g, were obtained from Sinar Scientific, Seri Kembangan, Selangor, Malaysia.

Assignment of Animals:

Female Balb/c mice were randomly divided into 4 groups comprising of 5 animals each (n=5). All the animals were identified by marking with black stain on the tail to ease observation. The animals were housed 5 in each polypropylene cage under standard animal housing conditions with controlled lighting (12 h dark-light cycles), temperature (25 ± 2^{o}C) and relative humidity (75 ± 5%).During the entire experimental period, the animals were provided with food and water *ad libitum*, except for the short fasting period where the drinking water was still in free access but no food supply was provided. The animals were allowed to acclimatise for 5 days before the experiment started. The experiment procedures were conducted after the approval by faculty ethical research committee of UCSI University (Proj-FAS-EC-14-038) and were in strict accordance with animal ethical committee guidelines for the care and use of laboratory animals.

Test Substance Administration:

The test substances were administered in a single dose by using oral gavage needle for mice and the animals were fasted 3 h prior to dosing. Following the period of fasting, animals were weighed and

orally treated with a single dose of test substances at 2000 mg/kg bw. The composition of experimental treatments is listed in **Table 1**. First group was served as a control group which received dimethyl sulfoxide (DMSO) as the vehicle. The treatment groups received CE (Group 2), α-TOH (Group 3) and a mixture of CE/α-TOH which combined at a ratio of 1:2(Group 4), dissolved in 100%DMSO respectively. The administration volume was 10 mL/kg bw of the animal. The quantity of the test substance was calculated based on the body weight of the animal and prepared in DMSO before administered directly to the mice. After the administration of test substance, the animals were fasted for 2 h (only food was withheld but not water).

TABLE 1: EXPERIMENTAL TREATMENTS OF RESPECTIVE GROUPS

Group	Treatment (2000 mg/kg body weight)
1	Dimethyl sulfoxide (Control)
2	*A. crassna* leaves crude extract (CE)
3	α-tocopherol (α-TOH)
4	*A. crassna* leaves crude extract + α-tocopherol (CE/ α-TOH mixture)

Observation Period:

All the animals were closely observed *via* cage-side observation during the first 30 min after treatment, followed by observation during the first 4 h for mortality, moribund or ill health, observed periodically for the next 24 h and once daily for the next 14 days [14]. The animals were observed daily for the purpose of recording any signs of intoxication including changes in the skin and fur and behavioural changes.

Body Weight:

Body weight (g) of the female mice was individually weighed and recorded on day-0, day-3, day-7 and day-14 by using Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland).

Organ Weight:

At the end of the experiment, the animals were sacrificed to obtain the relative organ weights. At day-14, all the animals were overnight fasting for 16 h. At day-15, the animals were sacrificed by cervical dislocation and measured the organ weights (g) namely liver, kidney, heart, spleen and lungs. The organs obtained from each treated mouse were grossly examined to see the changes compared to the control group and the relative organ weight (ROW) of each organ was then calculated.

Statistical Analysis:

The results were expressed as mean ± standard deviation (SD). Comparisons were made between the treatment groups with the control group using the one-way analysis of variance (ANOVA) followed by Dunnett's test using Minitab 17 software (Minitab Inc., USA). In all cases, a *p*-value of < 0.05 was considered as significant difference when compared to the respective control group.

RESULTS: Based on the cage-side observation, all the female mice in treatment and control groups did not show noticeable toxic signs which included changes on skin and fur, abnormal behaviour pattern and coma post 24 h of treatment as well as during 14 days observation duration. No mortality was observed in any of the mice during this duration. In additional to that, there were gradual increases in body weight of treated and control mice on 0-14 days, as shown in **Table 2**. The changes in body weights and the relative organ weights of the treated mice (CE, α-TOH and CE/α-TOH mixture) were not significant difference ($p>0.05$) as compared to the control mice (**Table 2 and 3**). Based on macroscopic observation, gross examination of all the organs from the 3 treatment groups did not reveal any abnormalities or changes in the colour of the internal organs compared to the control group.

