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Toxicological Evaluation of Syrup from *Rhizophora mucronata* Hypocotyls Ethanolic Extract in Sprague–Dawley Rats

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Abstract

Rhizophora mucronata hypocotyls extract is known to be a new antioxidant and hepatoprotector supplement syrup. Thus, its toxicological profile needs to be studied. At present, the syrup from *R. mucronata* hypocotyls ethanolic extract was evaluated for the 28 day repeated dose (subacute) toxicological evaluation. The *Sprague dawley* rats were treated with with three dose (15, 105, and 735 mg/kg bw) syrup and administered orally for a period of 28 days in rats. The effects on body weight and clinical biochemistry were studied. There were no significant differences in the body weight between control and treated animals. There were no significant changes that occurred in the blood chemistry analysis including total bilirubin, blood urea nirogen, creatinine, SGPT, and SGOT in experimental animals. The syrup from *R. mucronata* hypocotyls ethanolic extract was found safe in 28 day repeated dose toxicity studies.

Keywords: *Rhizophora mucronata, sub-acute toxicity, syrup.*

1. Introduction

Indonesia is ranked second for having the widest of mangrove forests (5.5 million hectare in the 2011) in the world after Brazil (State Minister for The Environment 2013). Traditionally, the mangrove fruits and some edible plant parts are commonly eaten fresh or cooked as vegetable supports for main dishes and also served as one of the ingredients in local products include fruit juice, syrup, sugar, and vinegar. Mangrove species with medicinal properties are also harvested as herbal remedies by coastal communities in some countries (Baba *et al*. 2013).

Rhizophora mucronata is one of mangrove species in Indonesia, belongs to the genus *Rhizophora* spp. and it is commonly known as bakau hitam in Indonesia. The extract of *R. mucronata* reported to have anti-diabetic activity in *Long Evans* rats (500 mg/kg bw) (Haque *et al*. 2013); anti-bacterial activity in *S. aureus* and *E. Coli* bacteria (Nurdiani *et al*. 2012) and potential use in the treatment of gastrointestinal motility such as diarrhea with the most effective concentration was 0.30% (Puspitasari *et al*. 2012). Ethanolic extract of *R. mucronata* hypocotyls has hepatoprotective effect of carbon tetrachloride (CCL_4)-induced hepatotoxicity in Sprague Dawley rats (Sukarno 2014). It is also showed strong antioxidant activity with IC_{50} = 0.7201 ppm. The phytochemicals assay showed that extract contained flavonoids, tannins, hydroquinone, and saponin (Purwaningsih *et al*. 2014).

However, *R. mucronata* extract has bitter taste, so it is not preferred by consumers. To overcome that, it can be preserved as a supplement syrup product in order to obtain a more acceptable flavor and taste. The oral use of liquid pharmaceuticals like syrup was generally ease of administration to those individuals who have difficulty in swallowing solid dosage forms. In spite of the widespread efficacy of *R. mucronata* hypocotyls extract, evaluation of its toxic effects for selecting a safe dosage is lacking. Therefore, the present study was undertaken to provide data on the safety of syrup from *R. mucronata* hypocotyls ethanolic extract on the 28-day (4 weeks) subacute toxicity study of given orally to Sprague Dawley rats.

2. Materials and Methods

2.1 Plant Material

Hypocotyls of *R. mucronata* were collected from Untung Jawa Island, Jakarta, Indonesia. The material was determine in the Herbarium Bogoriense Indonesian Institute of Sciences (LIPI) , Bogor.

2.2 Preparation of syrup from R. mucronata extract

The ethanolic extract of *R. mucronata* was prepared by adding the crushed plant hypocotyls with ethanol 96% as solvent (ratio sample : solvent = 1:5) for 24 hours at room temperature and stirred it at 175 rpm in orbital shaker. The extract was separated by filtration and concentrated on rotavapour at 70 °C.

The syrup from *R. mucronata* hypocotyls ethanolic extract was prepared by adding sucrose (64 g) to purified water and heated until it dissolved with occasional stirring, then cooled at room temperature. The ethanolic extract of *R. mucronata* hypocotyls (0.15 g) was added to the above mixture. Sufficient boiling water was added to produce 100 ml syrup.

2.3 Experimental Animals

Adult male rats *Sprague dawley* strain (180-220 g) were obtained from Veterinary Research Institute (BALITVET), Indonesia. These rats were fed with rat feed (Indofeeds, Indonesia) and water ad libitum. All animals were acclimatized to laboratory conditions for 7 days before the experiments were started. The animals were maintained under standard laboratory conditions, housed under conditions of 12-h day/night cycle with controlled temperature (26 ± 2°C).

2.4 Experimental Design

Sprague dawley male rats (180-220g) were randomly divided into 4 groups (n=3). Rats were given with syrupus simplex (control group) and different concentration of syrup from *R. mucronata* hypocotyls ethanolic extract 15, 105, 735 mg/kg bw respectively, for 28 days. Body weight of all rats were measured on day -7, 0, 7, 14, 21, 28. The experimental protocols

were approved by Animal Care and Use Committee, Verterinary Teaching Hospital, Bogor Agricultural University (01-2014 RSH-IPB).

2.5 Clinical Biochemistry

On day 29, all surviving animals were anesthetized for blood collection from the right ventricle. Blood samples were collected into tubes. The serum was separated from blood with centrifuge and was assayed for total bilirubin, blood urea nitrogen, creatinine, serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT) using an autoanalyzer (Clinical Chemistry Analyzer Selectra Yunior 69.154).

2.6 Statistical Analysis

Statistical evaluation was performed using SPSS version 16. All results are presented as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test. p value <0.05 were regarded as significant.

