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Acute and Subchronic Toxicity Tests of Papaya Leaf (*Carica papaya linn*) Methanol Extract on Wistar Strainwhite Mice

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ABSTRACT

Papaya leaf (*Carica papaya linn*) is one of the plants used for the treatment of servical cancer. Papaya leaf contains papain and carpaine compounds. Methanol extract (*Carica papain L*) has an inhibitory activity against the DNA enzyme Topoisomerase II, an enzyme that plays an important role in the replication, transcription, DNA recombination processes, and the proliferation of cancer cells. The purpose of this study was to know the potential acute and chronic toxicity on papaya leaf extract. This research was experimental study with *post test only design*. The researchers determined the maximum dose of papaya leaf extract that can be used so that it is safe for management of cervical cancer cases. In previous research, the researchers used papaya leaf extract at doses of 250 mg / kg up to 750 mg / kg in experimental animals with cervical cancer. The toxicity test method used is acute and sub-chronic toxicity test. Acute toxicity test was made for 24 hours and sub-chronic toxicity test was done by calculating lethal dose value (LD50). The doses used in this study were ranging from doses of 200 mg, 400 mg, 800 mg, 1600 mg and 3200 mg / kg. Acute toxicity test results in all doses showed that no animals died and no change in behavior among experimental animals. Further treatments were continued for 2 months (60 days) to see the sub-chronic toxicity test. After 60 days of treatment all the mice did not die, then the SGOT and SGPT levels of blood samples were examined. The average SGOT level in all doses were in range of 111-327 U / L and the average SGPT levels in all doses were in range of 51-267 U / L. The average SGOT level was 45 U / L and SGPT level was 35 U / L in the control group. The results of statistical tests Kruskal –Wallis showed that SGOT level was significant and SGPT level was not significant. Methanol extract of *Carica papaya linn* did not show any toxicity in model mice. The results of this study recommends that *Carin papaya linn* methanol extract is safe for use in human.

KEY WORDS: *Carica papaya linn*, Toxicity test, LD50, SGOT, SGPT

INTRODUCTION

Papaya is one of nutritious plantsthat grows in tropical and subtropical regions, including India, Ceylon, Malaysia, the Philippines, South America, South Africa, Hawaii, and Indonesia. Papaya leaf contains quite a lot of secondary metabolite of alkaloid compared with fruit.In addition, papaya leaf also contains papain enzymeso it is often used to soften the meat and some people use it to treat cancer [1,2].

Methanol extract of papaya leaf (*Carica papaya L.*) has inhibitory activity against DNA Topoisomerase II enzyme, an enzyme that plays an important role in the process of DNA replication, transcription and recombination and thereby thecancer cellproliferationmay also increase. With the inhibition of the enzyme activity, there will be the longer binding between the enzyme and DNA thereby the Protein Linked DNA Brake (PLDB) occurred andended with death by apoptosis [3].

Some studies showed thatpapaya leaf chloroform fraction has anticancer activity against myeloma cells and can induce apoptosis by staining method of ethidium bromide and acridine orange. Papaya leaf methanol extract has cytolytic activityagainst myeloma cell culture [4].

This research was done by carrying out acute and subchronic toxicity tests of papaya leaf methanol extract that is used as anti-cancer. Toxicity test is divided into acute, subchronic, and chronic toxicity tests. Acute toxicity test is designed to determine the Lethal dose or abbreviated as LD_{50} of a substance [5]. Acute toxicity test is carried out by administering the chemicalthat was tested onceor several times within a period of 24 hours [6]. Acute toxicity test is a preclinical test that aims to measure the degree oftoxic effect of a compound in certain time after administration ofsingle dose. Quantitative method usually used to express lethal doserangeon acute toxicity test is LD_{50} [7]. Medicinal plantmust go through various test processes for the safety of consumption, one of them is acute toxicity test [8, 9]. While the subchronic toxicity test is a test to determine the toxicity of a compound which is performed in experimental animal with at least three dose levels, usually within 60 to 90 days. Chronic toxicity test is a test to determine the toxicity of a compound performed in experimental animal in relatively long period of about 6 months[10].

