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# Proceedings

## International Conference On Medicinal Plants The Future of Medicinal Plants: *From Plant to Medicine*

Surabaya, 21-22 July 2010

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**PROCEEDING OF  
INTERNATIONAL CONFERENCE ON  
MEDICINAL PLANTS**

in occasion of

**the 38<sup>th</sup> Meeting of National Working Group on Indonesian Medicinal Plant**

**21-21 July 2010  
Surabaya, Indonesia**

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in collaboration with  
**National Working Group on Indonesian Medicinal Plants  
and German Academic exchange Service**

## PREFACE

Earth is perfectly made by God for His people to live. It consists of different bodies of land and water where thousands of species of plants and animals can be found. The human race is called to explore this order, to examine it with due care and to make use of it for the benefits of human being. Since very early in human history, people have relied on medicinal plants to cure them of their various ills. This can be partly attributed to the simple yet highly effective forms of traditional medicine. Knowledge of medicinal plants is a part of the Indonesian national heritage known as *jamu*. To facilitate networking, collaboration, exchange of information, experiences and knowledge in the key issues of medicinal plants development, the Faculty of Pharmacy of Widya Mandala Catholic University Surabaya in collaboration with National Working Group on Indonesian Medicinal Plants (POKJANAS TOI) and German Academic Exchange Service (DAAD) held the International Conference on Medicinal Plants on 21-22 July 2010 in Surabaya. The conference provided a evaluation in pharmacology, pharmacognosy, ethnobotany, standardization, cultivation, cell culture and chemistry for medicinal and aromatic plant species. There were over 250 participants, 8 plenary speakers, 101 contributed speakers in oral presentation, and 101 posters presented.

The papers contained in the first volume of the proceeding report the submitted papers on 'The Future of Medicinal Plants: From Plant to Medicine'. Keynote speakers and authors of selected contributed oral and poster presentations were given the opportunity to submit a manuscript for publication.

The conference organizers gratefully acknowledge the financial and other support from the following:

- National Working Group on Indonesian Medicinal Plants (POKJANAS TOI)
- German Academic Exchange Service (DAAD)
- PT. Landson
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- PT. Pasifik Sarana Cantik
- Herbal Plus

I hope that this publication will raise international awareness of the value of medicinal plants in Indonesia and hence makes a contribution towards promoting the proper use of medicinal plants.

Dr.phil.nat. Elisabeth Catherina Widjajakusuma  
Conference Chairman

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## TOXICITY AND TERATOGENIC TESTS OF ETHANOL EXTRACT OF *ARTOCARPUS CHAMPEDEN* STEMBARK

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**Abstract :** *Artocarpus champeden* (family Moraceae), known as "cempedak", is widely distributed in Indonesia and has been traditionally used in malarial remedies. Our previous study revealed that the ethanol extract of *Artocarpus champeden* stembark (EEAC) exhibited potent antimalarial activities against *P. falciparum* *in vitro* and *P. berghei* *in vivo*. Therefore, it is potential to develop EEAC as an antimalarial phytopharmaceutical product. The development of phytopharmaceutical product requires consistency in the efficacy, safety, and effectivity.

This research was conducted to evaluate the safety of EEAC as an active material for antimalaria phytopharmaceutical product. Toxicity test in mice after oral administration was carried out. Acute toxicity test was conducted using the highest dose of 21 g/kg body weight/day. The result showed that EEAC was relatively non toxic. Subacute toxicity test was expressed by the levels of ALT and AST activities in serum. The result showed that EEAC was relatively safe. In addition, there was no significant difference in the observed ALT and AST activities in serum. Histopathological changes due to degeneration and necrosis were observed after 30 days oral administration of EEAC at a dose of 1.90 mg/20 g body weight/day. The teratogenic test was also conducted using the highest dose of 254.80 mg/20 g body weight/day. The result showed that there was no significant morphological deformity of mice fetus at organogenesis phase after 10 days oral administration of EEAC.

