

A STUDY OF THE TOXICITY OF GNETUM GNEMON IN RATS

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ABSTRAK

Empat puluh ekor tikus putih sapihan dibagi menjadi 4 kelompok dan setiap kelompok terdiri atas 10 ekor hewan. Grup A diberi makan makanan standar dan dipergunakan sebagai kelompok kontrol. Grup B diberi makan percobaan yang mengandung 10% tepung *Gnetum gnemon* (melinjo). Grup C diberi makan percobaan yang mengandung campuran 7.5% *G. gnemon* (GG) dan 2.5% tepung beras. Grup D diberi makan percobaan yang mengandung 7.5% GG dan 2.5% susu skim. Makanan dan air minum diberikan secara *ad libitum* selama masa observasi. Lima ekor tikus dari setiap kelompok dibunuh pada akhir bulan pertama dan bulan kedua. Semasa observasi, gejala klinis yang tampak pada Grup B adalah bulu bulu menjadi kasar dan suram, kurang aktif, lemah dan menjadi kurus. Seekor nampak mengeluarkan cairan berwarna merah dari mata dan hidungnya. Empat ekor dari grup ini mati pada masa observasi bulan pertama. Dua ekor tikus Grup C nampak menjadi kurus dan mati pada hari ke-14. Semua tikus dalam Grup D nampak tumbuh kurang sempurna dan kerdil, tetapi tidak menunjukkan gejala klinis lain. Perubahan anatomis terdiri atas atrofi otot gerak, anemia dan perdarahan serta nekrosis di dalam hati.

INTRODUCTION

Melinjo or *Gnetum gnemon* is a common indigenous tree found throughout the Indonesian archipelago. It is called tulip tree¹ or jointfir tree^{2,3}. This tree has been described in detail by Heyne in 1950⁴.

In Java the young leaves, stems and seeds (fruits) are used in the traditional popular soup called "Sayur Asam" (Tamarind soup) and "Sayur Lodeh" (Mixed vegetables coconut soup). The ripe seeds can be consumed after boiled or roasted, as a snack. The seeds can also be processed into "emping melinjo", which is a dry thin circular chip with diameters varyins from 2 to 10 centimeters after flattened into thin layers, dried and

finally deep fried. It is frequently consumed as a snack or side dish just like potato chips. The young leaves, stems and fruits can be harvested all year round.

Since "melinjo" seed is quite popular, relatively cheap and its products are commonly consumed, it is important to know more about its side effects to health. The present report will discuss about the toxicity of melinjo seeds fed into young rats.

MATERIALS AND METHODS

Experimental Animals : Forty 30-day-old weanling rats were used for this

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experiment. These animals were kept in individual suspended wire mesh cages. Ten rats were allotted to each group.

A total of 5 rats in each group were killed for necropsy at the end of the first and second month of feeding period (Table 1).

Table 1. Mortality and Number of liver Damage in Rats Fed *Gnetum gnemon*

Group	No. Animal	Diet	No. Dead		No. Anim. Kill		No. Liver Lesion	
			1st Mo	2nd Mo	1st Mo.	2nd Mo	1st Mo	2nd Mo
A	10	10% S.M.	0	0	5	5	0	0
B	10	10% GG	4	0	1	5	0	2
C	10	7.5% GG + 2.5% Rice	2	0	3	5	NE	o
D	10	7.5% GG + 2.5% S.M.	0	0	5	5	0	0

GG = *Gnetum gnemon*

S.M. = Skim Milk

NE = Not examined

Necropsy was performed and tissues such as heart, lung, liver, kidneys, digestive tract and muscle tissues were collected and fixed in 10% buffered formalin solution. Fixed tissues were then processed by standard routine fixatives and stained with hematoxyline and eosin for microscopic examinations.

Experimental Diets : Group A : Rats were fed on standard basal diet which was used daily by the Nutrition Research Unit Diponegoro and served as the control group.

Group B : Rats were fed on basal diet which contained 10% *G. gnemon* (GG) powder.

Group C : Rats were fed basal diet

which contained a mixture of 7.5% GG powder and 2.5% rice powder.

Group D : Rats were fed basal diet containing 7.5% GG powder and 2.5% skim milk.

All animals were provided food and water *ad. libitum* through out the entire experimental feeding period.

