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TOXICITY TEST OF PHYSIC NUT (*Jatropha curcas* L.)" *WANGI*" VARIETY ON WHITE RAT (*Rattus norvegicus*)

Eko Widaryanto

Faculty of Agriculture, University of Brawijaya, Malang of Indonesia

ABSTRACT

The effort to improve economical value of Physic nut (*Jatropha curcas* L.) is by selection of non-toxic physic nut variety. The benefit is expected from other plant organs beside seeds (leaf and seed milk as a feed) and Crude Jatropha Oil (CJO) as the edible oil. The objective of this experiment was obtain toxicity level test of Wangi variety and local physicnut on the white rats (*Rattus norvegicus*). The methods used in this experiment are 1) observation of the rat's liver tissue damages on the level of food mixture of 0.5 g, 0.75 g and 1 g/rats respectively for 2 physic nut varieties.. This observation is done on the rat's liver tissue damages after 1 and 2 weeks of treatments. Toxicity level of **Wangi** variety's seeds on white rats (*Rattus norvegicus*) is lower compare with local variety. White rats with local physic nut feed have more *pycnosis* and *hyperchromatic* (liver tissues damages) compared with rats feed by **Wangi** variety under the same dosage. This similar phenomenon showed on each level of dosage given to the white rat. From this experiment, It is concluded that **Wangi** variety has smaller toxicity level on animal experiment compare to the local variety, however, more advance research is needed on Wangi variety to determine its potential to use as the alternative of edible oil.

Key word: *Jatropha curcas "Wangi variety", toxicity, Rattus norvegicus*.

INTRODUCTION

Physicnut (*Jatropha curcas* L.) can be found in Central and South America, Africa, India, and South East Asia. It is considered as wild and semi-culture plant [1][2]. Weight of the physicnut seed is about 0.75 g in average, containing 27-32% and 58-60% of protein and fat, respectively [3]. Either as oil source, a non-toxic physic nut has also reported as feed. The protein content of the physic nut was reach about 53-58% of crude protein [4].

The potential physic nut as traditional medicine plant has also been found by local people in Mojokerto, East Java, Indonesia to reduce uric acid syndrome. Such physicnut was called Wangi variety since it has a *Pandanus sp*.-like aroma (local language of wangi means aromatic). Widaryanto [5] stated that Wangi variety is different with local based on protein molecular marker. **Wangi** variety has protein molecular marker of 70.4 kDa, which is not detected in local variety. Conversely, the local variety has protein molecular marker of 31.3 kDa, which is not appear in *Wangi* variety.

The toxicity test on animal showed that the physic nut seed is toxic [6][7][8]. The physic nut seed is toxic when it applied on both small and big white rats. Liberalino *et al.* [8] found that raw material and the processed result of the physic nut seed contains high toxicity level. It is found that all feeds for big rats contain different fraction of seed in which the rats died within 23 days following the administration of raw materials, 68 days for the processed seeds, and 14 days for the seeds fried without oil. Ahmed and Adam [6] fed six calves using the physic nut by the dosages of 2.5, 1.0 and 0.25 g/kg and two other calves using 0.025 g/kg within 14 days. The clinical symptoms of diarrhea, dyspnea, dehydration, as well as deteriorated condition have close correlation with the pathological findings. There was an improvement in aspartate aminotransferase, ammonium, as well as potassium and decreasing in total protein and serum of the calves which were being poisoned by the physicnut.

The toxicity level of the seeds is caused by some components, such as saponins, lectin (curcin), phytates, protease inhibitors, curcalonic acid, and phorbol ester. Phorbol ester which activated the important cellular target of protein kinase C (PKC) was the most active component that must be removed if the seeds utilized as nutritive sources for both animals and human. The physic nut's oil and **phorbol ester** have insecticidal and molluscicidal activities over wide range of organism, Physic nut recommended as bio-rationale pesticides for mollusca controller for crops (against water snails which spread parasite as

***Corresponding Author:** Eko Widaryanto, Faculty of Agriculture, University of Brawijaya, Malang of Indonesia.
Email: eko.widar@ub.ac.id

schistosomes or tapeworm). Phorbol ester also identified as stimulus of tumor, but it is neither mutagenic nor carcinogenic [9].

It is expected that **Wangi** variety, which has been used as medicine material, can be cultured and developed as an alternative livestock's food sources. The objective of this experiment was to obtain toxicity level on Wangi variety and local physic nut by white rats liver tissue (*Rattus norvegicus*) feeding assessment.

