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Research Article

ACUTE ORAL TOXICITY STUDY OF *ARECA CATECHU LINN*. AQUEOUS EXTRACT IN SPRAGUE-DAWLEY RATS

LIZA MEUTIA SARI[1], SUYATNA FD[2], SRI UTAMI[3], CHAIRUL CHAIRUL[4], GUS PERMANA SUBITA[5], YUNIARDINI S WHULANDHARY[5], ELZA IBRAHIM AUERKAURI[3]*

[1]Department of Oral Medicine, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia. [2]Department of Pharmacology and Therapetic, Faculty of Medicine, Indonesia University, Jakarta, Indonesia. [3]Department of Oral Biology, Faculty of Dentistry, Indonesia University, Jakarta, Indonesia. [4]Phytochemical Laboratorium, Indonesian Institute of Sciences, Research Center for Biology, Cibinong, Indonesia. [5]Department of Oral Medicine, Faculty of Dentistry, Indonesia University, Jakarta, Indonesia. Email: zazalukman@yahoo.com

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ABSTRACT

Background: Areca catechu Linn. (A. catechu L.), commonly known as "biji pinang" by the locals, belongs to botanical family Palmaceae. This plant is traditionally used in Indonesia as traditional ceremonial cultural role. The evaluation of toxic properties of A. catechu L. is crucial considering that this nut is one of predisposing factor in aetiology of oral cancer and its exposure may cause undesirable effect on health.

Objective: The purpose of the study was to test the acute oral toxicity of the extract of the plant.

Methods: The acute oral toxicity of A. catechu L. nuts extract was investigated in rats, as per OECD Guidelines 423 for acute protocols. The body weight, possibility of death, and activity parameters were measured for 14 days to ascertain the median lethal dose (LD50) of the extract. At the end of the study, all the animals in all the treated group were sacrified.

Results: The LD50 was found to be >15.000 mg/kg body weight. There was significant weight increase ($p<0,05$). No mortality was observed during the course of whole 14 days study period. No detectable alterations were found in activity parameter in treated group when compared to control group.

Conclusion: Overall, the results suggest that oral administration of aqueous extract of A. catechu L. did not produce any significant toxic effect in rats. Hence, the extract can be utilized for pharmaceutical formulations.

Keywords: Areca catechu Linn, Acute oral toxicity, Lethality.

INTRODUCTION

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoid, which have been found in vitro to have antioxidant properties. Plants remain the most common source of antioxidant agents. Any substance that reduces oxidative damage (damage due to oxygen) are caused by free radicals [1]. Antioxidants may possibly reduce the risks of cancer and age-related macular degeneration [1]. Areca nut (Areca catechu L., Palmaceae) is one of popular traditional herbal medicines used in Indonesia; it is called biji pinang. A. catechu L. can be chewed and it is a common masticatory in tropical and subtropical countries [2]. In Indonesia, these plants grow naturally and sometimes they are used as ornamental plants. In Aceh, a province that is located at the northern end of Sumatra, A. catechu L. is chewed with betel for traditional ceremonial cultural role. In some regions of Indonesia, stews made of the seeds from A. catechu L. are often being used to treat prolonged bleeding, such in menstruation, epistaxis and ulceration, to treat diphtheria, parasites' infections, diarrhea, and dysentery.

A. catechu L. had been found to contain mineral, fiber, 50-60% sugars, 15% lipids (glyceride of lauric, myristic, and oleic acid), 15% condensed tannins (phlobatannin, catechin), polyphenolics (flavonoids and tannin), and 0.2-0.3% alkaloids (arecoline, arecaidine, guvacine, and guvacoline) [2,3]. The activities of areca seed are antioxidant [1-9], antihelmintic [3], antidiabetic [10], antidepressant [2], antifungal [3], antibacterial [11], antimicrobial [12], antimalarial [13], anti-inflammatory [8], insecticide, psychoactive, hepatoprotective [14], lavicidal [15]. A. catechu seed was also used in anti-aging effect and cosmetic [16], hypolipideamic [17], hypoglicemic [18]. Although a lot of literatures showed that A. catechu L. had a strong antioxidant activity, this nut is also known as one of etiology of oral cancer besides betel quid chewing, heavy alcohol drinking, and dietary micronutrient deficiency [19]. This issue invokes the need to acknowledge the safety of A. catechu L. aqueous extract using experimental animals.