**Keywords:** *Artocarpus champeden*, ethanol extract, toxicity test, teratogenic test

### Introduction

*Artocarpus champeden* (family Moraceae), known as "cempedak", is widely distributed in Indonesia and has been traditionally used in malarial remedies (Heyne, 1987). Previous study reported that prenylated stilbene from *Artocarpus integer* (syn *A. champeden*) exhibited antimalarial activities against *P. falciparum* (Boonlaksiri et al., 2000). Our preliminary test revealed that extract from *A. champeden* exhibited potent antimalarial activities against *P. falciparum* *in vitro* and *P. berghei* *in vivo* (Utomo, 2004; Hidayati, 2005; Ernawati, 2005). Several isolated compounds from this plant exhibited antimalarial activities. One of the isolated compound identified as heteroflavon C, a prenylated flavone, have an antimalarial activities higher than chloroquine (Widyawaruyanti et al., 2007<sup>a</sup>). Standarized ethanol extract of *A. champeden* stembark (EEAC) also exhibited potent antimalarial activities against *P. falciparum* *in vitro* and *P. berghei* *in vivo*. Therefore, it is potential to develop EEAC as antimalarial phytopharmaceutical product. The development of phytopharmaceutical product requires consistency in the efficacy, safety, and effectivity (Widyawaruyanti et al., 2007<sup>b</sup>, Widyawaruyanti et al., 2008). Therefore, it is need to study the safety of EEAC.

This research was conducted to evaluate the safety of EEAC as an active material for antimalaria phytopharmaceutical product. Safety test includes acute toxicity, sub acute toxicity and teratogenic test.

### Materials and methods

#### Plant and materials

The stem bark of *A. champeden* were collected from Bogor, West Java, Indonesia. A voucher specimen was identified and deposited at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia.

#### Preparation of extract

Extraction of 1 kg *A. champeden* stem bark with 80% ethanol at 40°C in rotavapor, yielded 74.64 g of crude extract.

#### Acute Toxicity Test

Male Balb-C mice (25-30 g body weight, 2-3 month ages) were used in this experiment. Mice were divided into groups of five mice per group. This test was conducted using the highest dose of 21 g/kg body weight/day that relatively harmless based on toxicity rating (Dorelanko and Holinger, 1995). Each group of mice was treated per orally with EEAC at dose of (D1) 21.00, (D2) 10.50, and (D3) 5.25 g/kg body weight/day respectively for 7 days, and untreated group were given CMC-Na 0.5%.

#### Sub acute toxicity test

Male Balb-C mice (25-30 g body weight, 2-3 month ages) were used in this experiment. This test was conducted using the dose of EEAC that equal to 25.48 mg dried stem bark/20 g body weight/day (1.90 mg EEAC/20g body weight/day). Mice were divided into groups of ten mice per group. Each group of mice was treated per orally with EEAC at a dose of (D1) 1.90, (D2) 9.50, and (D3) 19.00 mg/20g body weight/day respectively for 30 days, while untreated group were given CMC-Na 0.5%. The levels of AST and ALT were determined. Data were analysed using anava  $\alpha$  0.05 and Duncan's Multiple Range Test (DMRT). Macroscopic examination of liver was carried out. Liver were placed in 10% formalin to prepare histological slides. The slides were stained by haematoxylin-eosin and observed. The observation using scoring system as described below. Data were analysed using Kruscal Wallis Test and Z 5% Test (Daniel, 1990).

Table 1. Scoring of histopathological changes of mice liver

Histopathological changes	Score
Normal	0
Low Degeneration (less than 50%)	1
Mid Degeneration (approximately 50%)	2
High Degeneration (more than 50%)	3
Low Necrosis (less than 50%)	1
Mid Necrosis (approximately 50%)	2
High Necrosis (more than 50%)	3