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Determination of LD_{50} is important to assess the acute potential of papaya leaf extract. There are three methods to calculate the value namely method of profit graph, Weil C.S, and Indonesian Pharmacopoeia III.

METHODS

This research used experimental design with posttest only design approach. This research was done in Bioscience Institute of Brawijaya University of Malang from April until September 2017.

Selection and Preparation of ExperimentalAnimal

Experimental animals used were 30 healthy male wistar strain mice with agile movement activity, clean fur, aged 2-3 months, and body weight of 20-30 grams.

Preparation and Treatment of ExperimentalAnimals

Papaya leaf extract treatment at5 doses of 200 mg, 400 mg, 800 mg, 1600 mg, and3200 mg/kgBW.Each dose consists of 5 experimental animals. Controlgroupwas only treated with standard diet and without papaya leaf extract.

Acute toxicity test used Thomson and Weil method.Before being treated with the administration ofpapaya leaf extract, mice wereadapted for1 week, feed and drink were givenad libitum.All mice were not given with feed and drink for 24 hours before treatment.Observation of mice death was done for24hours using CCTV cameras.

For subchronic toxicity test was carried out for 60 days withthe samedose if there was no lethal dose during the acute toxicitytest. Data was taken from mice that shows abnormal symptom after the administration of papaya leaf extractcompared withcontrol group. LD_{50} data was taken from the number of mice that died and still alive in each group.

Papaya Leaf ExtractPreparation

Sampling of the fifth blade of slightly dark green papaya leaf (*Caricapapaya L.*) was done between 10.00 to 12.00 a.m. with sunny weather condition, this is because the plant contains nutritious ingredients when it is performing the photosynthesisprocess.

Processing of the harvested papaya leaf (*Carica papaya L.*) sample was performed by removing the dirt or foreign materials. Then the leaves were washed with running water, cut into small pieces and dried under the sun (indirectly) coatedwithblack fabric until dry, then dry sorted and smoothed withblender.

Sample extraction used methanol solvent. To obtainpapaya leaf (*Carica papaya L.*) chloroform fraction, then 350 grams of papaya leaf (*Carica papaya L.*) powder was macerated first with hexane solvent to remove the fat content (defatted). Maseration was performed until the extract shows clear color. Dregs of hexanemaceration was macerated again with methanol in acidcondition (pH 3) with the addition of 1% tartaric acid, this was done repeatedly until the extract was clear colored. The next stage was to alkalinize the acid methanolextract with 5% NH4OH until pH 9. This stage aims to hydrolyze alkaloids in salt form into its baseform so it can be drawn by organic solvent such as chloroform. The chloroform fraction obtained was then evaporated with rotary evaportant to obtain result of chloroform fraction.

Karpaintesting was done by weighing ± 0.1 gr of karpain and then 10 mL methanol was added. Sonication was done for 10 minutes at the speed of 4500 rpm. Supernatant was filtered with 0.2 micron PTFE filter. Filtrate inserted in vial bottle and 2 µl volume of sample injection was analyzed in LC-MS/MS.

Test of SGPT and SGOT Enzyme Levels on Male Mice after the Administration of Papaya Leaf Extract

After the administration of papaya leaf extract for 60 days, surgery for blood sampling from heart was done. Blood drawn was inserted in sterile tube, it was then centrifugedwith the speed of 3000 rpm for 10 minutes. Separate serum was taken and inserted in other clean, dry, and closed tube. If the serum was not immediately checked, then it must be stored in the refrigerator at temperature of 2-8ºC for maximum of 4 days. If it was stored more than 4 days, it will experience activitydegradation by 10%.

Measurement of SGOT and SGPT enzyme activity was performed by collecting 50 µl serumand added 500 µl of reagent solution then it washomogenizedand wait for 1 minute before measuring. After 1 minute, its absorbance was measured with spectrophotometer at wavelength of 340 nm, and the decrease in its absorbance every minute for 3 minutes was recorded. Preparation of reagent solution by dissolving SGPT and SGOT reagent tabletsin buffer solution with ratio of 1:10.

