Journal of Chinese Pharmaceutical Sciences http://www.jcps.ac.cn A subchronic toxicity test of 5

ethyl acetate extract

from endophytic fungus Penicillium sp. of kunyit putih (Curcuma zedoaria) 3

against Swiss albino mice Muharni*, Heni Yohandini

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Abstract: Ethyl acetate extract of endophytic fungus from Penicillium sp. of kunyit putih showed antibacterial activity in vivo but no acute toxicity. However, the extract

may have toxic effects on major organs for long-term consumption. This study was carried out in order to test sub-chronic toxicity of the 1

ethyl acetate from endophytic fungus Penicillium sp. of kunyit putih against mice (Mus musculus). A total of 50 male mice

were divided into five groups. Groups I, II, III and IV were orally administered with ethyl acetate extracts of 250, 500, 1000 and 2000 mg/kg body 1
weight (BW), respectively. Group V was used as a control without extract treatment. A toxic symptom was observed by analyzing several parameters,
namely change in BW, hematologic and

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sdn1Tepg2thh..uo3eesKx–ary0rPucet8-ens2wuny'liihecttsiiyglpdlshiuhrtutoomibhxwe.ystewpT-d.5eho'teemhfnakecttuotrhtenmhayyetpilret-oed2pu'wuan,3ntdai'dhss- n in almost all parts of the plant, which are able to w w w

dihydrofuran-3'-il (hydroxy) methyl-4-isopropyl-3- methyl-2- piran -2-on 3

and bis-ethylhexilphtalat[7]. Both produce certain secondary metabolites with their host plants[1,2]. For this reason, endophytic fungus is used as compounds were tested for anti-bacterial activity against source to search new bioactive compounds[3]. Several E. Coli (ATCC 25922), S. dysentriae, S.

aureus (ATCC 25923) and B. subtilis (ATCC 6633) and 9

displayed bacterial authors have reported that endophytic fungi possess unique ability to produce secondary metabolite compounds activity among all tested bacteria[8]. Active extract of that have high biological activity, which are even more Penicillium sp. had also antibacterial activity in vivo by mice active than compounds produced by their host plants[4]. (Mus muscullus). The lab mice were induced by diarrhea In addition, active compounds also have a unique basic bactery (E. coli and S. dysentriae) prior to the activity test. framework that is not related to the biosynthesis of Result showed extract had active antidiarrhea in vivo with its host compounds[5,6]. effective dose at 250 mg/kg·BW. This dose was equal to In our two previous works, two compounds have positive control of ciprolaxacin for S. dysentriae and been successfully isolated from ethyl acetate extract of chloramphenicol for S. typii at 10 mg/kg·BW. Acute toxicity test has indicated that the extract does not possess Received: 2017-11-13, Revised: 2017-12-20, Accepted: 2018-01-12. the acute toxic effects[9]. In this work, we studied subchronic

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toxicity test of ethyl acetate extract endophytic fungus http://dx.doi.org/10.5246/jcps.2018.02.014 Kunyit putih against mice (Mus muscullus). 2. Material and methods Health Guide

for the Care and Use of Laboratory Animals[13]. **2.** 1. Collection **of** 6

test endophytic fungi Toxic symptoms were observed by means of body Endophytic fungi Penicillium sp was collected and weight (BW) analysis, hematologic and biochemical prepared according to our previous study[7]. parameters, and macroscopic organs.

The behavior and motoric activity of the test **animals were monitored** 1

2.2. Preparation of ethyl acetate extract from

every day, and their BW was measured every 1

15 days. endophytic fungus Penicillium sp. After 30 and 90 d, three mice from each group were sacrificed. The mice were deprived of pellet food and Ethyl acetate extracts were prepared as previously reported[7,10,11]. only given

free access to drinking water at **24** 2

h prior to sacrifice.

Blood samples were collected through cardiac 2

2.3. Experiment animals puncture for haematological and biochemical analyses.
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out in accordance with current guidelines for the care 1

w w treated mice was then compared with control group[14]. of laboratory animals. 2.5. Statistical analysis 2.4. Subchronic toxicity test using mice (Mus muscullus) w Data analysis was performed to evaluate differences among groups using one-way analysis of variance, Briefly, 50 male swiss albino mice (3-month-old)

followed by Duncan New Multiple Range Test (DNMRT). 11

with BW of 20–25 g were evenly divided into five

The significance level was set at 5% (P<0.05). 1

groups. Four groups were treated with the extracts of ethyl acetate from endophytic fungus Penicillium sp. of kunyit putih, and the last one was the control group 3. Results without treatment. Groups I–IV were treated with ethyl 3.1. Effect of the extract on motoric activity and BW acetate extract at a