3. Results and Discussions

To determine the safety of plant products for human use, toxicological evaluation is carried out various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in human (Sateesh & Addepalli 2009). Ethanolic extract of *R.mucronata* hypocotyls may produce several biological activities in humans. Nevertheless, there were no scientific reports abotu the safety of the syrup from *R. mucronata* hypocotyls ethanolic extract in long term administration

3.1 Body Weight

In the repeated dose toxicity study, the syrup from *R. mucronata* hypocotyls ethanolic extract was given orally at doses up to 735 mg/kg bw in rats. There was no significant difference in body weight between control and the treated group (Table 1). The changes in body weight were used as an indicator of adverse effects of drug and chemicals (Safithri *et al*. 2012). The present results suggest that at the dose levels administered the syrup from *R. mucronata* hypocotyls ethanolic extract is non-toxic in rats. Another plant part of *R. mucronata*, the methanolic leaf extract of *Rhizophora mucronata*, showed that no significant difference in the body weight gain between control and treated group was observed after 28 days of treatment (Suganthy *et al*. 2013).

Table 1. The effect of 28 days treatment with syrup from *R. mucronata* hypocotyls ethanolic extract on rats body weight

Day	Avarage body weight (g)			
	Control	Syrup from *Rhizophora mucronata* hypocotyls extract		
	Syrupus simplex	15 mg/kg bw	105 mg/kg bw	735 mg/kg bw
-7	203.9 ± 8.3[a]	202.5 ± 12.2[a]	196.9 ± 6.5 [a]	210.8 ± 12.8 [a]
0	216.4 ± 9.1[a]	230.9 ± 10.3[a]	213.7 ± 18.7 [a]	223.5 ± 15.4 [a]
7	225.2 ± 8.9[a]	239.7 ± 7.4[a]	227.5 ± 13.0 [a]	238.4 ± 19.0 [a]
14	236.0 ± 8.8[a]	248.1 ± 7.6[a]	239.6 ± 12.0 [a]	248.5 ± 21.2 [a]
21	240.9 ± 9.8[a]	250.0 ± 6.9[a]	243.9 ± 9.2 [a]	253.8 ± 17.5 [a]
28	248.3 ± 7.9[a]	260.5 ± 5.9[a]	256.5 ± 13.8 [a]	261.1 ± 16.0 [a]

The same superscript letters indicated not significant differences (p > 0.05) in the same colom.

Table 2.The effect of 28 days treatment with syrup from *R. mucronata* hypocotyls ethanolic extract on rats biochemical parameters

Biochemichal parameters	Control	Syrup from *Rhizophora mucronata* hypocotyls extract		
	Syrupus simplex	15 mg/kg bw	105 mg/kg bw	735 mg/kg bw
SGPT (U/L)	87.33 ± 25.17 [a]	91.33 ± 10.69 [a]	71.33 ± 7.09 [a]	73.67 ± 7.02 [a]
SGOT (U/L)	200.33 ± 57.01 [a]	143.67 ± 9.24 [a]	166.67 ± 42.77 [a]	167.00 ± 23.43 [a]
Creatinine (mg/dl)	0.65 ± 0.17 [a]	0.65 ± 0.03 [a]	0.75 ± 0.13 [a]	0.56 ± 0.13 [a]
Blood urea nitrogen (mg/dl)	38.00 ± 4.58 [a]	36.33 ± 6.03 [a]	34.67 ± 0.58 [a]	32.33 ± 2.52 [a]
Total Bilirubin (mg/dl)	0.12 ± 0.06 [a]	0.13 ± 0.02 [a]	0.09 ± 0.06 [a]	0.09 ± 0.05 [a]

The same superscript letters indicated not significant differences (p > 0.05) in the same colom.

3.2 Clinical Biochemistry

The effect of repeated dose oral administration of syrup from *Rhizophora mucronata* hypocotyls ethanolic extract on biochemichal parameters is presented in Table 2. There were no significant differences observed in any of the biochemical parameters examined in either the control or treated group. However the value of biochemical parameters of both control and treated groups were within the normal reference range of animal used (Han *et al*. 2010; Petterino& Storino 2006).

The transaminases (SGOT and SGPT) are well-known enzymes used as good indicators of liver function and as biomarkers predicting possible toxicity. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood. SGOT found in the serum of both mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum (Gautam *et al*. 2012). SGOT and SGPT plasma level remained normal after administration of syrup from *R. mucronata* hypocotyls ethanolic extract for 28 days. This result represents a normal liver function with no impairment.

Renal function markers like urea and creatinine plasma level (Safithri *et al*. 2012) remained normal after administration of syrup from *R. mucronata* hypocotyls extract at all selected dose levels. Thus it can be stated that syrup from *R. mucronata* hypocotyls ethanolic extract does not show any renal toxicity.

Bilirubin is regarded as a member of the antioxidant family, even though it is known to have toxic effects at high concentrations. Bilirubin can act in

vivo as efficient scavenger of ROS and that bilirubin plays a key physiological role in cytoprotection against oxidant-mediated damage. In plasma, bilirubin can act synergically with vitamin E to protect lipid membranes from the peroxidation initiated within the lipid phase (Berrahal *et al*. 2007). Total bilirubin plasma level remained normal after administration of syrup from *R. mucronata* hypocotyls ethanolic extract for 28 days.

4. Conclusion

In conclusion, the oral dose tested of the syrup from *R. mucronata* hypocotyls ethanolic extract on administered for 28 consecutive days did not induce any biochemical, signs of toxicity and well tolerated in Sprague Dawley rats. Overall, this study provides valuable data on the subacute toxicity profile of syrup from *R. mucronata* hypocotyls ethanolic extract that should be useful for the planning of future preclinical and clinical studies of the formulation.

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