#### Teratogenic Test

Female and male Balb-C mice (25-30 g body weight, 2-3 month ages) were used in this experiment. Mice were divided into groups of eight mice per group. Impregnation was carried out before treatment. Female mice injected intraperitoneally with Pregnant Mare's Serum Gonadotropin Hormon (PMSG) and 48 hours latter, Human Chorionic Gonadotropin Hormon (HCG) was injected. Treated females were caged with untreated males for overnight mating (1 male : 1 female). The presence of copulation plug or sperm in the vaginal smears on the following morning was regarded as pregnancy day 0. Each group of pregnant mice was treated per orally with EEAC at a dose of (D1) 25.48, (D2) 127.40, (D3) 254.80 mg/20g body weight/day respectively for 10 days at organogenesis phase (day 6 until day 15), while untreated group were given CMC-Na 0.5%. All pregnant females were isolated and sacrificed at day 18 of pregnancy, and mice fetuses were observed. Observation includes number of total fetuses, number of alive and dead fetuses,

fetuses that resorbtion in uterus, fetuses weight and sizes, morphological includes head, extremity and tail. Data were analysed using anava  $\alpha$  0.05.

## RESULT AND DISCUSSION

### Acute toxicity test

The result of toxicity test is given in Table 2. This test was conducted using the highest dose of 21 g/kg body weight/day for 7 days and the mortality of mice was observed. The result showed that all mice were alive after treated with EEAC.

**Table 2. Mice mortality after treated with EEAC**

Groups	Number of mice	
	Dead	Alive
control	0	5
D1	0	5
D2	0	5
D3	0	5

### Sub acute toxicity

Subacute toxicity test was expressed by the levels of ALT and AST activities in serum. The result is given in Table 3.

**Table 3. Mice AST and ALT**

Groups	N	AST	ALT
control	1	139	369
	2	130	110
	3	136	61
	4	140	37
	5	102	46
D1	1	151	98
	2	186	94
	3	158	44
	4	126	51
	5	102	40
D2	1	181	52
	2	212	86
	3	264	78
	4	179	61
	5	263	64
D3	1	245	124
	2	227	44
	3	221	49
	4	243	62
	5	285	157

### Data Analysis

Data of AST and ALT were analysed statistically using anava  $\alpha$  0.05 and results are given in table below.

The anava result of AST data showed that there was no statistically different in AST value between groups. Duncan's Multiple Range Test (DMRT) showed that there were statistically different between control, D2 and D3 groups. Mean of AST control group was 129.4 IU/L, D2 = 219.8 IU/L, and D3 = 244.2 IU/L. While normal AST in mice is 70-400 U/L. It means that there was no influence of EEAC at dose D2 and D3 to the level of AST.

The anava result of ALT data showed that there were statistically different in ALT level between groups. It means that there was no influence of EEAC at dose D1, D2, and D3 to the level of ALT.

**Table 4. Mean of AST each groups**

Groups	N	Mean ( IU/L)	Std.deviation
control	5	129.4	15.8
D1	5	144.6	31.9
D2	5	219.8	41.9
D3	5	244.2	25.0

**Table 5. Mean of ALT each group**

Groups	N	Mean ( IU/L)	Std. deviation
control	5	124.6	139.5
D1	5	65.4	28.2
D2	5	68.2	13.6
D3	5	87.2	50.4

**Scoring of mice histopathological changes (liver cell alteration)**

Cell alteration that observed in mice liver obtained from microscopic observation of five different area, scored and processed using rank value. The result is given in table below.

**Table 6 Score of liver cell degeneration**

N	Control	D1	D2	D3
1	0	0	1	2
2	0	1	1	1
3	0	1	2	2
4	0	1	1	2
5	0	1	2	2

**Table 7 Score of liver cell necrosis**

N	Control	D1	D2	D3
1	0	0	1	2
2	0	0	1	1
3	0	1	1	2
4	0	1	1	3
5	0	1	1	2

Observation of histopathological changes was carried out by microscopic evaluation of mice liver after treated with EEAC. Based on observation result showed that there were histopathological changes due to degeneration and necrosis. Scoring data were analysed statistically using Kruskal Wallis test, the result showed that there were significant histopathological changes between treatment groups. Scoring data then analysed using Z test, the result showed that there were significant different due to degeneration and necrosis that occurred between control group and treatment groups, it means that EEAC can caused histopathological changes due to degeneration and necrosis by a dose of 1.90 mg/20g body weight/day for 30 days.