RESULTS

a. Body weight of experimental animal

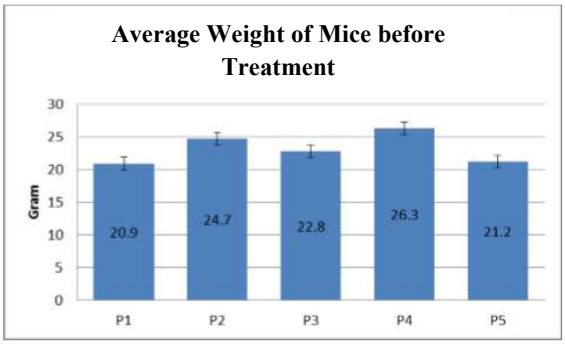

Figure 1: Average weight of experimental animal before treatment

b. Treatment of papaya leaf extract administration

Five groups of mice @ 3 micewere treated with test material at doses (200mg, 400mg, 800mg, 1600mg, and 32000mg/kg BW) by using single certain multiplication factor, and the material was administered orally by using feeding tube,the observation was performed for 24 hours by seeing the death of mice. This toxicity test was carried outto find out the acute toxicityof a material. If the mice did not die, then the subcronic toxicity test until 2 months (60 days) by administering the 5 doses will be carried out.

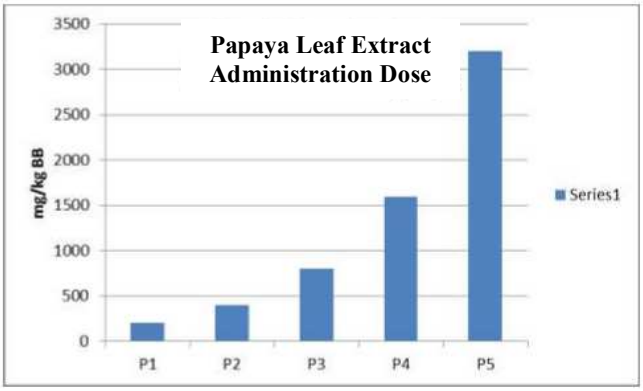

Figure 2: Treatment of papaya leaf extract administration

c. Toxicity Test Results

After the administration of papaya leaf extractat doses of 200 mg, 400 mg, 800 mg, 1600 mg, and 3200 mg/kg BW for 24 hours(acute toxicity test), there were no dead mice and behavioral changes in mice such as scratching, weakness, crooked tail, reduced movement.

In subchronic toxicity test with papaya leaf extract at doses of 200 mg, 400 mg, 800 mg, 1600 mg, and 3200 mg/kg BW for 60 days (8 weeks), there also were no dead mice and behavioral changes in mice. So the LD_{50} value was said to be 0 because there was no lethal dose.

Furthermore, surgery was done to draw blood serum samplefor examination of SGOT and SGPThepatic function.

