Preliminary Acute Oral Toxicity Study of White Tea Leaf (*Camellia sinensis* (L.) Kuntze) Ethanolic Extracts

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ABSTRACT

Background: White tea is a kind of tea which manufactured with minimal processing only drying without fermentation process. White tea prepared from very young tea leaves or buds of *Camellia sinensis* (L.) Kuntze, Theaceae, covered with tiny, silvery hairs, and dried immediately after picking to prevent oxidation and commonly used as a beverage and herbal medicine. **Objective:** The present study was aimed to evaluate the safety of the white tea leaf ethanolic extract (WTE) with acute toxicity tests. **Methods:** The acute oral toxicity of WTE performed at dose 1250, 2500, and 5000 mg/Kg BW of Deutschland, Denken, and Yoken (DDY) mice. The animals observation for any mortality, behavioral, body weight and feed-water consumption pattern during the 14-day study. The liver, kidney, and heart isolation performed on day-15 to observe macroscopic and relative organ weight (ROW). **Results:** No treatment-related toxic symptom or mortality observed for the first 4 hours and 24 hours after oral administration of WTE at a dose of 1250, 2500, and 5000 mg/kg BW. All the groups of mice did not show the significant changes in behavior, breathing, and motoric activity. **Conclusions:** This studies showed that the oral LD_{50} of WTE was greater than 5000 mg/kg BW and suggests that the WTE is practically non-toxic in a single dose of level 5000 mg/kg BW.

Key words: Acute Toxicity, *Camellia Sinensis* (L.) Kuntze, Safety, Teh Putih, Theaceae.

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INTRODUCTION

The tradition of drinking tea at this time has become one of culture in every community. Therefore, tea is the most popular beverage in the world[1], made from the leaves of the tea plant, *Camellia sinensis* (L.) Kuntze, Theaceae. One type of the tea is a white tea that manufactured with minimal processing only drying without fermentation process. White tea leaf, also known as 'teh putih' in Indonesia, prepared from very young tea leaves or buds covered with tiny, silvery hairs, then dried soon after collected, to prevent oxidation.[1,2] The high concentrations of tea polyphenols and catechins are higher in white tea compared to green or black tea.[2] The recent investigation associated with the bioactive compound like polyphenols-flavonoids-catechins of tea due to their antioxidant activities which contribute to human health benefits.[3,4] Pharmacological research indicates that tea leaf extract has pharmacological activities such as antidiabetic, anticarcinogenic, antiviral, antibacterial, antiinflammatory, anti-aging and immune boosting antioxidant activities which contribute to human health.[1,5-7] This activity associated with the efficacy of tea for the prevention and treatment of diasease.[8]

As the second biggest biodiversity in the world, Indonesia has a high number of indigenous medicinal plants.[9] Therefore, traditional medicine is one of the cultural heritage of ancestors and very well known in Indonesia. Although traditional medicine has been used for a long time but not completely safe, it is important to determine the potential acute toxicity of herbal medicines through LD_{50} value and the spectrum of toxic effects. The potential acute toxicity of herbal medicine can be used to assess the limits of safety or therapeutic index (LD_{50}/ED_{50}).

The efficacy and toxicity of tea leave assumed from their very long history of consumption in the world and their main functional ingredients studies,[7] but a systematic evaluation of the toxicity of white tea has been lacking. Therefore, the present study was aimed to evaluate the safety of the white tea leaf ethanolic extract (WTE) with acute toxicity tests in Deutschland, Denken, and Yoken (DDY) mice.

MATERIALS AND METHODS

Plant Material and Extraction

The white tea obtained from the Tea Plantation and Quinine Research Center in Gamboeng, West Java, Indonesia. White tea leaves (*Camellia sinensis* L. Kuntze) sorted and collected then dried under sunlight. Furthermore, the tea leaves withered with a dryer. The white tea leaves powder made by grinding dried white tea leaves by using a grinder. Extraction method conducted by reflux with ethanol 70% at 60ºC for 3 hours, then re-reflux for two times and evaporated using evaporator.