**Teratogenic Test**

This teratogenic test was carried out using female Balb-C mice because of it's estrus phase that relatively short, brief pregnancy time, human resemble reproduction cycle, high fertilization and easy to treated.

The day when copulation plug observed was regarded as day 0. Treatment at day 6 until day 15 was chosen because of its critical period which organogenesis phase was occurred. At that phase, differentiation, mobilization and organization of cells happen intensively. Therefore, treatment of teratogenic material at this phase will be able to observe the morphological changes that might happen. Treatment at day 0 until day 5 was not appropriate because the fission of embryo cell happened fast. The cell damaged because of teratogenic material will be able to be replaced and the teratogenic effect will not be observed.

Pregnancy time of mice usually takes 19 days. Caesarian operation was carried out on day 18 and mice fetus were observed. Observation includes fetus body weight, sizes, morphological changes of head (eyes), tail, extremity (hands and legs) to determine deformity.

Descent of fetus weight and sizes were minor effects of teratogenic agent and became sensitive parameters (Wilson, 1973). Although there were variations in body weight data, but statistically there was no significant difference between control and treatment groups. One of the reproductive and teratogenic toxicity parameters was the descent of fetus size (Lansdown, 1985). Normal fetus sizes showed that there was no gigantism and cretinism caused by material (Djunarko, 2003). Based on ANOVA analysis of fetus sizes, there was no significant difference between control and treatment groups. Observation of morphology includes head (eyes), tail, extremity (hands and legs) showed normal conditions. There were two eyes, and number of fingers (five fingers of hands and five of legs) and there were no deformities such as polydactyly, syndactyly, ectrodactyly, etc. Tail also occurred and there was no extreme deformity. No deformities were found in control and treatment groups. This result indicated that EEAC at the dose used in this study, did not impair reproduction in female mice. Data of teratogenic test is given in table below.

**Table 8. Teratogenic test result**

Groups	Mean (x ± Sd)					
	Total fetus	Dead fetus	Resorption embryo	Deformity	Weights	Sizes
control	9.25 ± 2.49	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.89 ± 0.12	18.68 ± 1.32
D1	9.50 ± 2.07	0.13 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.95 ± 0.09	19.28 ± 1.05
D2	9.13 ± 2.36	0.25 ± 0.46	0.00 ± 0.00	0.00 ± 0.00	0.94 ± 0.17	19.79 ± 2.57
D3	8.00 ± 2.78	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.02 ± 0.37	20.29 ± 3.89

### CONCLUSION

The acute toxicity was conducted using the dose of 21 g/kg body weight for 7 days that is relatively harmless based on toxicity rating. The result showed that there was no mortality occurred and concluded that EEAC was relatively non-toxic. Subacute toxicity test result showed that EEAC was relatively safe and there was no significant difference in the observed ALT and AST activities in serum. Histopathological changes due to low degeneration and low necrosis (less than 50%) were observed after 30 days oral administration of EEAC at a dose of 1.90 mg/20 g body weight/day. Total amount of EEAC used in this study was about 57 mg. It is important to note that 57 mg is high enough, compared to the amount of EEAC used in antimalarial treatment that is about 0.8-8 mg. This result indicated that lower dose was safe thereby confirming the usefulness of EEAC as an antimalarial product. The teratogenic test result showed that there was no significant morphological deformity of mice fetus at organogenesis phase after 10 days oral administration of EEAC at a dose of 254.80 mg/20 g body weight.

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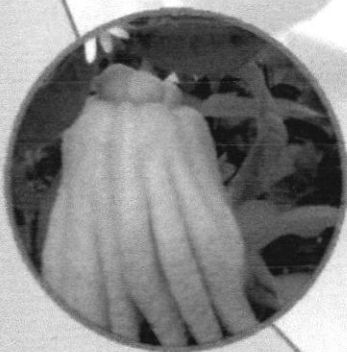
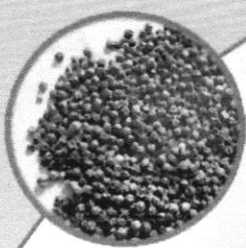
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