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From Plant to Medicine

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PROCEEDING OF INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

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FACULTY OF PHARMACY
WIDYA MANDALA CATHOLIC UNIVERSITY

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 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)
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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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CONTENT

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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 againts *P. falciparum* (Boonlaksiri et al., 2000). Our preliminary test revealed that extract from *A. champeden* exhibited potent antimalarial activities againts *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[a]). Standarized ethanol extract of *A.champeden* stembark (EEAC) also exhibited potent antimalarial activities againts *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[b], 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 stembark 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* stembark 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 devided 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 stembark/20 g body weight/day (1.90 mg EEAC/20g body weight/day). Mice were devided 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 α 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 haematoxoylin-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 devided into groups of eight mice per group. Impregnation was carried out before treatment. Female mice injected intraperitoneally with Pregnant Mare's Serum Gonadrotropin Hormon (PMSG) and 48 hours latter, Human Chorionic Gonadrotropin 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 α 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 α 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/I, D2 = 219.8 IU/I, and D3 = 244.2 IU/I. 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 necrocis

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 necrocis 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 cicle, 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 it's critical periode which organogenesis phase was occured. At that phase, differentiation, mobilization and organization of cells happen intensively. Therefore, treatment of teratogenic material at this phase will able to observe the morphological changes that might be happen. Treatment at day 0 until day 5 was not appropriate because the fission of embrio cell happened fast. The cell damaged becaused by teratogenic material will be able to replaced and the teratogenic effect will be not able to observed.

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

Descent of fetus weight and sizes were minor effect of teratogenic agent and became sensitive parameter (Wilson, 1973). Althought there were variation on body weight data, but statistically there was no significant different between control and treatment groups. One of reproductive and teratogenic toxicity parameter was descent of fetus size (Lansdown, 1985). Normal fetus sizes showed that there was no gigantisme and cretinisme caused by material (Djunarko, 2003). Based on anava analysis of fetus sizes, there was no significant different between control and treatment groups. Observation of morphological includes head (eyes), tail, extremity (hands and legs) showed normal condition. There were two eyes, and number of fingers (five fingers of hands and five of legs) and there were no deformity such as polidactily, sindactily, ectrodactily, etc. Tail also occurred and there was no extreme deformity. No deformity 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	Resorbsion embrio	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 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 about 0.8-8 mg. This result indicated that lower dose was safe thereby confirming the usefulness of EEAC as 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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Sehubungan dengan adanya International Conference On Medicinal Plants The Future of Medicinal Plants : Form Plant to Medicine pada tanggal 21 – 22 Juli 2010 di Fakultas Farmasi Universitas Widya Mandala, dengan ini Wakil Dekan II Fakultas Farmasi Universitas Airlangga menugaskan :

- Dr. Aty Widyawaruyanti,MSi NIP. 19620426 199002 2 001
- Dr. Achmad Fuad H.MS NIP. 19521212 198103 1 009

Untuk menghadiri acara tersebut.

Demikian Surat Penugasan ini untuk dilaksanakan dengan baik dan penuh tanggung jawab.

Surabaya, 20 Juli 2010

Dekan
a.n Wakil Dekan ,

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