THE NATURAL RODENTICIDAL EFFECT OF PAPAIN PAPAYA (*CARICA PAPAYA* L.) IN HISTOPATHOLOGICAL CHANGES OF ALBINO MICE ORGANS

EFEK RODENTISIDA NABATI PAPAIN PEPAYA (*CARICA PAPAYA* L.) TERHADAP PERUBAHAN HISTOPATOLOGI ORGAN MENCIT ALBINO

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ABSTRAK

Rodentisida dari papain pepaya untuk pengendalian tikus dan mencit masih jarang dilaporkan. Penelitian ini bertujuan untuk melihat perubahan patologi pada organ dalam mencit albino (lambung, ginjal, hati dan usus) kaitannya dengan efek toksisitas dari papain pepaya (*Carica papaya* L.). Penelitian dilakukan dengan desain quasi eksperimen dan rancangan *post-test only with control group design*. Tujuh puluh delapan mencit *BALB/c* dewasa (jantan dan betina) digunakan dalam penelitian ini dan dibagi menjadi 5 kelompok: 1 kelompok kontrol dan 4 kelompok perlakuan. Kelompok perlakuan diberikan pakan yang dikemas dalam bentuk blok yang berisi papain. Asupan pakan dan kematian mencit telah diamati pada penelitian sebelumnya. Pada akhir penelitian, jaringan mencit dinekropsi dan dilakukan pemeriksaan histopatologi. Hasil *post-mortem* menunjukkan kelainan berupa hepatomegali dan perdarahan gastrointestinal. Hati dan lambung mencit terlihat lebih menghitam. Perubahan histopatologi berupa nekrosis terjadi pada semua organ mencit perlakuan (lambung, ginjal, hati dan usus). Hati mencit mengalami bengkak keruh dan pada ginjal mengalami dilatasi. Degenerasi lemak terjadi pada lambung, hati dan usus. Kongesti juga terjadi pada ginjal dan hati. Asupan pakan yang berisi rodentisida nabati papain dengan dosis 16-32% memberikan efek kerusakan pada lambung, ginjal, hati dan usus mencit perlakuan. Dosis papain tertinggi (40%) tidak memberikan efek kerusakan yang lebih parah dibandingkan dengan dosis papain yang lebih rendah.

Kata Kunci : rodentisida nabati, papain, histopatologi, mencit

ABSTRACT

Reports on the rodenticide effect of papain papaya to control rats and mice are still limited. The aim of this study was to determine pathological changes in albino mice internal organs (gaster, kidney, liver, and intestine) associated with the toxicity effect of papain papaya (*Carica papaya* L.). A quasi-experimental with post-test only with control group design was used as the research design in this study. Seventy-eight adult BALB/c mice of both sexes were divided into 5 groups: 1 control group and 4 treatment groups that were given papain feeding block. Feed intake and mortalities have been monitored in the previous study. At the end of the experimental period, tissues of mice were harvested for necropsy and histopathological examination. The abnormalities of post mortem examination were hepatomegaly, gastrointestinal bleeding. The dark discoloration was founded in liver and gaster. Histopathological changes founded in all treatment albino mice were necrosis in gaster, kidney, liver, and intestine. Cloudy swelling occurred in the liver and dilatation in the kidney. Fatty degeneration occurred in gaster, liver, and intestine. In addition, the kidney and liver were congested. Feed intake of 16 to 32% papain natural rodenticide gave effect to the organs of studied mice, i.e gaster, kidney, liver and intestine.

Keywords: natural rodenticide, papain, histopathological, mice

INTRODUCTION

Commensal mice affected significant economic and health risk to people, as they can damage houses, destroy crops, contaminate food, and directly spread eleven diseases and indirectly spread fifteen diseases that threaten people's health and lives (Centers for Disease Control and Prevention, 2012). They are well-known reservoirs and hosts for a number of infectious diseases (e.g., plague, leptospirosis, leishmaniasis, salmonellosis, and viral hemorrhagic fevers) and play an important role in their spread and transmission (Rabiee *et al*., 2018). The number of leptospirosis cases rose drastically to 830 in 2016 and declined back to 640 in 2017. Significant decrease in the number of leptospirosis cases occurred in DKI Jakarta (from 39 cases in 2016 to 1 case in 2017). Meanwhile, significant increase occurred in Central Java (from 164 cases in 2016 to 316 cases in 2017) (Ministry of Health of the Republic of Indonesia, 2018). This in consequence, controlling them as reservoir of several diseases is considered as a priority.

