ACUTE AND DELAYED TOXICITY STUDY OF CASSYTHA FILIFORMIS DEFATTED ETHANOLIC EXTRACT

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ABSTRACT
Defatted ethanolic extract of Cassytha filiformis exhibits a potential antihypertensive activity based on our previous study. The objective of this study is to evaluate the acute and delayed toxicities of the extract. Determination of LD$_{50}$ and delayed toxicity were conducted on 90 mice (divided into 6 groups of 15 mice each) at doses of 25, 50, 100, 200, 400 and 800 mg/kg. Data was analyzed by Probit analysis to determine LD$_{50}$, while Two Way ANOVA was used to analyze the change of animal body weight, food and water intakes. Results showed that the 1, 2, 3, 24 and 48 hours LD$_{50}$ of this extract were 0; 875.83; 313.63; 52.28 and 2.17 mg/kg, respectively. Animal body weight, food and water intakes were affected by the dose of the extract and the time of measurement and interaction of those variables significantly (p<0.05). In this situation, food and water intake decreased up to fifteen days after the single dose of this extract, especially at high dose (400 mg). These led to reducing animal body weight. There was no significant change (p>0.1) in the heart, liver and kidney weight ratios at the end of this study (14 days after single dose of extract). These indicate that the defatted ethanolic extract of C. filiformis is toxic and produces delayed toxicity.

KEYWORDS: Ethanolic extract, cassytha filiformis, toxicity, acute, delayed.

INTRODUCTION
Cassytha filiformis has been reported to have antioxidant activity (Mythili et. al., 2011 Dhanalakshmi et. al., 2012) and vasorelaxant (Tsai, et. al., 2008), increase the bleeding time on mice (Armenia, 2007), decrease the body weight on high fat diet mice (Fitri et al., 2011)
and reduce blood glucose if it is used for a longer period. The extract showed a low toxicity (Babayi et al., 2007). According to our previous work, the ethanolic extract of this plant reduced blood pressure of hypertensive rats induced by prednisone-NaCl (Yuliandra et al., 2013). We recently compared the hypotensive effectiveness of defatted crude extract, BuOK and EtAct fraction of this plant on prednisone-NaCl and prednisone-NaCl-LNAME induced hypertensive rats, where on the last hypertensive rats model, the hypotensive effect is greater and the effectiveness of crude defatted extract was better (Armenia et al., 2014).

The World Health Organization estimates since the 80th century that approximately 80 percent of the world’s population rely primarily on traditional medicines as sources for their primary health care (Farnsworth, et al., 1985). The reason is that a complex pathogenesis of many diseases makes a mono-substance therapy dogma change to multi-substances therapy to obtain multi-target therapy. This means that a combination of medications that should be prescribed by a physician could be replaced by multi-component content of an herbal medicine.

It is often assumed that herbal medicine is safer than conventional medicine due to relatively small side effect if it is used properly. However this is not always true (Alam et al., 2011). Yet, no category has been established to judge the safe use of plant for medicinal purposes (Griggs, 1981). Herbs contain chemicals that, when they are pharmacologically active, will potentially produce toxic effect, especially when the higher dose is used. That’s why toxicity study is compulsory to every herbs and their product prior to their use for medicinal purposes.

Not many information available regarding the toxicity of *C. filiformis*. Previous study on the water extract described that this plant is relatively save (Babayi, 2007). But, when we tested the defatted extract for its hypotensive effect at 20 mg/kg (data is not shown here), some rats were died. The present study is conducted to evaluate acute and delayed toxicities of the defatted ethanolic extract of *C. filiformis*. This may lead other researchers to follow up the research in any fields, such us natural product chemistry, pharmacology or clinical pharmacy etc.

