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ANTIHEPATOTOXIC ACTIVITY AND TOXICITY OF ETHANOLIC EXTRACT FROM STEM BARK OF HOPEA MENGARAWAN

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Abstract: The antihepatotoxic activity and toxicity of ethanolic extract from stem bark of Hopea mengarawan were investigated. The in vivo antihepatotoxic activity test was performed by using male rats induced by CCl4. The levels of liver damage were evaluated by microscopic analysis and quantified based on the concentration of GPT from blood serum comparing with control group (without CCl4 and test sample). Treatment with a dose of 10, 30, and 50 mg/kg body weight by oral administration (p.o.) showed to be active with 30, 68.4 and 93.5%, respectively. Further, the acute and subchronic toxicity of this extract on male mice and Swiss rats were investigated and LD50 on mice was 2.290 mg/kg body weight, indicating that extract can be categorized as "little toxic". For subchronic toxicity, the test was carried out by giving the extract to male rats by p.o. for 3 months continuously as well as the body weight and feed consumption were observed every week, including behavior appearance and all abnormality, SGPT, histopatologis test of rat organs. The results showed all rats still alive and there were no significant differences between experiment and control groups.

Introduction

Antihepatotoxic activity test *in vivo* of aceton extract of stem bark of *H. odorata. H. mengarawan* and *H. nigra* can degrease concentration of SGPT (Serum Glutamat Piruvat Transaminase) of rats, that induced by CCl_4 and also eliminate of necrocis of the lever [1-3]. In that research have be known five substances as antihepatotoxic actvity, that were balanocarpol (1), heimiol A (2), vaticanol B (3), and vaticanol G (4)[1]. The extract of stem bark of plant *Hopea*, specially *H. mengarawan* can be develop to phytopharmaca which can be used as a new drug of antihepatotoxic. The antihepatotoxic must be safe, high quality, and high efficacy. That extract must be tested as antihepatotoxic activity test, and toxicity test (acute and subcronic toxicity test). That activity test can be used as the basic to clinical test at human. In this research will be studied the antihepatotoxic activity and toxicity of ethanolic extract from stem bark of *Hopea mengarawan*

Materials and Methods

The Antihepatotoxic Activity

The antihepatotoxic activity test conducted by using male rat of Wistar strain, with the age around 7 weeks, and the body weight around 118-124gram. Every cage contain 6 rats. The rat feed are E22-FT (water 12%, protein 61%, lipid 13.5%, fiber 5%, ash 6.5%, calcium 1.1%, and phosphor 0.9 %, and drink water from water pump. The test was carried out by giving the extract to male rats by p.o, with a dose of 10, 30, and 50 mg/kg body weight. The treatment at each group of the rats (6 rats in each group) can be seen at table 1.

Table 1. The treatment at each group of the rats

Group	Treatment				
	First day	Secound day	Third day	Fourth day	Fifth day
I	Na-CMC 0,5%	Na-CMC 0,5%	Na-CMC 0,5%	Na-CMC 0,5%	Blood and liver
II	Na-CMC 0,5%	CCl_4 25%	CCl_4 25%	Na-CMC 0,5%	
III	Extrct 10 mg/kg bw	Extrct 10 mg/kg bw + CCl_4 25%	Extrct 10 mg/kg bw + CCl_4 25%	Extrct 10 mg/kg bw	
IV	Extrct 30 mg/kg bw	Extrct 30 mg/kg bw + CCl_4 25%	Extrct 30 mg/kg bw + CCl_4 25%	Extrct 30 mg/kg bw	
V	Extrct k 50 mg/kg bw	Extrct 50 mg/kg bw + CCl_4 25%	Extrct 50 mg/kg bw + CCl_4 25%	Extrct 50 mg/kg wb	

The Acute Toxicity test

The acute toxicity test conducted by using male mice of Swiss strain with the age around 2-3 months, healthy and the body weight around 30-32 g. The mice used in this research is adapted environmentally at the research place

To determine the variation of dose require to be conducted by orientation test to know highest dose is which technically admit of passed to a animal test, while lowest dose is minimum dose which can generate effect. Dose got from orientation used as a highest dose, after that searched by the its fold factor and determined its variation dose to each group.