Toxicology is the important aspect of pharmacology that deals with the adverse effect of the bioactive substance on living organisms prior to the use as drug or chemical in clinical use [20]. Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of the battery of toxicity tests that are used [21]. The main aim of our study was to evaluate the extract for their toxic effects before it can be used for applications that are of importance to the public. Even though the A. catechu L. are used for various medicinal treatments, to our knowledge no literature exists on its toxicity profile. This study, therefore, designed to evaluate the acute oral toxicity effects of the aqueous extract of A. catechu L. The limit test dose of 15.000 mg/kg body weight was used following Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemical Section 4, health effects, 1981 [22].

METHODS

Plant material and extraction

1 kg of A. catechu L. fresh nut was collected from A. catechu L. plantation in Aceh Besar province, Indonesia, in September 2013. The nuts were identified and verified by Indonesian Institute of Sciences, Research Center for Biology, Cibinong, Bogor. The seeds of A. catechu L. were gathered and cleaned from the dirt and pulp (wet sorting process).

Seeds were washed under running water, and then cleaned and drained. Afterwards, these seeds were initially dried in the open air, protected from direct sunlight, and then continued to the drying process in the oven at 50°C. The dried simplicia were crushed into powder using a blender and sifted with 20 mesh of sieves. The powder was kept in cleaned and sealed containers. The extract was prepared by diluting the powder in water indirectly administered orally to the rats.

Experimental animals

The experiments were performed using healthy young adult female Sprague-Dawley rats, nulliparous, non-pregnant, and weighing 150-160 g, age 3-4 months. Female rats were chosen because of their sensitivity to treatment [21]. They were procured from histology laboratory of Faculty of Medicine, Indonesia University, Jakarta, Indonesia. All animals were put in stainless steel, open-mesh cages in a room maintained under environmentally controlled conditions of 25-27°C, a 12 hrs light-dark cycle, and 40-60% relative humidity. The rats were acclimated for at least 7 days in the laboratory. The animals were fed with standard laboratory animal food pellets with water ad libitum. After the acclimation period, the rats were categorized into two groups (each contains five rats), received the same handling conditions as during the acclimation process, and put into individual cages. During the 17th-20th hrs of the fasting period, the rats were fed only with plain water. All procedures were conducted in accordance with the European Community Guidelines (EEC Directive of 1986; 86/609/EEC) and were approved by the Animal Ethics Committee of the Faculty of Medicine, Indonesia University.

Assignment of animals

The animals were randomly divided into two groups each containing five rats. They were identified by the marking using yellow stain. They were marked on head, body, tail, head and body, body and tail, to ease the observation.

Mode of administration

The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animal was fasted 3 hrs prior to dosing (only food was withheld for 3 hrs but not water).

Administration dose

Following the period of fasting, animals were weighed, and test substance was administered orally at single dose 15.000 mg/kg daily. The concentration was made by 15.000 mg of dry extract powder mixed with aqueous up to 10 mL. The administration volume was 1 mL/kg of the animal body weight. After the administration of test substance, food for the animals was withheld for 2 hrs.

Test substance administration volume

The administration volume was 1 mL/kg body weight of the animal. Based on the body weight of the animal on the day of the treatment, the quantity of the test substance was calculated.

Observation period

Animals were observed individually after at least once during the 1, 2, and 4 hrs after the administration. The observation was continued daily thereafter for a total of 14 days. All the rats were observed daily with the purpose of recording any symptoms of ill-health or behavioral changes. Individual body weights of animals were recorded before the administration of the drug on 1st day of the study and thereafter on the 14th day of the experiment. Changes in the weight of individual animals were calculated and compared with that control animals as stated in paragraph 26 of OECD guidelines 423 [22].

Pathological observation

On the last day of observation, all rats were decapitated and examined macroscopically. Anomalies in the internal organs were documented and examined microscopically. After these thorough examinations were done, the remaining rats and tissue were sacrificed and discarded.

Statistical analysis

Statistical analysis of lethal dose (LD_{50}) value was performed using Thompson-Weil with 95% confidence interval. Comparisons were made between before and after treatment by the use of t-paired test. A p value of 0.05 or less ($p<0.05$) was considered as significant. All data were expressed as mean±standard error of the mean.