Various methods of controlling mice have been employed in times past. These methods range from the ancient biological means, which is by keeping of cats, snakes, and so on, to act as predators. Physical methods of control are the use of baited traps and rat-proof construction. Meanwhile, the most widely employed method of control is the use of rodenticide (Taiwo *et al*., 2008). Rodenticides are pesticides designed to kill rodents, including mice. Synthetic rodenticides cause adverse health effects humans and ecosystems. Many investigators suggested that the basic research must be directed to the discovery of new safe types of pest control agents in order to ensure high production and preservation of agriculture products (A. Abou-Hashem, 2013). Therefore, the application of natural rodenticides has been increased as an alternative to synthetic insecticides (Mossa *et al*., 2018). The advantages of that rodenticides were relatively safe, environmentally friendly, inexpensive and easy to find (Priyadi, 2014).

Papain is an endolytic plant cysteine protease enzyme which is isolated from papaya (*Carica papaya* L.) latex (Amri *et al*., 2012). The concentration of the compounds varies in the fruit, leaves, and roots (Milind *et*

al., 2011). The greener the fruit, more active is the papain (Amri *et al*., 2012). The anti-fertility, uterotonic activity, nephroprotective, anti-inflammatory, anti-hypertensive, hypoglycemic and hypolipidemic, anthelmintic, wound-healing, anti-amoebic, anti-bacterial, anti-tumor, free-radical scavenging, anti-sickling and diuretic activities of the plant parts have been documented (Milind *et al*., 2011).

Scientific documentation on the rodenticide effect of papain from papaya is still limited, but ripe fruit has been used to control rat pests in rice fields. It has great potential to control rats because the papaya sap contains proteolytic enzymes such as papain (Ahmad *et al*., 2018). The previous study has shown that papain natural rodenticide killed laboratory mice within 2-7 days (Pramestuti *et al*., 2018). The onset of the *signs* of *poisoning* may not be evident until 24 hours following the ingestion. The effects of these rodenticides were chronic exposure. Hence this study conducted to determine the histology of laboratory mice organs (gaster, kidney, liver, and intestine) for pathological changes associated with the toxicity effect of papain papaya (*Carica papaya* L.).

METHODS

Study Design

The research design used in the study was quasi-experimental with post-test only with control group design. It was conducted from April to December 2016.

Animals and Rodenticide Treatment

We used seventy-eight adults BALB/c mice of the same weight, 18–20 g, of both sexes which obtained from Rodentology Laboratorium of Health Research and Development Unit of Banjarnegara, Central Java, Indonesia. The animals were kept under standard laboratory conditions. They were acclimatized for a week in the observation room before the experiment conducted. The animals were divided into 5 groups i.e 1 control group and 4 treatment groups (3 mice per group). This research was conducted in six replications. Treatment mice were fed with rodenticides in the composition of containing papain of 16%, 24%, 32%, 40% of the “X” brand product. The “X” brand compositions are papain, salt, and sugar, caramel, corn, wheat, soybean powder, paraffin, corn oil, and sugar.

The rodenticide was made in block shape. After a week of acclimatization, the normal diet for the experimental group was replaced by a formulated feed block which contains papain natural rodenticide. Animals in the control group were given feed block without papain in a similar manner. Feed intake and mortalities of mice treatment have been monitored in a previous study (Pramestuti *et al*. 2018).

Post mortem and histopathological examination

The certain signs of post mortem examination were exuded from anus and peritoneum, evaluation of liver, kidney, gaster and intestine abnormalities. At the end of the experimental period, mice of each treatment group were necropsied. Part of gaster, liver, kidney, and intestine of these mice were fixed in 10% neutral buffered formalin. The sections were cut at 5µm with a rotary microtome. The sections were stained according to Haematoxylin and Eosin (H and E) technique for microscopical examination. (Azubuike, Okwuosa, & Achukwu, 2015) The sections were evaluated based on the severity of the pathological changes, scores were given to lesions observed in the sections in each animal in different groups.

Gaster: score 1 (mild inflamatory cell/mild degeneration/mild necrosis; score 2 (moderate inflamatory cell/moderate degeneration/moderate necrosis); score 3 (severe inflamatory cell/severe degeneration/severe necrosis); score 4 (total necrosis). Kidney: score 1 (tissue damage <25%); score 2 (tissue damage 25 - < 50%); score 3 (tissue damage 50 - <75%); score 4 (tissue damage ≥ 75%). Liver: score 1 (very mild: cloudy swelling 25%); score 2 (mild: cloudy swelling 50%, hydropic/fatty degeneration and necrosis 25%); score 3 (moderate: hydropic/fatty degeneration and necrosis 50%); score 4 (severe: hydropic/fatty degeneration and necrosis 75%). Intestine: score 1 (mild inflamatory cell/degeneration/necrosis); score 2 (moderate inflamatory cell/degeneration/necrosis); score 3 (severe inflamatory cell/degeneration/necrosis); score 4 (total necrosis).

The result was calculated from observations of each part of organs in the 5 quadrants. Photographs of the sections were taken with an Olympus

photo microscope for observation and documentation of histopathology.