**MATERIALS AND METHODS**

**Acute Toxicity Study**

A number of 90 mice were divided into 5 groups, each group treated with ethanolic defatted extract of *C. filiformis* at doses of 25, 50, 100, 200 and 400 mg/kg. The number of animal
death at 1, 2, 3, 24 and 48 hours were recorded to calculate LD$_{50}$ on each times. Animal behavioral changes such as motoric activity, respiratory rate, and diarrhea were monitored. *Delayed Toxicity Study.*

The survived animals from death on the acute toxicity study were monitored for the symptom(s) associated with the delayed toxicity, their body weight, 24 hour food intake and water intake for 14 days. At the end of experiment, the animals were sacrificed and their liver, heart and kidney were taken to measure their ratios to the body weight.

**RESULTS AND DISCUSSION**

**Acute Toxicity study**

There were several predominant behavioral changes demonstrated by the animal treated with *C. filiformis* defatted ethanolic extract, such as decrease of motoric activity, respiratory alteration and diarrhea.

The LD$_{50}$ at 1, 2, 3, 24 and 48 hours after single dose of defatted ethanolic extract of *C. filiformis* were 0; 875.8; 313.6; 52.3 and 2,171 mg/kg (Table 1).

**Table 1. The number of death due to *Cassytha filiformis* L. defatted ethanolic extract treatment and LD$_{50}$ values at 1, 2, 3, 24, and 48 hours.**

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>N</th>
<th>Number of death at hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>LD$_{50}$ (mg/kg)</td>
<td></td>
<td>875.8</td>
</tr>
</tbody>
</table>

**Table 2. Behavioral changes following *Cassytha filiformis* L. defatted ethanolic extract administration on acute study**

<table>
<thead>
<tr>
<th>No</th>
<th>Symptoms</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Motor Activity</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Respiration</td>
<td>-</td>
</tr>
</tbody>
</table>
Delayed Toxicity
Administration of single dose of defatted ethanolic extract of *C. filiformis* produced delayed toxicities, indicated by the decrease in animal body weight, alteration of water and food intake, especially at high doses (100 and 400 mg/kg) (p<0.05). Animal body weight at 25 mg extract was not significantly influenced (p>0.1) (Figure 1). The average changes of body weight of the control animal, the animal treated with extract at doses of 25, 50, 100, and 400 mg/kg were 1.83±0.183; 2.03±0.177; -0.86±0.177; and -4.60±0.177 g, while the average animal body weight on day 0, 2, 4, 6, 8, 10, 12 and 14 were 0±0.251; -0.87±0.251; -0.76±0.251; 0.98±0.251; -0.74±0.251; -0.53±0.251; 0.20±0.251 and 0.50±0.251 g.

Like the body weight, food and water intake of the animals treated with extract were also altered significantly (p<0.05) (Figure 2 A and B). It is shown that food intake of the animal decreased 2 days after treated with the extract but then tent to increase by time, especially on the groups treated with low and medium doses (25 and 100 mg/kg). The average food intake of animals treated with extract were lower as compared to control group, especially at dose of 400 mg/kg. The average food intake of control and the groups treated with extract at dose of 25, 100 dan 400 mg/kg were 3.91±0.067; 3.65±0.067; 2.86±0.067 and 2.69±0.067 g respectively, while the average food intake on day 0, 2, 4, 6, 8, 10, 12 and 14 were 3.37±0.094; 1.41±0.094; 3.49±0.094; 3.36±0.094; 3.43±0.094; 3.83±0.094; 3.89±0.094 and 4.21±0.094 g respectively (Figure 2A).

On the other hand, water intake of the animal treated with extract was lower at the beginning of treatment but then increased even higher than shown by control animals, especially at low
dose (p<0.05). The average water intake of control and groups treated with extract at dose of 25, 100 dan 400 mg/kg were 3.39±0.108; 3.36±0.108; 3.10±0.108 and 2.87±0.108 mL respectively while average water intake on day 0, 2, 4, 6, 8, 10, 12 and 14 were 3.20±0.152; 1.88±0.152; 3.15±0.152; 3.01±0.152; 3.46±0.152; 3.37±0.152; 3.51±0.152 and 3.78±0.152 mL (Figure 2B)

Figure 2. Food (A) and water (B) intakes changes 14 days after single dose administration of Cassytha filiformis L. defatted ethanolic extract on the survived animals.