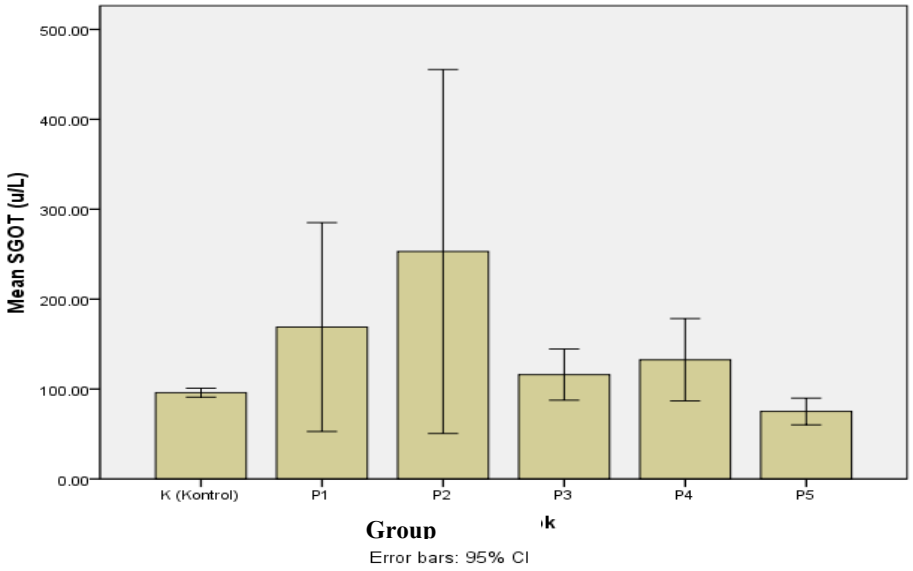

Figure 3: SGOT levels after treatment of papaya leaf extract for 60 days

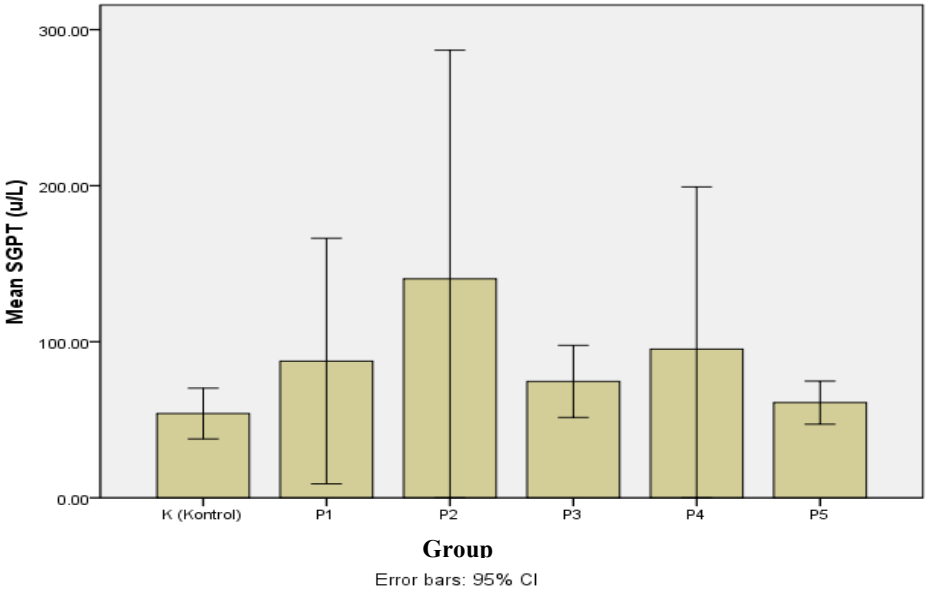

Figure 4: SGPT levels after treatment of papaya leaf extract for 60 days

Table 5: Results of Kruskal-Wallis Statistical Test

PARAMETER	Kruskal-Wallis Test		
	Chi-square Test Statistic	p-value	Information
SGOT	14,754	0.011	Significant
SGPT	10,291	0.006	Not significant

From the results of kruskal-wallis statistical test, p-value of 0.011 for SGOT level and p-value of 0.067 for SGPT level were obtained. Thus it can be concluded that there was difference in effect between treatment group at SGOT variable only compared with control group. While for SGPT level, there was no difference between treatment group and control group.

DISCUSSION

Administration of papaya leaf extract atdoses of 200 mg, 400 mg, 800 mg, 1600 mg, and 3200 mg/kgBW for 24 hours for acute toxicity test and 60 days for subchronic toxicity test did not indicate the lethal dosein male wistar mice. This proves thatpapayaleaf extract did notcause toxicity effects when consumed.The results of this research were supported by several previous researches such as research, which used papaya leaf ethanol extractfor 90 days in experimental animalatdose of 1gram/kg BW. In this previous research, there were no dead mice and abnormality of liver function and renal function in mice[11].