RESULTS

One hour after the administration of 15.000 mg/kg body weight of the A. catechu L. aqueous extract orally, the rats were becoming less active for 30 minutes. Weight loss was observed on the 2nd day, but the weight increased again in the following days. Afterward, weight gain was observed until the end of observation. No mortality was observed during 14 days after treatment with aqueous extract of A. catechu L. A significant difference on the weight gain, pre and post administration of A. catechu L. aqueous extract is shown on Table 1. There were no abnormal findings from gross pathological examination of all internal organs at necropsy in the group. Based on these results, the oral LD_{50} of A. catechu L. aqueous extract is suggested to be >5000 mg/kg body weight for female rats and this extract should, therefore, be labeling as unclassified non-toxic in the hazard category according to globally harmonized system. (OECD-hazard). In the end of the observation period, all rats were decapitated. From the autopsies, no macroscopic anomalies were seen in the internal organs.

DISCUSSION

Products from traditional medicinal plants have become popular in primary health care, particularly in developing countries, and some products may have been mistakenly regarded as safe just because they are a natural source [23]. In recent years, herbal drugs are exclusively used for the treatment of various diseases and are still practiced in rural communities [24]. However, in some circumstances, the usage of herbal medications may also create some adverse reactions. With the resurgence of the use of medicinal plants, scientific studies have become imperative to validate the folkloric use. As we know that A. catechu L. nut had been used in many developing countries as a mixture of betel quid which may cause oral submucous fibrosis, but it seemed that this condition may appear depending on duration and frequency of the habit, increased susceptibility due to deficiency iron and vitamin B12, the site of constant irritation, and tobacco use [25,26].

Many of incidents show that areca nut is also a carcinogen which causes oral squamous cell carcinoma. The carcinogenic effect comes from nitrosamines that occur during chewing areca seed. However, from this study shows that areca nut has no strong acute toxicity in rats that could be considered as a natural antioxidant for medicinal uses. Although, the incident of causing oral cancer must be kept in mind and used it with awareness.

Free radicals or reactive oxygen species (ROS) have been associated with the pathogenesis of several degenerative diseases and cancer. Antioxidants can retard or stop the uncontrolled generation of ROS,

Table 1: Effect of aqueous extract of *A. catechu L.* on Sprague-Dawley rats at 15.000 mg/kg body weight

Groups	Treatment	Body weight g		p value	Remarks
		Before treatment M1±SD1	After treatment M2±SD2		
Control	Water for injection	151.60±2.19	158.80±1.09	0.000	S
Oral	15.000 mg/kg of extract	152.00±2.00	160.80±1.78	0.000	S

M1, SD1, M2, and SD2 are mean weight and SD, respectively, for 1 (before treatment) and for 2 (after treatment), $p<0.05$: Statistically significance.
S: Significant, Areca catechu: A. catechu, SD: Standard deviation

thus help to reduce oxidative stress-induced diseases [27]. The present study shows that apart from the increased of body weight, there were no significant changes in the activity parameters used for evaluation of toxicity. All the animals are alive at the end of the observation. As there was no significant body weight difference between the test and control groups before and after the test period, it is concluded that the administration of the extract also does not affect the growth of animals.

Wetwitayaklung et al. (2006) demonstrated the presence of phytochemical like flavonoid and alkaloid in an aqueous extract of various parts of A. catechu L. Flavonoids are polyphenolic compounds that are categorized according to chemical structures into flavonols, flavones, flavonones, isoflavonoves, catechins, antocyanidins, and chalcones [5]. Flavonoids are strong antioxidant, and the active reaction mechanisms of flavonoids are through scavenging or chelating process [4]. These metabolites are generally used in various pharmaceutical and cosmetic products, which are an indication that these metabolites may be non-toxic [15].

CONCLUSION

Our results had demonstrated that the aqueous extract of A. catechu L. possesses non-toxicity effects as indicated in Sprague-Dawley rats. No deaths or signs of toxicity were observed in the rats that received the extract up to an oral acute limit dose of 15.000 mg/kg body weight. However, since the A. catechu L. had been known as an oral squamous cell carcinoma predisposing factor, therefore, it is recommended that a comprehensive study should be conducted to ascertain the toxicity and safety effects of A. catechu L. extract.

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