TABLE 2: ACUTE EFFECT OF *AQUILARIA CRASSNA* LEAVES CRUDE EXTRACT (CE), α-TOCOPHEROL (α-TOH) AND CE/α-TOH MIXTURE ON BODY WEIGHT CHANGES IN FEMALE MICE

Grouping	Body weight changes (g)				Lethality
	Day-0	Day-3	Day-7	Day-14	
Control	16.3±0.5	17.0±0.6	17.8±0.3	20.2±0.6	0/5
CE	17.2±0.6	17.8±0.5	18.2±0.5	20.5±0.6	0/5
α-TOH	17.0±1.9	17.6±1.8	18.2±1.7	19.6±2.0	0/5
CE/α-TOH mixture	16.7±1.0	17.3±1.0	18.1±0.3	20.0±0.7	0/5

Note. CE/α-TOH= mixture of *A. crassna* leaves crude extract and α-Tocopherol (1:2).
Value = mean ± standard deviation; n=5. Analysed using Dunnett's test

TABLE 3: ACUTE EFFECT OF *AQUILARIA CRASSNA* LEAVES CRUDE EXTRACT (CE), α-TOCOPHEROL (α-TOH) AND CE/α-TOH MIXTURE ON RELATIVE ORGAN WEIGHTS IN FEMALE MICE

Grouping	Relative organ weight (g/100g body weight)				
	Liver	Kidney	Heart	Spleen	Lung
Control	4.39±0.52	1.14±0.15	0.41±0.03	0.49±0.05	0.74±0.07
CE	4.32±0.14	1.11±0.06	0.43±0.03	0.46±0.09	0.73±0.07
α-TOH	4.39±0.33	1.09±0.06	0.44±0.02	0.52±0.02	0.73±0.05
CE/α-TOH	4.59±0.50	1.10±0.17	0.40±0.01	0.48±0.08	0.71±0.05

Note. CE/α-TOH= mixture of *A. crassna* leaves crude extract and α-Tocopherol (1:2).
Value = mean ± standard deviation; n=5. Analysed using Dunnett's test

DISCUSSION: In present study, acute toxicity of CE, α-TOH and CE/α-TOH mixture were evaluated in female Balb/c mice. Female mice are commonly used in toxicity test because they are small, easy to handle and relatively economical to obtain [15]. Additionally, female is more sensitive to see the effect of treatment compared to male [16]. Prior to dosing, the animals were fasted for 3 h to prevent food and other chemicals in the digestive tracts in affecting the absorption and reaction of the compound.

Generally, the changes in body weight gain and internal organ weights of mice would reflect the toxicity after exposure to the toxic substances [17]. The relative organ weight is used as one of the parameters to examine the severity of toxicants on the targeted organs. The heart, liver, kidney, spleen and lungs are the primary organs affected by drugs and chemicals ingested through the oral route [17-18]. Normally when the test substance is toxic, in acute response, it will damage liver and kidney, causing the relative organ weight for liver and kidney to increase due to swelling [19]. Based on the result obtained, an increase in the body weight of the animal and insignificant effect (p>0.05) on the relative organ weights indicate that the administration of CE, α-TOH and CE/α-TOH mixture has negligible level of toxicity on the growth of the animals. This finding is in agreement with the result reported by Kamonwannasit *et al.* who demonstrated that no sign of toxicity or death was observed in mice treated with aqueous extract of *A. crassna* leaves at the doses of 2000 and 15000 mg/kg [6]. Additionally, α-TOH was reported to have LD_{50} greater than 2000 mg/kg bw in mouse [20].

Lethal concentration (LD_{50}) is normally shown in acute toxicity study as the dose which kills 50% of the animals. The higher the LD_{50} value, the lower the toxicity. According to the OECD 423 guideline method, a high starting dose of 2000 mg/kg bw was suggested in order to determine the maximum dose which is most likely to produce mortality in some of the treated animals. At this dose, no deaths, hazardous signs of toxicity or organ damages were recorded in these animals during the 14 days period of observation after acute treatment through the oral administration. This clearly indicates that the CE/α-TOH mixture does not cause any acute toxicity effect in mice and the same effect is likely to be demonstrated in human as well. As a result, it is concluded that the LD_{50} of the CE/α-TOH mixture was greater than 2000 mg/kg in mice.

Due to the high price of naturally derived antioxidants, a new food additives trend where blending of antioxidants to cut costs has emerged. Young leaves are discovered to have higher antioxidant activity than mature leaves. The

synergistic antioxidant interaction between *A. crassna* young leaves CE and α-TOH allows suppliers to partially replace the natural antioxidants with *A. crassna* young leaves to minimise the cost. With no sign of acute oral toxicity, CE/α-TOH mixture is valuable to the food industry as natural antioxidants and can potentially be incorporated into novel functional foods or beverages with optimum health benefits. However, acute toxicity studies have limitation in detecting the toxic effects on vital functions of cardiovascular, central nervous and respiratory systems which should be evaluated prior to human exposure[21]. Hence, further studies are recommended to assess the long-term safety of CE/α-TOH mixture using repeated dose-toxicity and safety pharmacology studies.