Ethical Approval

The experiment was conducted upon clearance from the Ethical Committee for Medical Research of National Institute of Health Research and Development, Ministry of Health Republic of Indonesia (Number: LB.02.01/5.2/KE.236/2016).

RESULTS AND DISCUSSION

Post mortem examination

Eighteen mice were used for each treated groups. During the research, some of the mice either venture out into open space were 2 mice in group A (papain 16%), 1 mouse in group B (papain 24%), and 3 mice from group C (papain 32%). Post mortem examination of all groups were shown in Table 1. Post-mortem studies indicate that abnormalities were seen in laboratory mice feed with papain formulated feed block. These abnormalities were hepatomegaly and gastrointestinal bleeding. The dark discoloration was founded in liver and gaster some of the treated mice. The observed changes of the organs are similar those documented in a previous report which revealed dark discoloration in the liver, kidney, and intestine of the treated albino rat after intoxication with ethanolic Oshar extract (A. Abou-Hashem, 2013).

Table 1. Post mortem examination in treatment mice after feed intake of papain natural rodenticide

Organ	Sign	Percentage of albino mice (%)				
		Papain 16% (n=16)	Papain 24% (n=17)	Papain 32% (n=18)	Papain 40% (n=15)	Control (n=6)
Anus	yellowish fluid	0.0	5.9	0.0	6.7	0.0
Peritoneum	yellowish fluid	0,0	0.0	11.1	0.0	0.0
Liver	hepatomegaly	6.3	17.6	22.2	6.7	0.0
	dark discolorati	0.0	17.6	22.2	0.0	0.0

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	oily and more brittle	0.0	5.9	33.3	0.0	0.0
Gaster	yellowish fluid	0.0	0.0	0.0	6.7	0.0
	bleeding	0.0	11.8	33.3	6.7	0.0
	dark discolorati on	0.0	0.0	5.6	0.0	0.0
Small intestine	bleeding	0.0	17.6	27.8	13.3	0.0
	gas	0.0	0.0	16.7	13.3	0.0

Histopathological in different organs

Level of damage in the different organ of laboratory mice after feed intake of papain natural rodenticide was presented in Table 2. It seems that the highest dose of papain (40%) did not give a strong effect compared to the lower dose of papain contents (Table 1 and 2). This was because the mice consumption rate to the block contain papain up to 40% were reduced as the effect of wary to the poison effect on the active substance in papain. This wary is one of the mice's habits and become a rejection mechanism if they found a new food that still unusual (Herawati *et al*., 2009)

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Table 2. Level of damage in the different organ of treatment mice

The section of organs	Percentage of albino mice (%)															
	Papain 16%				Papain 24%				Papain 32%				Papain 40%			
	score				score				score				score			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Liver	38	25	25	13	22	22	11	44	18	9	45	27	43	29	0	14
Kidney	33	44	0	22	33	67	0	0	25	75	0	0	71	14	14	0
Gaster	33	50	17	0	25	13	50	13	36	27	9	27	83	17	0	0
Intestine	14	57	14	14	0	14	86	0	2	9	45	27	67	17	17	0

Histopathological changes observed in all the experimental albino mice are almost similar, irrespective of the dose of papain. Necrosis occurred in the internal organs (gaster, kidney, liver, and intestine) of mice compared with control animals. Moreover, total necrosis occurred in the intestine of mice. Cloudy swelling occurred in the liver and dilatation occurred in the kidney compared with control. Fatty degeneration in this study occurred in the gaster, liver, and intestine. The photomicrographs of gaster, kidney, liver, and intestine tissue sections from control and experimental mice stained with hematoxylin and eosin were shown in Figure 1

