ABSTRACT

Background: Medication using herbal medicine is one of the alternatives chosen to reduce the adverse effects caused by synthetic drugs. Betel nut (Areca catechu) usually used as an alternative drug such as anthelmintic and others.

Aim: The study aims to determine the chronic toxicity effect of betel nut. Method: Five hundred grams of simplicial powder was macerated using 95% ethanol in a container, then sealed and let them for three days. They were protected from light while repeatedly stirred. Rat was given Betel nut extract for three weeks. The histopathological examination was observed under the microscope.

Result: The mean GOT serum, GPT serum, BUN, and creatinine serum levels between control (no extract given) and each dose, i.e., 0.2 cc, 0.4 cc, and 0.8 cc, showed insignificant results (p > 0.05). According to the histopathological observation, there was no difference between control and treatment.

Conclusion: Various orally doses of betel nut extract did not cause toxicity in white rats (Rattus norvegicus).

Keywords: betel nut, herbal medicine, kidney histopathology


INTRODUCTION

The use of synthetic drugs cannot be separated from new problems they give, including environmental pollution – drug residues in livestock product that often cause resistance – which lead to decrease in the effectiveness of the drugs.

Medication using herbal medicine is one of the alternatives chosen to reduce the adverse effects caused by synthetic drugs. Betel nut (Areca catechu L) usually used as an alternative drug such as anthelmintic and others. Betel nut consists of alkaloids (arecoline, arecaidine, arecaine, guvacoline, guvacine, and isoguvacine), red tannin, and fat. Arecoline is believed to have a beneficial effect as anthelmintic.

Toxicity in herbal medicine may occur when given excessively. As regard with prolonged use, herbal medicine can accumulate in tissues or organs such as liver and kidneys which can damage the organs. Liver is the biggest gland in the body which has many functions, one of them is drug metabolism. The detoxification process from various components of drugs produces components with higher toxicity which often cause pathological changes. Histopathological assessment, GOT serum, and GPT serum tests are generally used to determine liver health.

Kidney is an essential organ in the body, which has functions to discard metabolism wastes and poison in the form of urine. The kidney is vulnerable to the effect of chemical compounds because of its role in filtering metabolism residues from the blood; thus the possibility of pathological changes is very high. Histopathological examination, creatinine, and BUN test can be performed to determine kidney function. The in vivo administration of betel nut in pigs infected with Ascaris suum showed no pathological changes in liver, kidney, brain, and heart.

METHODS

Extraction

Five hundred grams of simplicial powder was macerated using 95% ethanol in a container, then sealed and let them for three days. They were protected from light while repeatedly stirred. After three days, the solution was strained, and the crude extract was squeezed. Twenty-five parts of 1.3L (ethanol 95%) solvent was added to the crude extract then mixed and strained until it obtains 100 parts. The container was closed then placed in a cool place and covered from the light for two days. The deposit was separated, and the liquid extract was obtained. Afterward, the extract was evaporated using rotary evaporator at 30-40°C and concentrated using a water bath to get the concentrated extract of betel nut.

Toxicity

Toxicity test using 20 white rats (Rattus norvegicus). Betel nut extract was applied for three weeks.
Histopathological examination of liver and kidney

The making of histopathological preparations was conducted according to Kierman method. The liver was fixated using 10% neutral buffered formalin and then cut. Afterward, the dehydration process was conducted using 70%, 80%, and 90% alcohol absolute I and absolute II. Purification was done using xylol and placed into paraffin blocks which were cut using microtome. The results of the cut next were floated in the water bath. The preparation was placed in object glass and stained with HE (Hematoxyline and Eosin). Finally, those specimen was examined under the microscope.

GOT serum, GPT serum, creatinine serum, and BUN examination

Both GOT and GPT serum was examined using Reflovet Plus. So did both creatinine serum and BUN. The first step was to place sample drops on the kit. The blood was taken by using pipette according to pipette size, then put onto GOT serum kit (GOT) to measure GOT serum level and GPT kit (GPT) to measure GPT serum level in blood. As against the same procedure, the creatinine kit (CREA) measured creatinine level and also BUN kit (BUN) measured urea level in blood. The second step was the kit insertion test. Insert GOT and GPT kits as well as CREA and BUN kits into measurement space of Reflovet Plus and close the area. The third step showed the results. After 2-3 minutes, Reflovet Plus will print and confirmed the findings.

RESULTS AND DISCUSSION

The GOT serum results of the rats who administered with betel nut extract at dose 0.2 ccs, 0.4 ccs, and 0.8 ccs were showed in Table 1. So did GPT serum results were confirmed in Table 2.

The results of ANOVA in both GOT and GPT serum mean levels between control and each dose showed insignificant results (p > 0.05). Both GOT and GPT serum acts as a parameter of liver damage, which can be performed of changes in enzyme activities in blood by observing compounds formed by the liver. Both GOT and GPT serum levels were within normal standard due to betel nut administration. The result may be due to liver cells were still in good condition. This function was performed by Kuffer cells which have phagocytosis ability. Kuffer cells are filtration tool against germs and foreign objects entering the liver through the veins.

Study of toxicity in betel nut extract in white rats for 28 days with 100, 250 and 500 mg/kg body weight dose resulted in both normal GOT and GPT serum levels.