Doses of defatted ethanolic extract of C. filiformis did not affect liver, heart and kidney weight ratios significantly (P>0.1) (Table 3)

Table 3. Kidney, heart and liver weight ratio of animal treated with single dose of C. filiformis defatted ethanolic extract.

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Organ weight ratio ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>Control</td>
<td>0.68±0.039</td>
</tr>
<tr>
<td>25</td>
<td>0.78±0.175</td>
</tr>
<tr>
<td>100</td>
<td>0.67±0.000</td>
</tr>
<tr>
<td>400</td>
<td>0.72±0.000</td>
</tr>
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</table>

DISCUSSION
Evaluating the LD$_{50}$ of a compound makes it possible to determine its therapeutic margin and the margin between its effective and toxic dose. Compound with a wide margin of safety allows it to be further developed. Therefore, a compound can be classified as very toxic, toxic, medium toxic, less toxic and non-toxic (Frank et. al., 1995).
The defatted ethanolic extract of *C. filiformis* can be categorized as toxic material, with the 24 hours LD$_{50}$ of less than 1 g. This finding is not in agreement with one reported earlier by Babayi *et. al.* (2007), who described that the water extract of this plan is non-toxic. The difference between these findings is easy to understand, since the different solvent in the extraction process will produce different contents of active compounds. In this situation, ethanol is a good solvent which can solve almost all kind of chemicals available in nature, while water can only solve very limited amount of compounds. These give impact to their different pharmacological effectiveness to the individuals. (Tomsone *et. al.*, 2012)

A decrease of motoric activity, alteration of respiration and diarrhea are associated with the parasympathetic/sympatholytic activities of the compounds contained in the extract. As already described in the most of pharmacological books, stimulation of parasympathetic nerve will produced several effects, such as vasodilation, diarrhea, muscle relaxation, asphyxia etc. (Goodman & Gilman's, 2011). This finding is in agreement with Babayi (2007), who found that water extract of *C. filiformis* also proceed similar effect as seen in this study. In addition, parasympathomimetic effect that manifested in this research are vasodilation and antihypertension effects that were reported by Chuakul *et. al.*, (2000), Tsai *et. al.* (2008) and Yuliandra, *et. al.* (2013).

Delayed toxicity discovered in this study is associated with the availability of active metabolite compound(s) of extract which remain in the animals (Donatus, 2000). Decreased body weight seen in this study is associated with a decrease of food and water intakes. Our previous study also described similar result (Fitri, 2011). This may be due to satiety center depression in the hypothalamus (Guyton, 2006) due to extract administration.

The decrease of food and water intake of the animal found in this study, especially at hight dose of *C. filiformis* extract, occurred only 2 or 3 days after extract administration. This may be related to muscle relaxation effect due to parasympathetic nerve activation as described above. Furthermore, when the extract has been eliminated from the body, the food and water intakes of the animal returned to normal, except on those treated with the high dose of the extract, where these two parameters remain low, leading to decreased body weight of the animals.

The weight ratio of vital organs, such as liver, kidney and the heart of the animal treated with a single high dose of *C. filiformis* extract were not significantly changed even though they
seemed to increase. These may be due to a reduction of animal body weight, since organ ratio is calculated by dividing organs weight with the body weight of the animal. Nevertheless, further study is needed to evaluate the histology and functions of these vital organs under the influence of the extract, both in acute and sub-chronical administrations.

From the above information, it can be concluded that the defatted ethanolic extract of C. filiformis is toxic and exhibit some delayed toxicity to cardiovascular, respiratory and gastrointestinal system. This extract also proceed a delayed toxicity indicated as a decrease in animal body weight, food and water intake significantly (p<0.01), but do not influence the weight ratio of liver, heart, and kidney significantly (p>0.1).

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REFERENCES