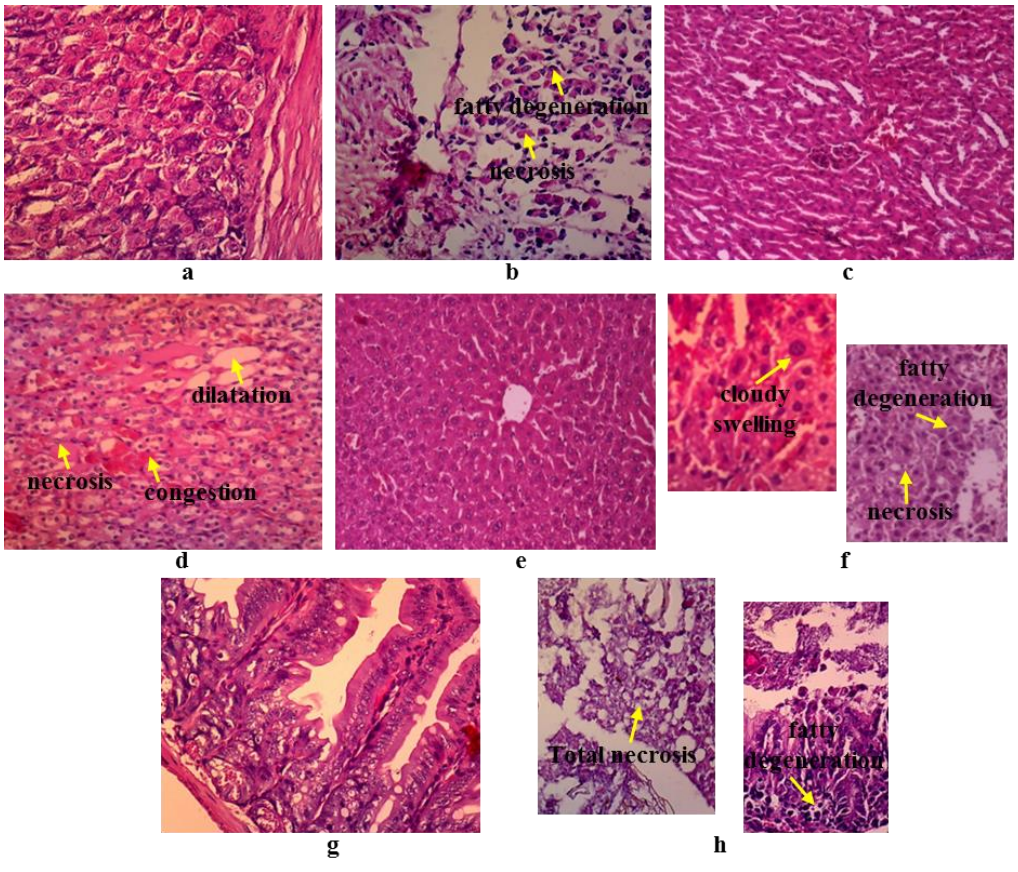

Figure 1. Photomicrographs of the sections of albino mice organs: a) gaster of control mice, b) gaster of treated mice, c) kidney of control mice, d) kidney of treated mice, e) liver of control mice, f) liver of treated mice, g) intestine of control mice, h) intestine of treated mice

In addition, congestion was noticed in the kidney and liver. Kidneys are responsible for the elimination of metabolic waste and the control of the amount and composition of the body fluids. In addition, they are important in the regulation of arterial blood pressure and produce, modify, or degrade substances which affect red blood cell production, calcium balance, and carbohydrate metabolism (Afshar *et al*., 2013). Moderate necrosis and dilatation in proximal tubules of the kidney are similar to those documented in a previous report after intoxication with aqueous extract of *Carica papaya* seeds (Naggayi *et al*., 2015). In our work, cloudy swelling observed in liver and congestion in the kidney and liver. These results are in line with the research by Abou-Hashem (2013) dan Abou-Hashem (2012). Congestion is a manifestation of inflammation due to injury. Toxic injuries were due to the reaction between toxic substances and molecules in the body. Dilatation of capillaries occurred after injury. This is caused by stimulation of vasodilators so that vascularization of the lesion widens and blood becomes blocked/congestion (Muljadi *et al*., 2014).

The most severe damage of mice organs occurred in the liver. The liver plays a major role in nutrition and maintenance of the body. The main function of the liver is the production of bile, maintenance of the blood sugar level, regulation of fat metabolism, detoxification, the formation of plasma protein and blood coagulants (Revathi *et al*., 2006). Fatty degeneration occurred in the gaster, liver, and intestine. This process is reversible and caused by the inhibition of transfer of lipid from the cell and an imbalance of synthesis and release of triglycerides by parenchymal cells into the circulation (Muljadi *et al*., 2014). These changes can be caused by disorders of oxygen supply such as disorders of blood flow or intoxication in gastric, liver and intestine.

Papain was the most obtained enzyme detected in papaya. These enzyme found in the latex of green papaya fruits (Milind *et al*., 2011) and papaya leaf (Welde *et al*., 2018).

Papayas contain the proteolytic enzyme papain, which has the ability to break down protein. This enzyme would cause damage to the cell that causes death for animals. Cell damage occurred by releasing of ribosomes from the endoplasmic granular reticulum, polysomes will spread to the plasma, which causes more acidophilic cytoplasm (Harjana, 2009). Adding salt to natural rodenticide could escalate the damage of mice's internal organs. Wahyuni *et al*., (1995) noticed that hypertrophy occurs in almost all organs of rats which given salted fish with a high level of salt.

CONCLUSION AND RECOMMENDATION

Feed intake of 16 to 32% papain natural rodenticide gave effect to the organs of studied mice, i.e gaster, kidney, liver and intestine. Therefore, further research work is needed to find feed block with right proportion with papain active ingredients as a natural rodenticide to control domestic rats.

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