MATERIALS AND METHODS

The experiment was conducted at the Biochemical Laboratory, Faculty of Science, and University of Brawijaya, Indonesia from September to October 2007. Materials of this experiment are two kinds of physic nut seeds (*Local* and *Wangi*) (Appendix 1), male white rat (*Rattus norvegicus)* Wistar strain with initial weight is 120 g/rat.

Feeding preparation

Making of physic nut seed (kernel) extract of 0.5 gram dosage is started by weighing 7 g of physic nut seed and put it into 250 ml beaker glass that contains 150 ml distilled water. The process after that is by boiling it under temperature of 80^{o}C for $\pm$ 3-4 hours. The physic nut extracted by omits the residue. For 0.75 g dosage, the seed is weighed of 10.5 g and 14 g for 1.0 g dosage. Treatment over the seed is similar to the previous one, the making of 0.5 g dosage.

Rat feeding

Eighty Male of white rats separated into six groups, fed with one time daily dose of 0.0; 0.5; 0.75 and 1 g physic-nut seed extract from two varieties of physic-nut (*Local* and *Wangi).* Body weight of each rat used in this experiment was about 120 g in each. Feeding treatment was conducted for one and two weeks. The liver assessment was conducted to see the toxicity level of each variety.

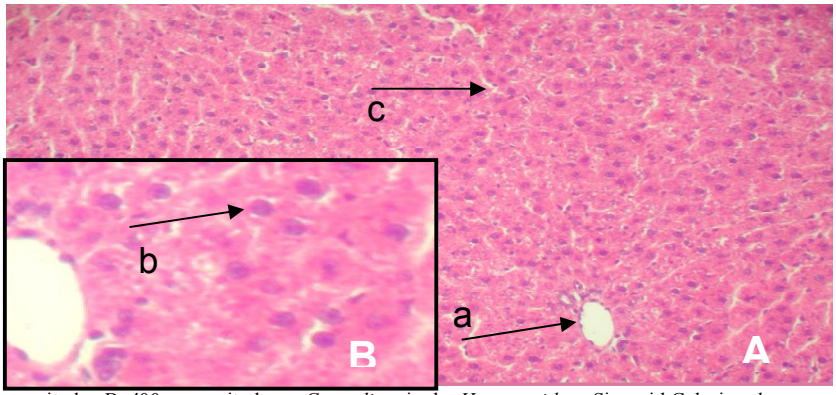
Blood smear test

A piece of rat liver tissue has been prepared and coated with xylol for 5 minutes, then dried for few minute. This step was repeated three times consecutively. Dehydration has been started by soaking tissue within 99.5 % ethanol (PA) and followed by 95%, 90%, 80%, and 70 % ethanol for 5 minutes in each. Distilled water was applied in the final wash. Prepared tissue was then dyed using hematoxylen for 10 minutes followed by washing with tap water. After rinsing with distilled water, dying has been fixed with eosin for 5 minutes and soaked into distilled water. Further, tissue was soaked into 70%, 80%, 90%, 95% ethanol, and absolute ethanol, successively for 10 minutes in each. The final step was re-coating tissue with xylol for 5 minutes then dried in ambient temperature. Glass mounting was done for microscopic observation.

RESULTS AND DISCUSSION

Toxicity level test of the physicnut seed on white rats

Microscopic observation on normal rat's liver cell showed the *radier*, intact sinusoid, nucleus of *normochromatic*, similar form of the cell (*isocytosis*) and no sign of abnormality of the cytoplasmic (Figure 1).



Notes: A. 100 x magnitude ; B. 400 x magnitude ; a. *Centralis* vein ;b ; *Hepatocoid* ; c. Sinusoid Coloring: *hematoxylin*-eosin