In this research, the extract gift to mice by p.o and [illegible] dose, (the dose killing the mice less

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than 10%, 50%, and more than 90%). The observation conducted by during 14 days, covering autonomic, behavioral, sensoric, neuromuscular, cardiovasculer, inhalation, eye, gastrointestinal, and skin. histopatologis test of rat organs at the end of the research.

The subcronic toxicity test :

The subcronic toxicity test of ethanol extract *H. mengarawan* are conducted to male rats wistar strain, age of 2-3 month, body weight around 150-200 g. For subchronic toxicity, the test was carried out by giving the extract to male rats by p.o. for 3 months continuously as well as the body weight and feed consumption were observed every week, including behavior appearance and all abnormality, SGPT, histopatologis test of rat organs. In this research selected three doses, namely high enough dose to generate sign toxicity, but insufficient high to kill a large part of that animals, low dose expected not give toxic effect.

The treatment of the each group of the rat (10 rats in each group):

Group I : (control group) 1% NaCMC
Group II : dose of extract 75 mg/kg bw
Group III : dose of extract 150 mg/kg bw
Group IV : dose of extract 300 mg/kg bw

Results and Discussion

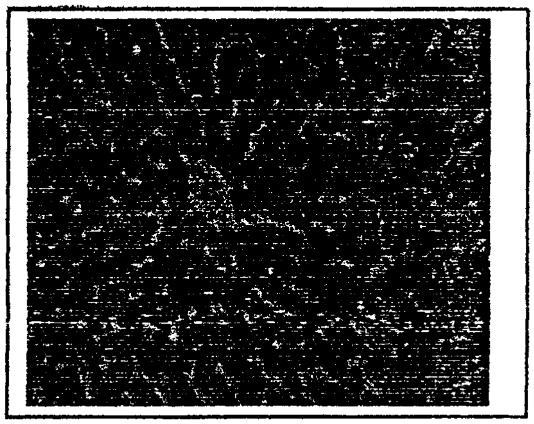
Antihepatotoxic activity

At the fifth day, the blood and the liver have been taken for SGPT and microscopic analysis (table 2).

Table 2. Concentration of SGPT and Histopatologis

Gro-up	Treat-ment	Concen-tration SGPT (U/l)	% Antihepa-totoxic activity	Histopa-tology	Degree of damage
I	Na-CMC	46 ± 0,1	-	Normal	-
II	CCl_4 + Na-CMC	4004 ± 77,4	-	N 14, D>>	+++
III	CCl_4 + Na-CMC + extract 10 mg/kg BW	293,7 ± 16,1	30 %	D>>	++
IV	CCl_4 + Na-CMC + extract 30 mg/kg BW	158,1 ± 13,4	68,4 %	D> less	+
V	CCl_4 + Na-CMC + extract 50 mg/kg BW	69,0 ± 0,25	93,5 %	D< less	+

There were antihepatotoxic activity of the extract ethanol *H. Mengarawan*, 30 % from dose extract 10 mg/kg bw, 68.4% from 30 mg/kg bw, and 93.5% from dose 50 mg/kg bw. The variation of the dose extract could be inhibition of necrosis centrolobuler (NC), degeneration of lipid (D) (figure 1).



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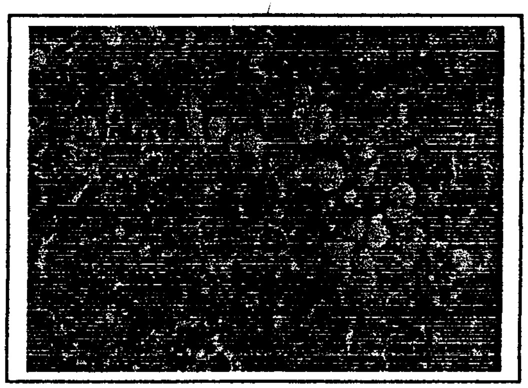