dose of 250 mg/kg·BW, 500 mg/kg·BW, 1000 **mg/kg·BW** and 2000 **mg/kg·BW,** 4

respectively. Subchronic

toxicity test was used **to evaluate** effects **The** 16

treatment was given by oral administration once a of

repeated or continuous administration of a test day **for** 1

90 d[12]. During the treatment, the mice were fed material on animals. Here, we first analyzed the with normal food and drinking water. All procedures motoric activity and BW of mice to observe treatment in the study were performed according to Ministry of effects (Tables 1 and 2). Muharni. et al. / J. Chin. Pharm. Sci. 2018, 27 (2), 123–130 3.2.

Effect of the extract on biochemical **parameters** 2

3.3.

Effect of the extract on haematological **parameters** Table 3 presents **the effect of** subchronic **administration of** ethyl acetate **extract** 2

from endophytic fungus Penicillium sp. on biochemical parameters (SGOT and SGPT). Biochemical parameters (SGOT and SGPT) showed abnormal values for the treatment group. Table 4

shows the effects of ethyl acetate **extract on the hematological parameters** (Hb) **in** subchronic **study.** 2

Hb parameter

showed no significant changes and remained within physiological range after the 90 **-day treatment period.** Table 1. **The** 2

toxic symptoms of tested mice after extract injection. Group Treatment extract dose (mg/kg·BW) Number of rats Motoric activity I 250 10 Normal II 500 10 Normal III 1,000 10 Normal IV 2,000 10 n Normal BTBWWaGb::rIIIlBoBIIVeluoo2pdd.yyEwwfefeTieiggcrhhettta..otmfeenthtyexltara12Ccce2500ot5000tnad0000ttoreoselex(mtrga/cktgf·BroWm) endo22222p23323h.....055553it±±±±±(iC11111c.....o27055fn55000u.tnrogl)ujs22222P21213e.....88393nc1±±±±±i5c21111i.....l85505l00500iumpsp22222.22114o.....n092113±±±± ±0Bs21121.....W0755005000ch.anBg22222Wea54625s.....121022(04ig±±±±±n5)11111d.....m75757a00055yiccse.2222244425.....397846±±±± ±022211c.....50075000502222244424.....000057±±±±±511121N.....55707o00505rmal V 0 90 Table 3. SGPT and SGOT levels for mice. w I 23.5±0.75 23.5±1.50 23.5±1.55 22.2±1.75 V w w 24.0±1.50 Group Treatment extract dose (mg/kg·BW) SGOT level (U/l) SGPT level (U/l) Day-31 Day-91 Day-31 Day-91 1 250 162±17.35b 211±11.53b 2 500 198±3.46c 351±11.00c 3 1000 251±2.65d 354±11.53c 4 2000 267±4.36e 374±5.29d 5 Control 69±2.65a 72±4.00a Note: Different superscript (a, b, c, d, e) in the same column indicate significant difference on Duncan test at level 5%. 68±4.58b 89±5.57b 103±4.36c 136±4.00c 156±4.00d 176±5.29d 173±2.65e 189±3.61e 46±1.73a 44±2.00a Table 4. The results of hematologic evaluation. Group Treatment extract dose (mg/kg·BW) Hb level (g/dL) Day-31 Day-91 1 250 10.6±0.10ab 2 500 11.9±0.36b 3 1000 12.9±0.53b 4 2000 12.2±0.35b 5 Control 12.2±1.57a 11.9±0.53ab 10.6±0.53bc 12.6±0.44cd 9.9±0.53e 11.2±0.52a Note: Different superscript (a, b, c, d, e) in the same column indicate significant difference on Duncan test at level 5%. Table 5. ROWs of tested and control mice. Dose of treatment (mg/kg·BW)

ROW of liver (g) **ROW of heart** (g) **ROW of kidney** 1

(g) Day-31 Day-91 Day-31 Day-91 Day-31 Day-91 0 2.362±0.15a 2.615±0.25a 0.170±0.03a 0.235±0.05a 0.505±0.06a 0.634±0.08a 250 2.217±0.20ab 2.474±0.12b 0.187±0.02a 0.287±0.03a 0.594±0.11a 0.594±0.07a 500 2.032±0.09bc 2.274±0.06b 0.146±0.01a 0.180±0.01a 0.523±0.84a 0.656±0.03b 1000 1.796±0.18cd 2.171±0.15bc 0.132±0.01a 0.132±0.01b 0.413±0.08a 0.413±0.05b 2000 1.711±0.15d 1.796±0.22c 0.141±0.073a 0.141±0.01c 0.465±0.04a 0.465±0.04b Note: Different superscript (a, b, c, d) in the same column indicate significant difference on Duncan test at level 5%. 3.4. ROWs and macroscopic test