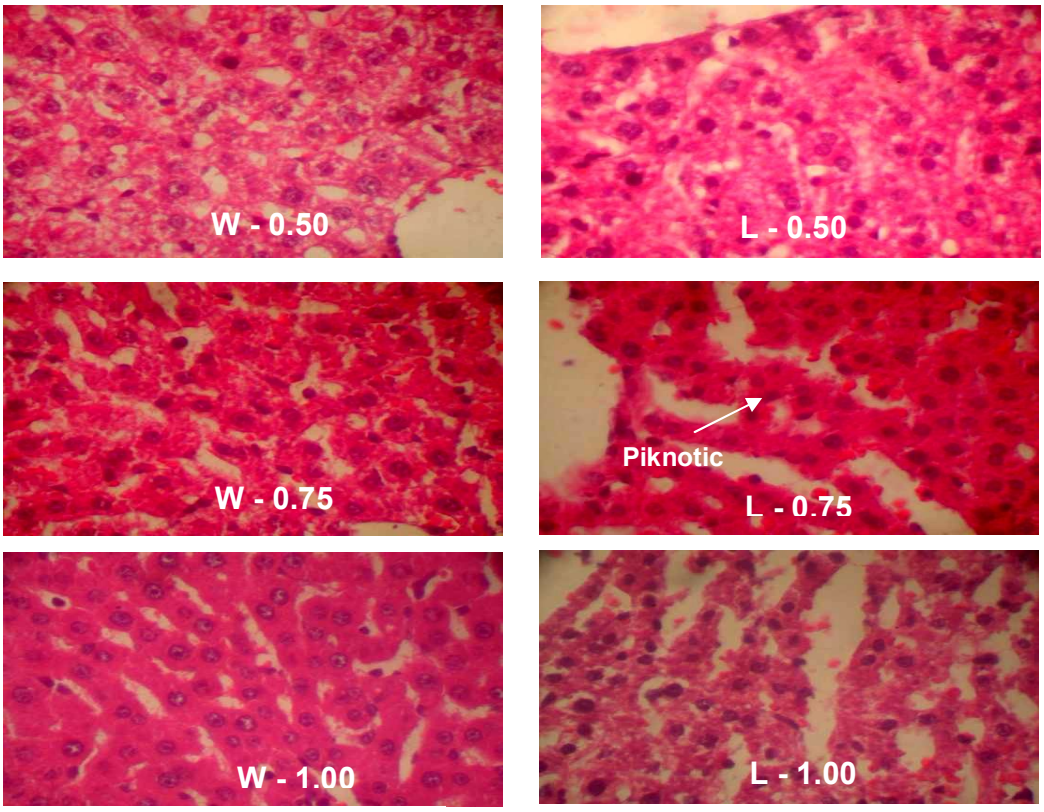
Figure 1 Normal Structure of the Liver.

On the other hand, the treated liver showed a change on the liver cells from normal to necrosis and disorganized surrounding tissues. Damages on the liver tissues were usually showed by the ***pycnotic*** (the cell is having *pinocytosis*) and ***hyperchromatic*** (more colored than normal) from the nucleus (Figure 2).

Treatment using 0.5 g, 0.75 g, and 1.0 g dosages showed *pycnotic* and *hyperchromatic* nucleus on all treatment dosages one week after treatment. The toxicity level was increased with higher dosage of the physic nut feeding. Smaller abnormality showed on liver cell treated with Wangi variety (W sign), Hence, local variety showed more abnormality (L sign).

