The Safety of Areca Seed Ethanolic Extract as Potential Chemopreventive Agent is Proven by Acute Toxicity Test

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Abstract

Areca (Areca catechu L.) seeds ethanolic extract (AE) exhibits antiproliferative activity and induces apoptosis on T47D and MCF-7 cells. This study aimed to verify AE safety using acute toxicity test to support its development as chemopreventive agent. Male Sprague Dawley Rat (Rattus norvegicus) age 8 weeks divided into five groups, one group of control treated with 0.5% CMC-Na only and four groups for treatment. Single dose in oral administration was done to test animal with various dose of AE starts from lowest dose to highest dose expected toxic to all of test animal (0.1; 0.72; 5.36 and 10 gram/kgBW). Observation was done during 24 hours and continued for 14 days. The observation criteria were toxic symptoms, appearance and mechanism of toxic effect and pathology of vital organ. Histopathology analysis of some vital organs was done with Haematoxylin&Eosin (H&E) staining. Toxic effect did not appear either on treatment groups or control group. Treatment of single dose of areca ethanolic extract, even in highest dose, did not cause the death of the animals. Therefore, observation extended to 14 days and terminated by necropt of the animals. All of groups did not show histopathological alterations in microscopic observation. Category of the potential toxicity of AE is practically non-toxic, ie 10 g/kgBW. The result shows the safety of areca seed ethanolic extract which is important for its development as chemopreventive agent.

Keywords: Areca catechu, acute toxicity, rat.

INTRODUCTIONS

Acute toxicity is the degree of toxic effect of a compound on a specific test animals which occur within a short time after the single dose administration (Ecobichon, 1997). Areca catechu L., a family member of Arecaceae, is potential as chemotherapeutic agent because of its antioxidant and antimutagenic activity (Wang and Lee, 1996; Lee and Choi, 1999). Areca seeds contain alkaloids, such as arecoline (C8 H13 NO2), arecolidine, arekaïn, guvakolin, guvasine and isoguvasine. Ethanolic extract of areca seed contains condensed tannins, hydrolyzed tannins, flavan, phenolic compounds, gallic acid, gum, lignin, volatile and non-volatile oil, and salt (Wang and Lee, 1996). Areca seeds contain proanthocyanidin, a condensed tannin belonging to the flavonoids (Nonaka, 1989). Proanthocyanidin has antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilation activity (Fine, 2000). Based on the previous studies, areca seed ethanolic extract and its fractions have cytotoxic effects and induce apoptosis against WiDr, T47D and MCF-7 cancer cells (Handayani, et al., 2008; Rahmi, et al., 2008; Meiyyanto, et al., 20081; Meiyyanto, et al., 20093). In addition, the ethanolic extract of areca seed is also potentially synergistic when combined with chemotherapeutic agents (Meiyyanto, et al., 20082; Meiyyanto, et al., 20093). Therefore, these extract is expected to have maximum pharmacological effects and minimum toxicity effects. In this study, we observe the safety of areca seed ethanolic extract using acute toxicity test in vivo in normal mice.

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MATERIALS AND METHODS

Animals
Normal and healthy male albino rats (Sprague Dawley), eight weeks old, each weighing 130-160 g. Animals were obtained from the Unite of Laboratory Animals, Faculty of Pharmacy, Gadjah Mada University.

Chemicals
Areca seed powder was purchased from BPTO Karanganyar and was extracted using absolute ethanol, CMC-Na (Merck), aquadest, NaCl, 10% formalin.

In Vivo Experiments
The rats were classified into 5 groups, each consisted of 5 rats:
- Group I : was treated with 0.1 g/kg bw AE
- Group II : was treated with 0.72 g/kg bw AE
- Group III : was treated with 5.36 g/kg bw AE
- Group IV : was treated with 10 g/kg bw AE
- Group V : was treated with 0.5% CMC-Na (the vehicle of AE) as control group.

Oral administration (p.o.) of AE was done through a single dose for each animal.

Observations of Toxic Symptoms
Intensive observations were done during the period of 24 hours after single dose administration of AE. The observation criteria were included: physical observations, the number of dead animals and organ histopathology. However, if none of the mice died within 24 hours, observations were continued until 14 days later.

Histopathological Observation
In the end of observation (after 14 day observation), each animal was sacrificed and its vital organs (hearth, liver, lung, kidney, spleen, and intestinal) were taken for macroscopic and microscopic observation. Furthermore, organs were rinse in aquadest and NaCl, and were continued by preserved in 10% formalin for histopathological study. Treatment groups were compared with control group for all parameter.

Statistical Analysis
Rat body weight was observed and was analyzed every week until the end of study and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) and Tukey’s post-hoc test was used to assess the statistical differences. P values <0.05 was considered for statistically significance. The potency of toxicity of AE was determined using “lethal dose” 50% (LD50) parameter. The LD50 was stated according to the toxicity categories in Table I.

Table 1. The range of LD50 value based on its relative toxicity (Ecobichon, 1997).