Evaluation of blood parameters can be used to determine the levels **of negative effect of foreign** 2

Macroscopic test was conducted on vital organ compounds[17–19]. Evaluation of hematologycal and (liver, heart and kidney) to evaluate how extract toxicity changed the structure of test animal's organ[15]. There biochemical parameters was conducted to observe liver wbtdk4heii.arfdAtDofsweneufeiretsgaeyeechnrun(oscT1sieusg-atihtnbohwilnettfehariecse5aae)ton.xtmrbtterseaaedntcritmvftfoeeefdnrterttehinnaepcteeeterdhtihieonydawl,tnahedaceingedhtwacttsoenenioogetrfhxostthlirgeaonacfgirt.frtifolcriauvoa n endophytic fungus Penicillium sp. kunyit putih, mice

showed no decrease in motoric activity, no signs **of** 1

behavioral distress, no casualties and no observable w w SGOT and SGPT are two transaminases produced primarily by liver cells when hepatic cells are damaged.

Measurement of the enzyme concentration in the toxicity symptoms. **The** 1

experimented mice survived till w blood can determine

the levels of SGOT and SGPT, the completion **of**		14

the experimental duration at all levels providing important information regarding the liver of treatment. It indicated that the extract had no effect dysfunction[20]. Liver injury will also cause damage to on their motoric activity. In addition to motoric activity, the hepatocyte cell membrane, in which the permeability

BW is important to initially describe the **health of**		1

test of hepatic cells will increase and the enzymes will be animals. Change in BW is a highly sensitive indicator of released into the blood circulation. One-way variance health condition[16]. Table 2 shows that during 90-day analysis showed that the SGOT level of treated groups treatment, the average BW of mices was relatively (I–IV) was significantly different from the control stable. From statistical analysis, no significant differences group (V). However, the SGPT level was insignificantly in BW were observed between control and treated different (Table 3). The treatment dose caused an groups during this period. The significance value increase in the SGOT value beyond the normal value was greater than 0.05, suggesting that the extract for all given doses. Result of SGOT level on day 31 administration had no effects on BW and a negligible showed that the administration of extract to the test effect on the growth of animals. animals increased the SGOT level compared with the Muharni. et al. / J. Chin. Pharm. Sci. 2018, 27 (2), 123–130 control group (V). SGOT level of group I treated with toxicity of extract. Toxic effect caused liver necrosis, 250 mg/kg·BW was 162±17.35 U/I, which was much especially through its reactive metabolite. SGOT could higher than that of control group (69 U/I). Anova also cause scuffle during treatment period, resulting in analysis suggested that extract administration affected muscle trauma in the test animal. Another possible cause the SGOT level. Duncan test on SGOT value at P<0.05 was disease or abnormalities of liver, kidney and heart. concluded that the higher extract dose administered, Statistical calculation using one-directional variance the higher SGOT level obtained. Highest level of analysis at a 95% and P<0.05 showed that the SGOT SGOT was found from group IV, where the test animals change between five groups was significantly different but were administered by 2000 mg/kg·BW extract, and the not for SGPT. Administration of extract from endophytic SGOT level was increased to 267±4.36 U/I. Statistical fungus Penicillium sp. increased measurabe SGOT and calculations proved that SGOT at this level was SGPT levels. The high

levels of SGOT and SGPT were significantly **different from**		1

all other test groups. Same possibly due to toxic material intake, which triggered
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n compare with control group. SGPT level of group I was (68±4.58) U/I, which was 47% higher than group IV w w w Although low Hb indicated inappropriate condition, high Hb level could also indicate poor condition. Test (control) with an SGPT level of (46±1.73) U/I. Anova animals were mostly under stressful condition if they analysis followed by Duncan test concluded that the showed high level of Hb. Results of Hb measurement difference of SGPT level was significant. The highest showed that on day 31, the Hb level was within the SGPT level (173±2.65) U/I was observed in group normal range of 10–14 g/100 mL. This result designated administered with 2000 mg/kg·BW. The SGPT level

that there was no significant difference in Hb parameter **in group**		8

administered with 1000 mg/kg·BW was between treated group and control. According to Hb proportionally higher than that of groups administered test result, we concluded that the extract did not cause with 500