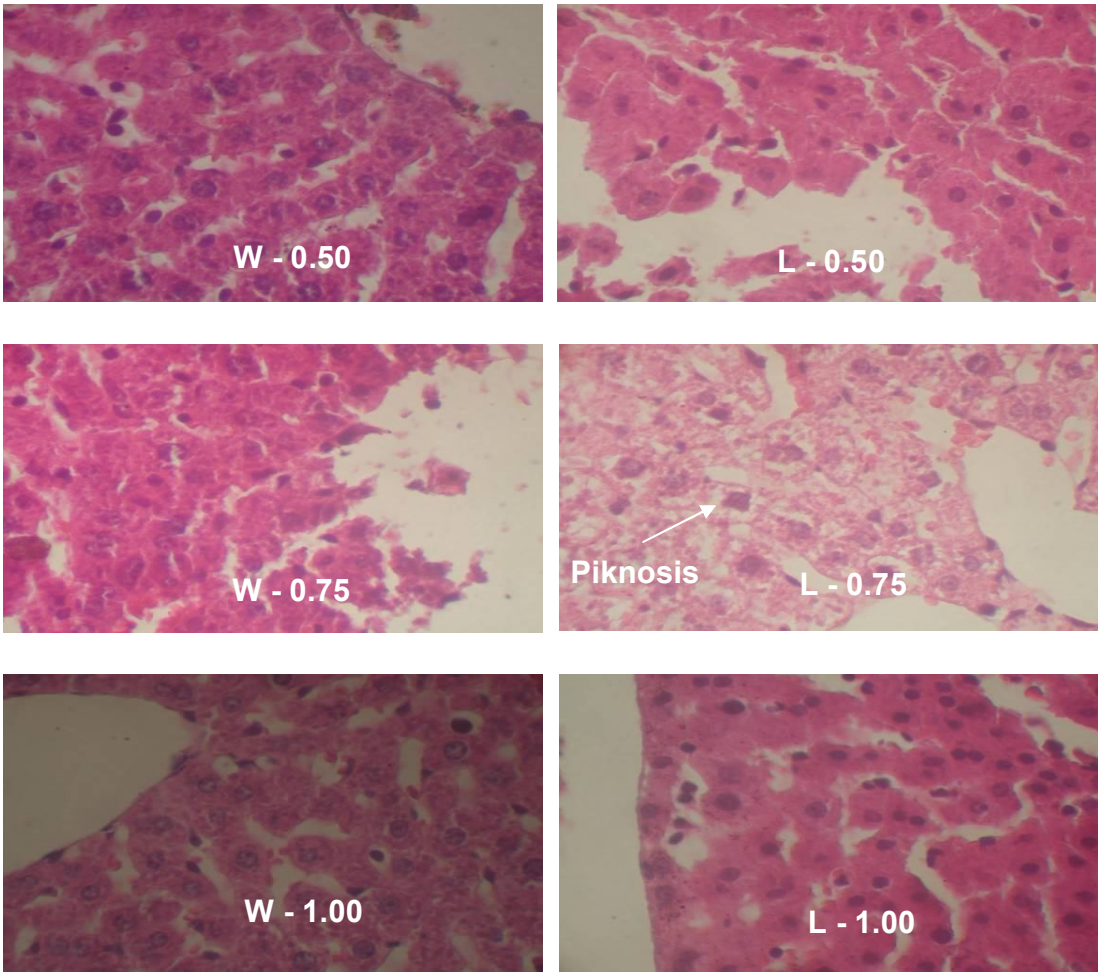
The feed stock both **wangi** variety and local physic nuts with the dosages of 0.5 g, 0.75 g, and 1.0 g for two weeks showed extended damage on the liver tissues compared with one week treatment (Figure 2 and 3). The tissue damage due to extended necrosis can be seen on all treatments. The cell damages start appeared on treatment of 0.5 g dosage and bigger damages in 0.75 g dosage and significant damage founded on the highest dosage of 1.0 g.

After 2 weeks application still no lethal effect on white rat. Therefore, it needs further research, particularly concerning with the separated influence of the press cake and CJO on the rat's liver damage. Based on those facts, the seed of **wangi** variety has smaller effect of cell damages compared with local physic nut in the same dosage of feeding; those revealed that **wangi** variety has potential to become the protein sources for fodder in the future. However, further research is still required concerning with its utilization as fodder. A research on toxicity test should be performed for the press cake of the physic nut's seeds for fodder after the oil removed from the seeds.



Notes:
W – 0.50: *Wangi* 0.5 g. rat^{-1}
W – 0.75 :*Wangi* 0.75 g. rat^{-1}
W – 1.00 :*Wangi* 1.00 g. rat^{-1}
L – 0.50 : Local 0.5 g. rat^{-1}
L – 0.75 : Local 0.75 g. rat^{-1}
L – 1.00 : Local 1.00 g. rat^{-1}
white rat weight: 110–120 g. rat^{-1}

Figure 2 The Rat's Liver Tissue during 1 Week Observation After application of the Physicnut's Seed on 400 x Enlargement



Notes:

W – 0.50: *Wangi* 0.5 g. rat^{-1}
W – 0.75 :*Wangi* 0.75 g. rat^{-1}
W – 1.00 :*Wangi* 1.00 g. rat^{-1}
L – 0.50 : Local 0.5 g. rat^{-1}
L – 0.75 : Local 0.75 g. rat^{-1}
L – 1.00 : Local 1.00 g. rat^{-1}
white rat weight: 110–120 g. rat^{-1}

Figure 3 The Rat's Liver Tissue during 2 Weeks Observation After application of the Physicnut's Seed on 400 x Enlargement

The treatments of both *wangi* and local varieties fodder with dosages of 0.5 g, 0.75 g, and 1.0 g for two weeks cause expanded damages on the liver in comparison with the treatments for a week (Figure 2 and 3). Damages of tissue are due to expanded necrosis found in all treatments. The damages were started in the treatment of 0.5 g dosage, and the damages get bigger and most significant during the treatment of 0.75 g and 1.0 g, respectively.