CONCLUSION: The normal behaviour of the treated animals during a period of 14 days suggests the non-toxic nature of the CE, α-TOH, and CE/α-TOH mixture since all the animals grow healthy and normal without being affected by the treatments. The single oral dose of the CE, α-TOH and CE/α-TOH mixture did not produce mortality or significant (p>0.05) changes in the body weight and relative organ weight of the animals. Hence, CE/α-TOH mixture at 2000 mg/kg bw exhibits no acute toxic effects in female Balb/c mice. Since acute toxicity study only provides preliminary data relevant to single exposure or over-dosage in human, further study on its chronic toxicity is essential for further support of this antioxidants mixture.

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REFERENCES:

1. Mir AH, Sexena M and Malla MY: An acute oral toxicity study of methanolic extract from *Tridex procumbens* in Sprague dawley's rats as per OECD guideline 423. Asian Journal of Plant Science and Research 2013; 3(1):16-20.
2. Lalitha P, Sripathi SK and Jayanthi P: Acute toxicity study of extracts of *Eichhornia crassipes* (Mart.) Solms. Asian Journal of Pharmaceutical and Clinical Research 2012; 15(4):59-61.
3. Naef R: The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: A review. Flavour and Fragrance Journal 2011; 26:73-89.
4. Pranakhon R, Pannangpetch P and 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.
5. Kakino M, Sugiyama T, Kunieda H, Tazawa S, Maruyama H, Tsuruma K, Araki Y, Shimazawa M, Ichihara K, Mori H and Hara H: Agarwood (*Aquilaria crassna*) extracts decrease high-protein high-fat diet-induced intestinal putrefaction toxins in mice. Pharmaceutica Analytica Acta 2012; 3:1-7.
6. Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S and Chudapongse N: Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus* epidermis by disruption of cell wall. Annals of Clinical Microbiology and Antimicrobials 2013; 12(20):1-7.
7. Dahham SS, Majid AMSA, Saghir SM and Suede FSA: Antibacterial, antifungal and antioxidant acivities of *Aquilaria crassna*. International Journal of Advances in Science and Technology 2013; 196-200.
8. Ray G, Leelamanit W, Sithisarn P and Jitratchariyakul W: Antioxidative compounds from *Aquilaria crassna* leaf. Mahidol University Journal of Pharmaceutical Sciences 2014; 41(4):54-58.
9. Thoo YY, Abas F, Lai OM, Ho CW, Yin J, Hedegaard RV, Skibsted LH and Tan CP: Antioxidant synergism between ethanolic *Centella asiatica* extracts and α-tocopherol in model systems. Food Chemistry 2013; 138:1215-1219.
10. Yin J, Becker EM, Andersen ML, Skibsted LH: Green tea extract as food antioxidant. Synergism and antagonism with α-tocopherol in vegetable oils and their colloidal systems. Food Chemistry 2012; 135:2195-2202.
11. Ghan SY: Regeneration of alpha-tocopherol in cooking oils by natural antioxidant (*Aquilaria crassna*) crude extracts and its effect on oxidative stability of the oil under accelerated storage condition. Master Thesis 2015; Malaysia: University College Sedaya International.
12. OECD/OCDE 423. OECD guideline for testing of chemicals.Acute oral toxicity-acute toxic class method.2001 Dec 17. 1-4.
13. Tay PY, Tan CP, Abas F, Yim HS and Ho CW: Assessment of extraction parameters on antioxidant capacity, polyphenol content, epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and iriflophenone 3-C-β-glucoside of agarwood (*Aquilaria crassna*) young leaves. Molecules 2014; 19:12304-12319.
14. P'ng XW, Akowuah GA and Chin JH: Acute oral toxicity study of *Clinacanthus nutans* in mice. International Journal of Pharmaceutical Sciences and Research 2012; 3(11):4202-4205.
15. Shayna CG: Animal models in toxicology. Taylor & Francis Group, Third Edition 2015.
16. Angeline M, Dewijanti ID, Hartati S and Meilawati L: Lethality of *Pterocarpus indicus* standardized ethanol extract. International Journal of PharmTech Research 2014; 6(2):676-685.
17. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY and Sasidharan S: Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Molecules 2011; 16:5268-5282.
18. P'ng XW, Akowuah GA and Chin JH: Evaluation of the sub-acute oral toxic effect of methanol extract of

Clinacanthus nutans leaves in rats. Journal of Acute Disease 2013; 29-32.
19. Chin JH, Akowuah GA, Sabu MC and Khalivulla SI: Sub-acute (28 days) toxicity study of methanol leaves extract of *Clinacanthus Nutans* in rats. International Journal of Pharmacy 2014; 4(2):61-69.
20. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin E.2013 April 23. 1-18.
21. Syahmi AR, Vijayarathna S, Sasidharan S, Latha IY, Kwan YP, Lau YL, Shin LN, Chen Y: Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq., (oil palm leaf) methanol extract. Molecules 2010; 15:8111-8121.

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