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 champedan* stembark (EEAC) exhibited potent antimalarial activity against *F.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 antimalarial 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 nontoxic. Subacute toxicity test was expressed by the levels of ALT and AST activities in serum. The results 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 degradation add necrosic 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 l0 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,2005l Emawati,2O05). 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). 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.,2007b, Widyawaruyanti et al., 2000). 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 antimalarial phytopharmaceutical product. Safety test includes acute toxicity, subacute 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 40° in rotavapor, yielded 74.64 g of crude extract.

Acute Toxicity Test

Male Balb/c mice (25-30 g body weight, 2-3 months 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 (Dorelenko and Holinger, 1995). Each group of mice was treated per orally with EEAC at dose of (D1) 21.00, (D2) 10.50, and (3) 5.25 g/kg body weight/day respectively for 7 days, and untreated group was given CMC-No 0.5%.

Subacute 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 divided into group 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 was given CMC-Na 0.5%. The levels of AST and ALT were determined. Data were analyzed using anava α

0.05 and Duncan's Multiple Range Test (DMRT). Microscopic examination of liver was carried out. Liver was placed in l0% formalin to prepare histological slide. 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).

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 group 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 later. Human Chorionic Gonadrotropin Hormon (HCG) was injected. Treated females were caged with untreated male for overnight mating (l male : 1 female). The presence of copulation plug or sperm in the vaginal smear on the following morning was regarded as pregnancy day 0. Bach group of pregnant mice was treated per orally with EEAC at a dose of (Dl) 25.48; (D2) 127.40; (D3) 254.80 mg/20g body weight per day respectively for l0 days at organogenesis phase (day 5 until day l5), while untreated group were given CMC-Na 0.5%. All pregnant females were isolated and sacrificed at day t8 of pregnancy, and mice fetuses were observed. Observation includes number of total fetus, number of alive and dead fetuses, fetuses that resorption in uterus, fetuses weight and sizes, morphological includes head extremity and tail. Data were analyzed using anava α 0.05.

RESULT AND DISCUSSION

Acute toxicity test

The result of toxicity test is given in Table 2. This test was concluded 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.

Subacute toxicity

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

Data Analysis

Data of AST and ALT were analyzed 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 Dl, D2, and D3 to the level of ALT.

Scoring of mice histopathological chances (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.

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 analyzed statistically using Kruskal Wallis test, the result showed that there were significant histopathological changes between treatment groups. Scoring data then analyzed 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 histopatological 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. Treament at day 6 until day 15 was chosen because of it's critical periode where organogenesis phase was occured. At that phase, differentiation, mobilization snd organization of cells happen intensively. Therefore, treatment of teratogenic material al 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 (hand and legs) to determine 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 size showed that there was no gigantisme and cretinisme caused by material (Djunarto, 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, extrimity (hands and legs) showed normal condition. There were two eyes, and number of finger (five fingers of hands and five of legs) and there were no deformity such as polidactily, sindactily, ectrodoctily, 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.

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 nontoxic. 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 l0 days oral administration of EEAC at a dose of 254.80 mg/20 g body weight.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7