Small mammal's liver has various functions. One of the functions is to detoxifying drugs and toxins. The influence of chemicals/toxic substance could induce damages on tissues. This phenomenon occurred due to blood circulation from *intestinum* to liver passes *portahepathyca* circulation system. The blood contains greater nutrient and lower oxygen. Futher, the blood contains *endotoxin*, (the remains of metabolism, chemicals absorbed by the *intestinum)*. The whole materials in the blood possibly get into the liver from the *intestinum* through *portahepathyca*, which will caused higher toxic risk on the liver [10].

Chamulitrat *et al*. [11] explain similar thing; from the mouth, toxic substance will reach high concentration in liver. Chemicals/toxic in blood within the liver sinusoid will go into the *hepatocyt* immediately. The entire metabolism in the liver, will transformed and the outcomes will be excreted through kidney or feces. Biotransformation in the liver occurs due to various enzymes that could metabolize strange chemicals or outputs produced by the body itself. However, this biotransformation process might be hazardous because it might result reactive/toxic compounds. Toxic/reactive metabolites would initiate a series of events, which will lead to liver damages.

The liver responses to toxic chemicals are categorized into: *Steatosis* (reversible); *Cytotoxicity,* which marked by swollen cell (reversible) during the initial stage and developed to necrosis (irreversible); *Cholestasis; Fibrosis and cyrosis* [12]. The treatments of physic nut for feeding in two weeks causes expanded damage (necrosis stage) within the liver in comparison with the one week feeding. However after two weeks still no lethal stage on white rat fed by physic nut.

Toxicity level of the seeds was caused by some components, for instance, *saponin*, *lectine (curcin), fitate, protease inhibitor, curcalonic acid,* and *phorbol esters. Phorbol esters*, which activate the target of important cellular protein kinase C (PKC), is the most active component that must be removed when oil and seed are utilized as the nutrient source for animals and human beings [9].

Both CJO and *phorbol ester* show insecticidal and *molluscicidal* for wide range of organisms, therefore, they are suggested to be applied in agriculture as *biorationale* pesticide and mollusks controller (against water snail, which can infects parasite, such as *schistosomes* or platyhelminthes). *Phorbol esters* is the impetus of tumor, but it is not mutagenic nor carcinogenic [9]. However, *J. curcas* **Wangi** variety showed less toxicity compared with local variety, hence it is potential to develop it as the edible CJO and feed.

Selection for specific type of non toxic physic nuts is required if there is a plan to develop physic nuts as an edible oil or feed. Chivandi *et al*. [13] revealed that the application of ***phorbol esters*** **detoxification method** in physic nut seed could detoxify the toxin. The physicnut of *wangi* variety for medicine is expected to be developed and maximize its potentials to obtain its aromatic volatile oil and alternative fodders for livestock's also protein source for human beings, as well as for edible oil.

On the near future *wangi* variety has a potential to develop as the multi products crop (as fodders, fish's feed, and raw material of volatile oil, traditional medicine, and greater possibility to be used as edible oil). Therefore, *J. curcas* wangi variety will give provide benefit for grower, specially on the marginal dry land.

CONCLUSSION

Normal liver of white rat's liver cell shows the *radier*, intact sinusoid, nucleus of *normochromatic*, similar form of the cell (*isocytosis*) and no sign of abnormality of the cytoplasmic. Treated mouse's liver by physic nuts (both local and wangi veriety) feed showed a change on the liver cells from normal to necrosis and disorganized surrounding tissues. Damages on the liver tissues were usually showed by the ***pycnotic*** (the cell is having *pinocytosis*) and ***hyperchromatic*** (more colored than normal). Abnormality and cell damages founds more on mouse's liver treated by Local physic nuts feed than Wangi variety. J. curcas Wangi variety is potential to develop as a protein source of feeding and edible oil due to less toxic than local variety

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Appendix 1. Pictures of *Jatropha curcas* L. Wangi variety and Mexico non toxic Variety

Jatropha curcas "Wangi" Variety

Jatropha curcas (Non-toxic) Mexico Variety