Analysis results showed that the average SGPT and SGOT levels of male mice administered with papaya leaf extractonce a day for 60 days differ significantly in the 5 treatments with significance level of α = 5% of kruskal-wallistest against SGPT level of control group mice. While in SGPT level, there was no significant difference compared withcontrol group. This was allegedly because papaya leaf extract in such dose range can affect SGPT enzyme level, especially the most prominent at dose 2. SGOT enzymenormal level is 23.2 - 48.4 u/L and SGPT enzymenormal level is 2.1 - 23.8 u/L. But in the control group of this research, the average of SGOT level was 96 u/L and the average of SGPT level was 54 u/L[12].

At dose 2 treatment, SGPT and SGOT levelsexperienced the greatest increase compared with other treatments with average of 252.97 u/L and average of SGPT levelatdose2 was 140.33 u/L. Different from treatment 3 in which SGOT and SGPT enzyme levels were decreased, but in treatment 4 the SGOT and SGPT enzyme levels were slightly increased again and in treatment 5 the levels were decreased again. This indicates that the concentration (dose) did not affect SGOT and SGPT enzyme levels.

Liver damage can be assessed by the increase in SGOT and SGPT enzyme levels. Increased levels of SGPT and SGOT enzymes 20-100 times above normal can occur in liver cell necrosis caused by drugs and toxins. In this research, papaya leaf extract level test was done by reviewing the effect of papaya leaf extract on SGPT and SGOT enzymelevels of male mice [13].

Results of this research indicated that the administration of papaya leaf extract in male mice did not affect SGPT and SGOT enzyme levels. Through kruskal-wallisstatistical analysis test, after administration of papaya leaf extract there was significantdifference on SGOT enzymeleveland there was no significant difference on SGOT enzymelevel, which means that papaya leaf extract has an effect on mice liver function especially at dose 2.

Abnormal liver function often indicates damage to the liver. In contrast, normal liver function testdoes not always show normal liver. For example, patientwith liver cirrhosis that carries out liver function test in which the testshows normal SGPT and SGOT enzyme levels despite the patient's liver damage. This indicates that SGPT and SGOT enzymes are not specific indicator of liver abnormality [14]. Besides found in the liver, SGOT enzyme is also found in the heart, skeletal muscle, brain, kidney, and pancreas. SGPT enzyme in small amount can also be found in the heart, skeletal muscle, kidney, and pancreas [6, 15]. Transaminase is sensitive as an indication of liver cell damage but it is not specific and can not be used to predict prognosis of liver disease.

Carica papaya extract did not cause toxicity symptom in Sprague Dawleymice at doses of 5, 50, 300, and 2000 mg/kg BW. This was known because there was no dead experimental animal after the 14-day treatment. But there were significantincreases in hemoglobin (HGB) level, hematocrit (HCT), red blood cell (RBC), and total protein that were allegedly due to dehydration[6].

Similar research has also been conducted on the safety of oral consumption of papaya leafwith subchronic toxicity test in Sprague Dawleyspecies mice. Doses administered were 0.01, 0.14, and 2 g/kg BW for 13 weeks. Research results showed that the administration of papaya leaf extract for 13 weeks did not cause death and behavioral changes in experimental animal. The results of this research also showed that there was no significant differencein hematological parameter, but there was significant difference in biochemical parametercompared withcontrol group. Biochemical parameters seen include high density lipoprotein(HDL), creatinine, total protein, and albumin. This research concluded that the administration of papaya leaf extract for 13 weeks with multilevel dosesdid not cause toxic effects [15, 16].

CONCLUSION

1. There was significant difference in SGOTenzyme level on the administration of papaya leaf extract at doses of 200 mg, 400 mg, 800 mg, 1600 mg, and3200 mg/grBW in male mice.
2. There was no significant difference in SGPTenzyme level on the administration of papaya leaf extract at doses of 200 mg, 400 mg, 800 mg, 1600 mg, and3200 mg/grBW in male mice.
3. SGOT enzyme level of male mice was greater than SGPT enzyme level.

4. Administration of papaya leaf extract at dose of 400mg/gr BW can increase SGOT and SGPTlevelsin male mice but did not cause toxicity on acute and subchronic toxicity tests.

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