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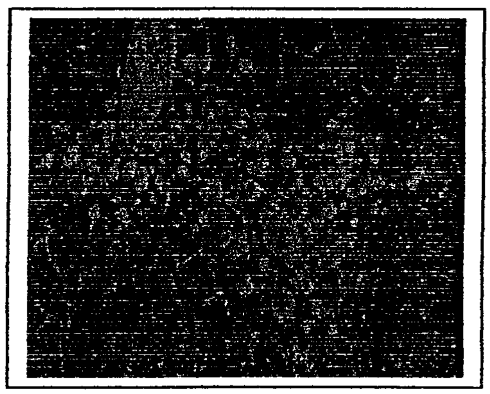

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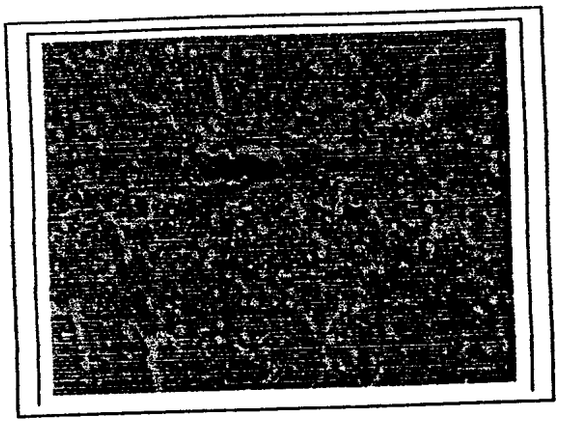

(d)

Figure1. Histopatology microscopic analysis of rat lever in each group

(a) control negative (CMC-Na); (b) control positive (CMC-Na and CCl_4); (c) treatment (CMC-Na , CCl_4, extract dose 10 mg/kg bw; (d) treatment (CMC-Na, CCl_4, extract dose 30 mg/kg bw

The Acute toxicity test

The variation of the dose of the acute toxicity test were 1,670 mg/kg bw; 2,460 mg/kg bw; 3,623 mg/kg bw; and 5,337 mg/kg bw. Observation conducted by during 14 days. The data of the death mice could be seen in table 3.

Table 3. Data of the death mice in acute toxicity test

Group	Dose mg/kg bw	Amount of mice	Amount of death	% of death	Probit
control	0,75 ml CMC-Na	6	0	0	-
D1	1,670	6	1	16	4.01
D2	2,460	6	4	66.67	5.43
D3	3,623	6	5	83.33	5.96
D4	5,337	6	6	100	8.09

From calculated by above tables of LD_{50} with the linear equation regresi (logarithm of dose of vs probit), the equation of regression is, Y = 7.594 X - 20.516, at the value of r = 0.974. From the equation obtained by value of LD_{50} 2,290 mg/ kg bw. The value of LD_{50} indicate that the extract of ethanol of stem bark powder of *H. mengarawan* have the character of a few/little toxic (Loomis, 1978). Observation to mice conducted after extract gift until 14 day, covering body weight, autonomic, behavior, sensoric, neuromusculer, cardiovasculer, inhalation, eye, gastrointestinal, and skin. Analysis of histopatologi conducted to all organ in mice covering heart, liver, right and left kidney, stomach, and spleen. The histopatologis data showed small difference condition at liver, lungs, and spleen but no significant differences between control and experiment group.

The subcronic toxicity test

In general condition, all of mice were like normal behavior. There were no sicnificant differences of general condition between control and experiment group. There were no sicnificant differences of body weight between control and experiment. There were significant differences SGPT between control and experiment. No carcinogenesis in organs of the rats.

Conclusions

The result of the research, the extract ethanol of *H. Mengarawan* with a dose of 10, 30, and 50 mg/kg body weight by oral administration (p.o.) showed to be active as antihepatotoxic activity with 30, 68.4 and 93.5%, respectively. Further, the acute toxicity was investigated LD50 on mice was 2,290 mg/kg bw, indicating that extract can be categorized as "little toxic". For subchronic toxicity, was investigated there were no significant differences between experiment and control groups.

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