mg/kg·BW and 250 **mg/kg·BW.** By comparing adverse **effect**		12

on hematology system of test animals. duration of extract treatment, group I exhibited increased The duration of extract administration for all given doses SGPT from day 31 to 91, from (68±58) to (89±5.57) U/I. based on T-test calculation also showed no significant The same pattern was observed in other doses. T-test difference (P>0.05). calculation revealed, however, that the difference was Kidney and liver

function analysis is highly useful in		2

not significant. At this point, we concluded that at dose

the toxicity screening of medicines **and plant extracts as**		2

of 250 mg/Kg, 31-day treatment showed symptom of

both are important for the survival of an organism[		2

21,22]. toxicity. Increased levels of SGOT and SGPT indicated The test was carried out by observing the physical condition of organs, such as visual appearance, color and 5%

significance level found **P(0.000)>0.05.** This **result** 13

weight. Histopathology test was performed on day 31 revealed that extract administration exerted no effect and day 91. The result was depicted on Table 5. on the organ. Duncan test confirmed that there was no Observation on sample of mice liver histology (Mus difference in kidney's average weight between treated musculus L) was conducted after mice were treated group and control group, suggesting no kidney damage with ethyl acetate extract from endophytic fungus in test animals. Visual observation on kidney with and Penicilium sp. of Kunyit putih for 31 d. The result without treatment showed the same condition, and no showed that liver become darker, thicker and harder difference was observed. Treatment duration and dose compare with control group. The same result also given also showed no effect on kidney's weight. T-test obtained from organs after 91-days treatment. These showed P>0.05, indicating no significant difference of findings showed that damage occurred on hepatocyte in treatment duration on weight of kidney. Histopathological forms of pyknosis and necrosis. Hepatocytes suffering observation on heart sample of test animal after 31-day fmhswBhhsrhueeeoleiprCpppt aimmhpanaachotttkbkeooorpaprmectccageyynyyynidekketctteeeennaidtsssholo.dersaseaainaSisasncnsmuobuydacbomaltlatollyvgesapeepseedocupli-maskmsicedsneeaeumeonlrsaenlfeemsatnhdditrohdaooesnlimaeldsenelsetfaodtroemmrhrgpacskcoaeeigraoeuzlnodtlmrenghieszchiorabeaslt.nluhitn.oitgdsPaothTgiganoneyiyhetrn[yki n increased the levels of SGOT and SGPT. Transaminase w w heart's weight. T-test indicated no significant difference level increase in serum was due to necrosis of transaminase (P>0.05) for treatment duration, hence we concluded rich cells (Verma and Ahmed, 2009). This result that extract administration duration did not affect weight indicated significant difference between treated group w of heart. and control group. Variation on test extract concentration Physical condition was used to evaluate how extract also gave significant impact on liver's weight. Duncan administration affected liver, kidney and heart in test on liver weight confirmed that the lowest weight addition to organ weight (Table 6). Table 6 shows a average was 1.711±0.15 U/l, which was detected in comparation between sample and control after extract 2000 mg/kg·BW group. However, decrease in weight administration started at an initial concentration of of an internal organ suggests toxicity resulting from 250 mg/kg·BW. On day 31, with all concentrations, exposure to toxic substances[24]. Table 5 shows the the liver became darker, thickened and harder compare effect of duration time of extract treatment on liver's with control group. Meanwhile, the heart and kidney weight. The weight of liver was increased as the exhibited similar physical condition compared with the treatment time was extended. T-test calculation proved control. Similar observation was found up to day 91. that there was significant difference between treated On day 91, extract concentration of

1000 mg/kg·BW group and **control group.** made **the** 15

liver smaller. These data confirmed the Analysis of variance calculation on kidney's weight at possibility of toxicity of the extract to the liver. Muharni. et al. / J. Chin. Pharm. Sci. 2018, 27 (2), 123–130 Table 6. The physical condition of organs. Dose of treatment (mg/kg·BW) Day-31 Liver Day-91 Day-31 Heart Day-91 Day-31 Kidney Day-91 0 – – – – – – 250 + + – – – – 500 + + – – – – 1000 + + – – – – 2000 + + – – – – Note : –: not affected +: affected. Based on data of hematology, blood chemistry and dalam Pengembangan Obat Herbal. Majalah Ilmu macroscopic test results, there was a significant difference Kefarmasian. 2005, 2, 113–126. in parameters of SGOT, SGPT and heart condition [6] Elfita, M.; Muharni, S. New pyran of an endophytic between treated mice and control. The difference

fungus Fusarium sp. Isolated from the leaves of brotowali 7

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