Animal Test

Adult healthy Deutschland, Denken, and Yoken (DDY) mice, weight 20-30 g and approximately six

weeks old, were used from Department of Pharmacology, University of Indonesia. All mice were acclimatized for seven days before the tests, then fasted four hours before administration of the WTE, while drinking still given.

Acute Toxicity Study

The oral acute toxicity study of WTE has conducted according to Organization for Economic Co-operation and Development (OECD) guideline with the limit test dose at 5000 mg/kg body weight (BW).[10]

The animals divided randomly into four groups; each consisted of 10 mice (five males and five females). The 1st group served as a control, while 2nd, 3rd, and 4th served as treated groups received orally WTE (dissolved in water) with a successive dose of 1250, 2500, and 5000 mg/kg BW. The WTE was administered only once (on day 0) at the beginning of the experiment.

The incidence of toxic effects in animals observed from the first four hours after the treatment period. The change of behavior, body weight, food intake, water intake, motoric activity, respiration and other death observed for 14 days. In the case of the mortality of the animals immediately dissected to see the possible causes. On the15th day, all of the animals were sacrificed by an anesthesia (ether) after an overnight fasting. The liver, kidney, and heart were isolated and weighed. The relative organ weight (ROW) determined to diagnose injuries that occur in organs during treatment and calculated as follows.[11]

Statistical Analysis

Statistical analysis determined as mean value ± standard deviation (SD). The data with normal distribution analyzed with one-way ANOVA followed by multiple comparisons using Bonferroni test. However, abnormal distribution analyzed with Kruskal-Wallis test. Probability level of less than 5% (p < 0.05) was considered significant.

RESULTS

No treatment-related toxic symptom or mortality observed for the first 4 h and 24 h after oral administration of WTE at a dose of 1250, 2500, and 5000 mg/kg BW. All the groups of mice did not show the significant changes in behavior, breathing, and motoric activity.

However, the mortality found at day-1, more over 24 hours after administration of WTE, one male mice from the Group 1 and two female mice from the Group 2, but not in Group 3. The animals then immediately dissected to see the cause of the death by obvious observation of liver, kidney, and heart. There were no differences in relative organs weight and apparent observation of their vital organ compare to all of the mice which still alive until the end of observation. The absolute and relative organ masses of all treatment groups showed no significant differences (P > 0.05) compared to control group, (Table 1, 2, 3, and 4). There were no significant differences on obvious observation of vital organs between treatment and control groups, and the appearance seems normal texture (Figure 1 and 2).

Table 1: Average absolute organ weight of male mice

Organ	Average weight *			
	Control	Group 1	Group 2	Group 3
Liver	2.10 + 0.85	2.00+ 0.08	2.08+ 0.81	1.90+ 0.49
Kidney	0.22+ 0.03	0.22+ 0.03	0.23+ 0.08	0.19+ 0.02
Heart	0.17+ 0.06	0.17+ 0.05	0.14+ 0.05	0.12+ 0.04
Average body on the sacrifice day	36.07+ 3.83	32.25+ 3.52	27.64+4.06	25.24+ 4.27

*P > 0.05 compared to control group

Table 2: Average absolute organ weight of female mice

Organ	Average weight*			
	Control	Group 1	Group 2	Group 3
Liver	2.2+ 0.44	1.92+ 0.43	1.63+ 0.38	2.2+ 0.44
Kidney	0.23+ 0.06	0.2+ 0.04	0.2+ 0.05	0.23+ 0.06
Heart	0.13+ 0.06	0.16+ 0.05	0.13+ 0.06	0.13+ 0.06
Average body on the sacrifice day	26.67 + 3.35	25.04+2.83	23.87+3.79	23.48+ 3.34