<table>
<thead>
<tr>
<th>Category of toxicity</th>
<th>LD50 (mg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high toxic</td>
<td>≤ 1.0</td>
</tr>
<tr>
<td>High toxic</td>
<td>1.0 – 50</td>
</tr>
<tr>
<td>Moderate toxic</td>
<td>50 – 500</td>
</tr>
<tr>
<td>Slightly toxic</td>
<td>500 – 5000</td>
</tr>
<tr>
<td>Practically non toxic</td>
<td>5,000 – 15,000</td>
</tr>
<tr>
<td>Harmless</td>
<td>≥ 15,000</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS

Qualitative Observation of Toxic Symptoms
Toxic symptoms were intensively observed in the first 3 hours and were continued until 24 hours. Toxic symptoms observation were included changes in behavior, movement licking, scratching, wrinkles, nervous, writhing, reacting to stimuli (hyperactivity, passivity), cerebral and spinal reflex, pupil size, secretions, breathing, cardiac palpitations, skin condition, hair condition, and death. The results of the qualitative toxic symptoms in male rats for 24 hours after administration of AE showed that visible toxic symptoms were not detected in group I, II, V, and VI. Nevertheless, some animal test in group III and IV showed behavioral changes such as biting the toes back, twitching, moving hyperactive then passive, irregular breathing and wheezing, gasping breathing, pyloerection, and arrhythmias (Table II). However, the toxic symptoms disappeared on the next days. The graphic of body weight during the observation in time period showed that the average weight growth of all of group was significantly not different among the test groups (p<0.05) (Fig.1).
Table II. Qualitative observation of toxic symptoms in male rats for 24 hours after oral 
administration of a single dose of AE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>N</th>
<th>Toxic symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.1 g/kg bw AE</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>0.72 g/kg bw AE</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>5.36 g/kg bw AE</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>10 g/kg bw AE</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>0.5% CMC Na</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) : toxic symptom was not detected; (+) : toxic symptom was detected

Figure 1. Effect of AE on the body weight growth. Rats were weighed on the first day, the 3rd, 9th, and 14th, then made an average. One way ANOVA was used to assess differences among treatment groups (p<0.05).

Histopathology Profile of Vital Organs
The macroscopic data of the vital organs of rats that were observed was the physical appearances and organ weights. Macroscopic observation after administration of AE showed that the treatment groups were not different compared to control group. All of vital organs were normal, but lung organ was the exception. Lung in all of group was red, indicating inflammation. Because lung inflammation was observed in all group, it was not due to the AE treatment.
Figure 2. Vital organs of rats in the high dose treatment group (group IV) showed microscopically almost not different compared to control (group V). (1A) spleen in group V, (1B) spleen in group IV, (2A) intestinal in group V, (2B) intestinal in group IV, (3A) gastric in group V, (3B) gastric in group IV, (4A) lung in group V, (4B) lung in group IV, (5A) heart in group V, (5B) heart in group IV, (6A) kidney in group V, (6B) kidney in group IV, (7A) normal liver in group V showed a normal blood vessel (a), (7B) liver vein in group IV, showed multifocal infiltration of inflammatory cells (b) of the blood vessels, (7C) liver in group V did not show fat in the vacuole, (7D) liver in group IV, showed fat in the vacuole.

The results of microscopic observation (Table III) showed that the spleen on treatment and control groups were seen a normal red pulp and white pulp. The intestinal, stomach, heart and kidneys appeared normal. Lung organ was seen an inflammatory infiltration cell that was showed a great number of lymphocytes cells (colored purple), but both the treatment and the control group were not have difference appearance (Fig. 2(4A) and 2(4B)). The difference was showed in the liver organ, whereas in the highest dose of AE there were multifocal infiltrations, the presence of inflammatory cells around the blood vessels and the presence of fats in the vacuole (Fig. 2(7B) and 2(7D); Table III). From the microscopic observation, histopathological alterations of organs in the treatment groups were hardly found compared to control.
Because the ethanolic extract of areca seed (AE) shows its potency as chemopreventive agent, especially for combination application with chemotherapeutic agent, toxicity testing to guarantee the safety of AE needs to be done. Acute toxicity test results showed that at doses 5.36 mg/kg bw (group III) and 10 mg/kg bw (group IV), some rats had difficulty breathing, passive and there are actually hyperactive. However, the situation improved in 24 hours later. Based on the observation, AE at high dose possibly affects the respiratory and somatomotor system of rat but can recover quickly. Since histopathological data of treatment groups showed hardly different than control, except liver at the highest dose, then AE shows its safety to be consumed. The observation also showed that none of the mice died during the treatment. Then, the LD₅₀ cannot be definitely stated. Therefore, the acute toxicity can only be expressed as “pseudo-LD₅₀”, ie the largest dose that technically can still be given to the test animals. In this study, pseudo-LD₅₀ of AE is 10 g/bw. In general, category of the potential toxicity of AE is practically non toxic according to table I, because pseudo-LD₅₀ of AE is in the range of 5-15 g/kg bw.

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REFERENCES


Table III. Microscopic observation of the vital organs of rats after oral administration of the single dose of AE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Gastric</th>
<th>Heart</th>
<th>Spleen</th>
<th>Intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>II</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>IV</td>
<td>+</td>
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<td>V</td>
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</tr>
</tbody>
</table>

(-) : normal; (+) : histopathological changed