The results of BUN level in white rats who given with betel nut extract at dose 0, 0.2 ccs, 0.4 ccs, and 0.8 ccs were showed in Table 3. So did the results of creatinine serum level were showed in Table 4.

ANOV A analysis results of both mean urea and creatinine serum levels between control and each dose showed insignificant results (p > 0.05). Both creatinine serum and urea levels are two crucial measurements in determining abnormalities in kidney function. Many conditions can affect kidney function, both acutely and chronically. Chemical substances often found in the kidney is suspected of causing kidney cell damage. Kidney function can be determined by observing both creatinine serum and urea levels. Creatinine is synthesized in

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Table 1  Average GOT serum levels

<table>
<thead>
<tr>
<th>Dose</th>
<th>Repetition</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control</td>
<td>115</td>
<td>113</td>
</tr>
<tr>
<td>0.2 cc</td>
<td>112</td>
<td>120</td>
</tr>
<tr>
<td>0.4 cc</td>
<td>103</td>
<td>118</td>
</tr>
<tr>
<td>0.8 cc</td>
<td>116</td>
<td>102</td>
</tr>
</tbody>
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Table 2  Average GPT serum levels

<table>
<thead>
<tr>
<th>Dose</th>
<th>Repetition</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control</td>
<td>70.9</td>
<td>57.2</td>
</tr>
<tr>
<td>0.2 cc</td>
<td>98.1</td>
<td>79.5</td>
</tr>
<tr>
<td>0.4 cc</td>
<td>83.4</td>
<td>107</td>
</tr>
<tr>
<td>0.8 cc</td>
<td>81.6</td>
<td>77.5</td>
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</table>

Table 3  Average BUN level

<table>
<thead>
<tr>
<th>Dose</th>
<th>Repetition</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>43.4</td>
</tr>
<tr>
<td>0.2 cc</td>
<td>41.1</td>
<td>40.5</td>
</tr>
<tr>
<td>0.4 cc</td>
<td>35.6</td>
<td>31.6</td>
</tr>
<tr>
<td>0.8 cc</td>
<td>48.5</td>
<td>35.2</td>
</tr>
</tbody>
</table>

Table 4  Average creatinine serum level

<table>
<thead>
<tr>
<th>Dose</th>
<th>Repetition</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control</td>
<td>0.501</td>
<td>0.578</td>
</tr>
<tr>
<td>0.2 cc</td>
<td>0.558</td>
<td>0.500</td>
</tr>
<tr>
<td>0.4 cc</td>
<td>0.501</td>
<td>0.500</td>
</tr>
<tr>
<td>0.8 cc</td>
<td>0.502</td>
<td>0.507</td>
</tr>
</tbody>
</table>
the liver from methionine, glycine, and arginine. Urea, also known as BUN, is the result of normal protein metabolism. A previous study showed no increase in creatinine and urea levels during kidney function examination. Studies regarding acute toxicity of betel nut ethanol extract in animal models showed that betel nut extract was classified as non-toxic material.

There was no difference between control and treatment groups (Figure 1, 2, 3, 4). Liver structure in control and treatment groups (0.2 ccs, 0.4 ccs, 0.8 ccs) were in normal condition. Entire treatment groups did not cause a toxic effect. Liver is a vital organ with various functions in the metabolism process, making this organ is often exposed to chemical substances. Those substances will undergo detoxification and inactivation to render them harmless for the body. In the liver, various important processes occur, which include energy storing, protein formation, and poison or drug neutralization. Drugs will undergo metabolism in the liver and chemical structure will change, which is catalyzed by an enzyme produced by hepatocyte microsome cells (biotransformation). Drugs are transformed into metabolites which are less active. A previous in vivo study of betel nut administration in pigs with Ascaris suum infection to determine therapeutic results and acute and subacute toxicity
tests showed no pathological changes in the liver, kidney, brain, and heart. Tannin in betel nut acts in the anti-inflammatory activity and can proceed as hepatoprotective.

According to the histopathological results of white rat kidney, there were no differences between control and treatment. Generally, kidney tissue structures in control and treatment (0.2 ccs, 0.4 ccs, and 0.8 ccs) were in normal condition. The entire procedure did not cause a toxic effect. Kidney is the main organ in the body, which functions in discarding metabolism waste and poison in the form of urine. It is vulnerable to the effect of chemical substances because kidney acts in filtering metabolism residue from the blood. Therefore, the possibility of pathological changes is very high. Indian Pam Masala containing betel nut for 16 to 90 days in white rats did not cause a harmful toxic effect to the kidney, heart, spleen, and liver. Histopathological study of betel nut extract administered orally with different doses for four weeks did not cause a toxic effect to the kidney.

CONCLUSION

Oral-administered betel nut (Areca catechu L.) extract with various doses, i.e., 0.2 ccs, 0.4 ccs, and 0.8 ccs did not cause a toxic effect in both kidney and liver of white rats (Rattus norvegicus).

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

Author has no conflict of interest regarding all element on this study.

AUTHOR CONTRIBUTIONS

A.A.Gde Arjana design and do the experiment, I Made Sukada collect the sample and do the experiment, and N.Adi Suratma analyse the data.

ETHICAL CLEARANCE

This research has got agreement ethical approval from animal ethics commission on Faculty of Veterinary Medicine Udayana University on Number 196a/KE-PH-Lit-3/VII/2015.

REFERENCES


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