*P > 0.05 compared to control group

Table 3: Relative organ weight of male mice

Organ	Relative organ weight*			
	Control	Group 1	Group 2	Group 3
Liver	6.00+ 3.07	5.98+ 0.79	7.68+ 3.37	7.43+ 0.99
Kidney	0.61+ 0.15	0.72+ 0.08	0.81+ 0.18	0.76+ 0.05
Heart	0.45+ 0.12	0.60 + 0.21	0.49+ 0.12	0.48+ 0.15

*P > 0.05 compared to control group

Table 4: Relative organ weight of male mice

Organ	Relative organ weight*			
	Control	Group 1	Group 2	Group 3
Liver	2.2 + 0.43	1.92 + 0.43	1.63 + 0.38	1.70 + 0.28
Kidney	0.23 + 0.06	0.2 + 0.04	0.20 + 0.05	0.16 + 0.05
Heart	0.13 + 0.06	0.16 + 0.05	0.13 + 0.06	0.10 + 0.00

*P > 0.05 compared to control group
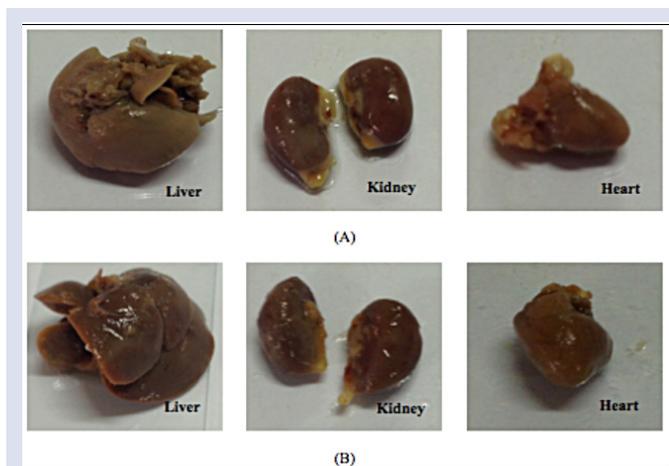
Figure 1 : Apparence of vital a Oran of male mice, (A) control, (B) group 3 (WTE 5000 mg/kg BW).
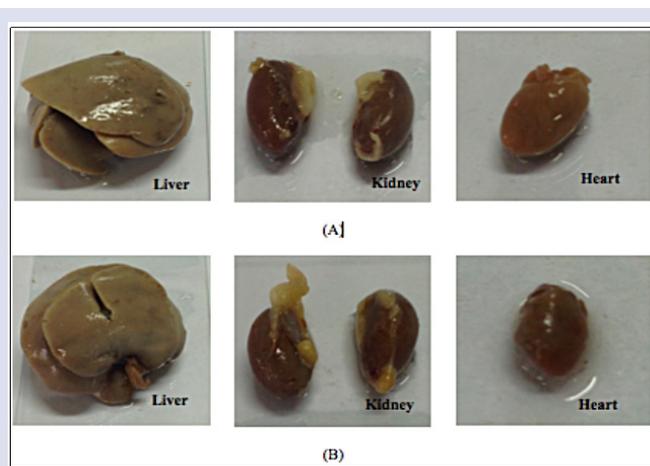
Figure 2 : Apparence of a vital origan of female mice, (A) control, (B) group 3 (WTE 5000 mg/kg BW).
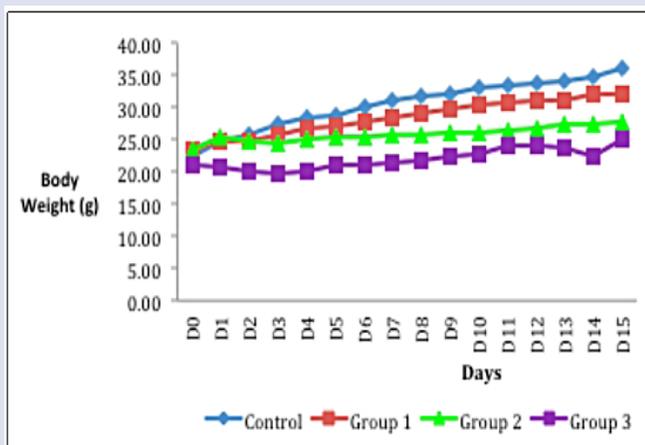
Figure 3: Mean body weight of the male mice.
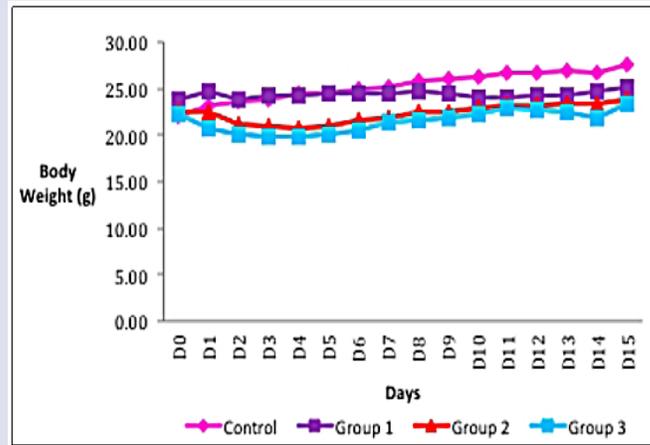
Figure 4 : Mean body weight of the female mice.

