ACUTE ORAL TOXICITY OF RAW ARECA NUT EXTRACT IN RATS

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ABSTRACT

The aqueous extract of raw areca nut extract was evaluated for its acute oral toxicity study in rats. Healthy male and female Wistar albino rats aged between 10 to 12 weeks and weighing 190 to 210 g adult were selected. The animals were fasted overnight and raw areca nut extract in graded doses was administered as a single dose by gavage using a gavaging tube. Careful general clinical observations were made for 14 days. The toxicity signs were depression, profuse salivation, tremors, muscular incoordination, convulsions, writhing movement of neck and severe diarrhoea. The LD$_{50}$ values of raw areca nut extract in male and female rats were found to be 2321.96 and 2257.52 mg/kg, respectively. The LD$_{50}$ value in female rats was slightly lower than that of the male rats.

Key words: Areca nut, LD$_{50}$ (median lethal dose), Wistar albino rats.

INTRODUCTION

Areca catechu is part of the Areceaceae family. Areca nut is a name given to the seed of the Areca catechu tree. Areca nut is an important agricultural product in many regions of the world. Raw areca extract is used for coloring the areca nuts in commercial marketing. In coastal belt of Karnataka, during the harvesting seasons of areca nut, raw areca extract is stored in open containers for drying in open field. Therefore, cattle will have an easy access for drinking it and end up in severe toxic reactions. Chemically, areca nut contains arecoline, which is a potent pharmacological agent resembling muscarine and pilocarpine in that it induces a range of parasympathomimetic effects (Farnsworth, 1976). Even though the clinical signs of the toxicity are similar to that of parasympathomimetic property of arecoline hydrochloride, the treatment with atropine sulphate was not successful. Hence the mortality in the large animals might be attributed to some other ingredients in the areca nut extract. (Shridhar and Narayana, 2003). Gilani et al. (2004) reported that acute toxicity study of petroleum and chloroform extracts of areca nut induced mortality in mice. The present study was undertaken to evaluate acute oral toxicity and to determine LD$_{50}$ values in rats.

MATERIALS AND METHODS

Extract preparation: Raw areca nuts were collected from the village Kuntinamadu, Chikkamagaluru, Karnataka during the month of November 2008. Twelve kg of raw areca nuts were separated from seed coat and mixed with 4 L of distilled water in the ratio of 3:1. The contents were boiled for six hours and later sieved to separate the filtrate. The collected filtrate was stored in refrigerator at 4°C. The dry matter content of raw areca nut extract was analyzed by drying the sample to a constant weight in a forced hot air oven at 105 °C (AOAC, 1995) and was found to be 125 mg/ml. This concentration of raw areca nut extract was used for the present toxicity study.

Pilot study: Pilot study for the raw areca nut extract was conducted in both male and female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class Method (OECD 423).

The animals were fasted overnight prior to the administration of the extract. The raw areca nut extract in graded doses were administered as a single dose to animals by gavage. The volume of...
administration was maintained to 2 ml/100 g through proper dilution of raw areca nut extract (Table 1).

**Main study:** Acute oral toxicity study for the raw areca nut extract was conducted in both male and female Wistar albino rats, based on the LD \(_{50}\) dose range obtained from the pilot study.

**Experimental animals:** The male and female Wistar albino rats aged 10 to 12 weeks weighing 190 to 210 g were procured from Indian Institute of Sciences, Bangalore and were acclimatized to the laboratory conditions for seven days prior to main study. The animals were randomly distributed into six groups (Control, Groups I, II, III, IV and V) of 10 animals each of either sex. The animals were maintained as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals standard guidelines (CPCSEA) and approval was obtained from Institutional Animal Ethics Committee (IAEC) with reference No.19/LPM/IAEC/2008 for laboratory animals.

**Administration of doses:** The median lethal dose was determined for both male and female rats separately (Table 2). The five doses were selected based on pilot study. The animals were fasted overnight and raw areca nut extract in graded doses were administered as a single dose to animals by gavage using a gavaging tube. The volume of administration was maintained to 2 ml/100 g through appropriate dilution of raw areca nut extract (Group I, II, III, IV and V). The control groups were administered with distilled water. The LD \(_{50}\) was calculated as per the probit analysis described by Finny (1971).

**Observation of animals:** Careful general clinical observations were made every day. All the animals were observed for effects on skin and face, eyes, mucous membranes, respiratory and circulatory systems, autonomic change such as salivation, central nervous system effects including tremors and convulsions, and changes in the level of activity, gait, posture, reactivity to handling or sensory stimuli, and altered strength, health conditions and mortality.

**Necropsy:** The necropsy was conducted on dead animals immediately after the death. The surviving animals were kept up to 14 days and on 15th day all the animals in control and treated groups were humanely sacrificed and subjected to detailed gross necropsy. The internal organs (lungs, heart, liver, intestine and kidney) were collected for histopathological study. The internal organs were processed for histopathology by routine paraffin embedding technique (Luna, 1968).

**Statistical analysis:** The data were subjected to statistical analysis. The data were analyzed by using two-way ANOVA, Bonferroni post-test. Mean values and standard error of mean were calculated and all the values are expressed as Mean±SEM using GraphPad Prism, 2007.

**RESULTS AND DISCUSSION**

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short term exposure by the oral rate. It is traditionally a step in establishing a dosage regimen in other studies by providing initial information on the mode of toxic action of a substance.

In the present study, dry matter content of raw areca nut extract was found to be 125 mg/ml. During the course of study, the clinical signs of toxicity observed were depression, profuse salivation, tremors, burrowing behaviour, hunched back, muscular incoordination, weakness, altered gait, convulsions, withering movement of neck and severe diarrhoea. At the terminal stage, the animals showed laboured breathing, gasping and death. The mortality pattern for each group is presented in Table 3 and 4. The acute toxicity study did not reveal any gross pathological changes. The histological features

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of male rats</th>
<th>Dose (mg/kg)</th>
<th>Group</th>
<th>No. of female rats</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3</td>
<td>50</td>
<td>Group V</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>Group II</td>
<td>3</td>
<td>300</td>
<td>Group VI</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Group III</td>
<td>3</td>
<td>2000</td>
<td>Group VII</td>
<td>3</td>
<td>2000</td>
</tr>
<tr>
<td>Group IV</td>
<td>3</td>
<td>3000</td>
<td>Group VIII</td>
<td>3</td>
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of the internal organs (lungs, heart, liver, intestine and kidney) of both male and female rats were apparently normal.

The observed toxic signs were indicative of gastrointestinal and respiratory tract involvement and were in accordance with Farnsworth (1976). The calculated LD$_{50}$ values of raw areca nut extract were 2321.96 mg/kg in male rats and 2257.52 mg/kg in female rats. The LD$_{50}$ value in female rats was slightly lower than that of the male rats. The LD$_{50}$ values recorded for male and female rats indicated that the raw areca nut extract is moderately toxic as per toxicity rating.

### CONCLUSION

Chemically, areca nut contains arecoline, which is a potent pharmacological agent resembling muscarine and pilocarpine in that it induces a range of parasympathomimetic effects (Farnsworth, 1976). The mortality observed in rats can be specifically attributed to dyspnea, hypotension and bradycardia caused by the raw areca nut extract.

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