DISCUSSION

Observation showed that there was no difference regarding of behavior during the treatment. Decreases or increases in mice body weight found in all of the group treatment and the relative organ masses showed that there were no injuries occur during the treatment. This condition caused by the presence of physiological adaptation response to the extract, especially the effects of the chemical content that causes a decrease in appetite and calorie intake of animal.[12] The food intake and water consumption all of the group were regular and consistent throughout the study. It is indicating no toxic effect in both controls and treated group.

At the end of studies, day-14 of observations, all animals are live in the group of 5000 mg/kg body weight as the highest dose and the other group on these study. Based on this observation, WTE seems to be safe at a dose level of 5000 mg/kg BW, and the LD_{50} is considered be > 5000 mg/kg BW. Although the safety data of WTE also support by the traditional uses of tea leaves as a beverage which have long history consumption in the world, this study provides data on the acute toxicity profile of WTE that should be very useful for any future *in vivo* and clinical study of this plant.

CONCLUSION

In conclusion, this studies showed that the oral LD_{50} of WTE was greater than 5000 mg/kg BW and suggests that the WTE is found to be safe in a single dose of level 5000 mg/kg BW. However, the safety of long-term uses of white tea leaves especially in the treatment of the chronic disease should be confirmed with sub- acute toxicity study.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ABBREVIATION USED

WTE: White tea leaf ethanolic extract; DDY: Deutschland, Denken and Yoken; BW: Body weight; OECD: Organization for Economic Co-opera-

tion and Development; ROW: Relative organ weight; LD_{50}: Lethal doses; ED_{50}: Effective dose; SD: Standard deviation.

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GRAPHICAL ABSTRACT
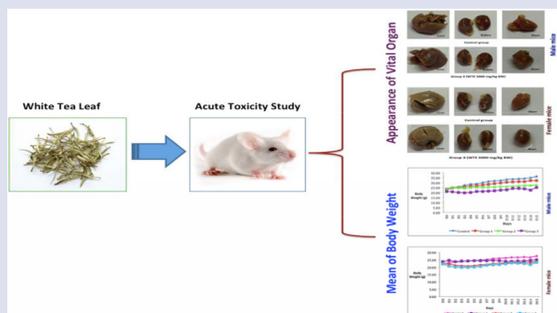
SUMMARY

- No treatment-related toxic symptom or mortality observed for the first 4 hours and 24 hours after oral administration of white tea leaf ethanolic extract in mice upto a dose of 5000 mg/kg BW.
- Both male and female treated mice showed no significant changes in behavior, breathing, and motoric activity.
- The oral LD50 of white tea leaf ethanolic extract was greater than 5000 mg/kg BW and suggests practically non-toxic in a single dose of level 5000 mg/kg BW.

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