RESULT

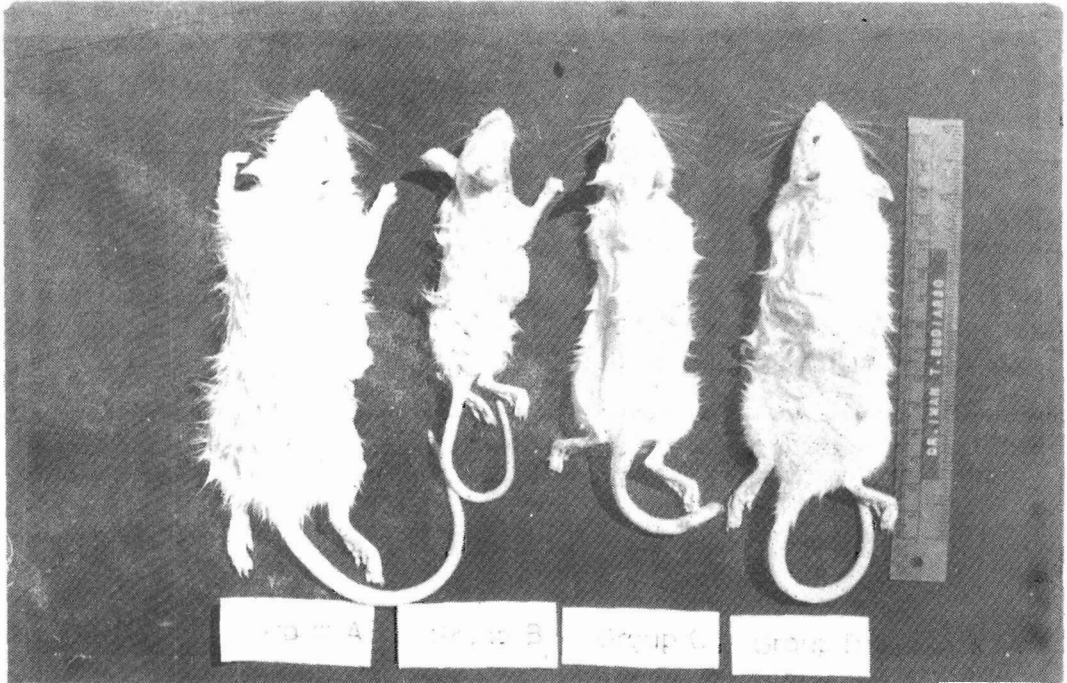
a. CLINICAL SIGNS

1. First Month Clinical Observation : Group A (Control) : Rats of this group had good apitite, appeared alert and were active. They were well and developed normally.

Group B : Some rats had rough hair coats and were less active. Their growth and development were very poor. They

seemed anorexic and did not eat well, subsequently few rats became obviously weak and emaciated (Figure 1). Two rats

Figure 1. Control rat (A) and treated *Gnetum gnetum* rats (A, B,C). Note the marked differences of their body size by the end of the first month feeding period.



showed generalised muscle atrophy of the hind legs and had posterior weakness. Sign of epiphora was observed in one rat by the evidence of redish brown dry crusted tears accumulation around the medial canthuses of the eyes and nares.

Group C : At the beginning they had lack of appetite and grew poorly. Two rats became progressively emaciated and eventually died at day 14. Besides rough hair

coats, no other clinical sign was observed.

Group D : Rats of this group, showed lesser body weith gain compared to the controls, but otherwise no significant clinical sign was noted.

II. Second Month Clinical Observation :

Group A (Control) : All rats continued to be alert, active and healthy as had been described in the first month. Their hair coats were shiny and thrifhty.

Group B : Three rats showed brownish discoloration of their hair coats, especially on the dorsal part of the body. Two rats had partial alopecia around the base of the tail and extended to the upper part of the thigh. In general all rats were unthrifty and had smaller body size and weight as compared to the controls. No other clinical sign was noted

Group C and Group D : Except for slight brownish discoloration as had been described in Group B, they had slightly smaller body size; in general they all were alert and active.

b. GROSS FINDINGS

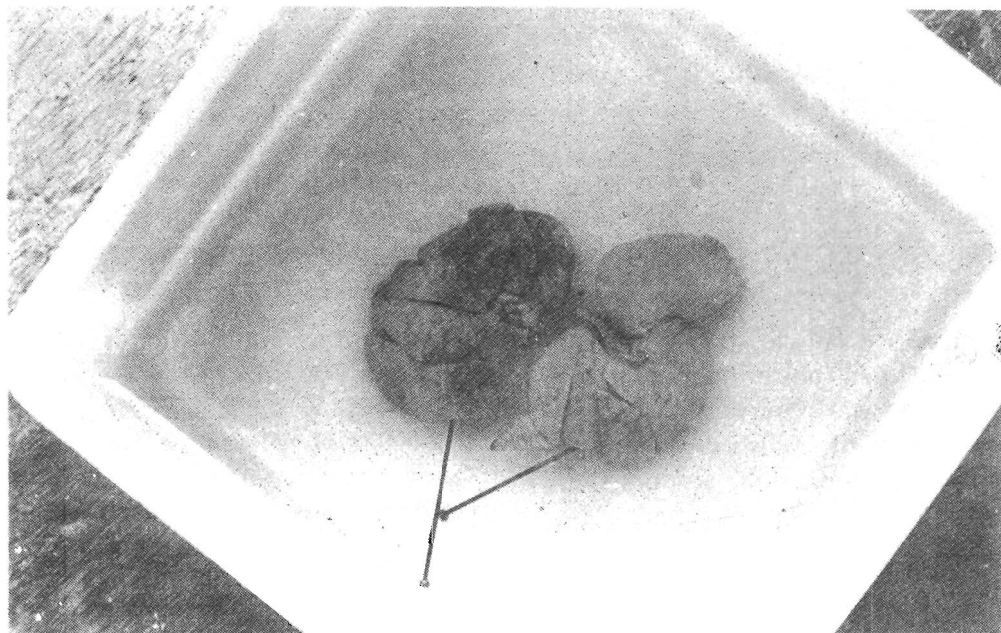
For convenience, the gross findings of all rats either had been killed at the

end of the first month or second month period, will be described together.

Group A : All rats showed excellent body condition by the evidence of abundance of fat deposits in subcutaneous tissue, omentum, mesentery, pericardium, and peri-renal regions. No lesion was found in the visceral organs.

Group B : In general all rats were smaller in size and their bodies showed lack of fatty deposition. Two rats which were killed at the end of the second month showed streaks and patches of haemorrhage in their liver, especially affecting the anterior surface of the lobes and concentrated in hylus area (Figure 2). All other organs showed no any significant change.

Figure 2. Livers of test rats fed 10% *Gnetum gnemon*. Note few to numerous streaks and patches of haemorrhage (darker areas) in the parietal surface of the liver lobes (Formalin fixed and immersed in water).



Group C : All animals in this group were unthrifty and had meager fatty tissue deposition in their bodies. Otherwise all visceral organs appeared normal.

Group D : The body condition and fatty deposition of all animals in this group were similar to those described in Group C. No other gross lesion was observed.

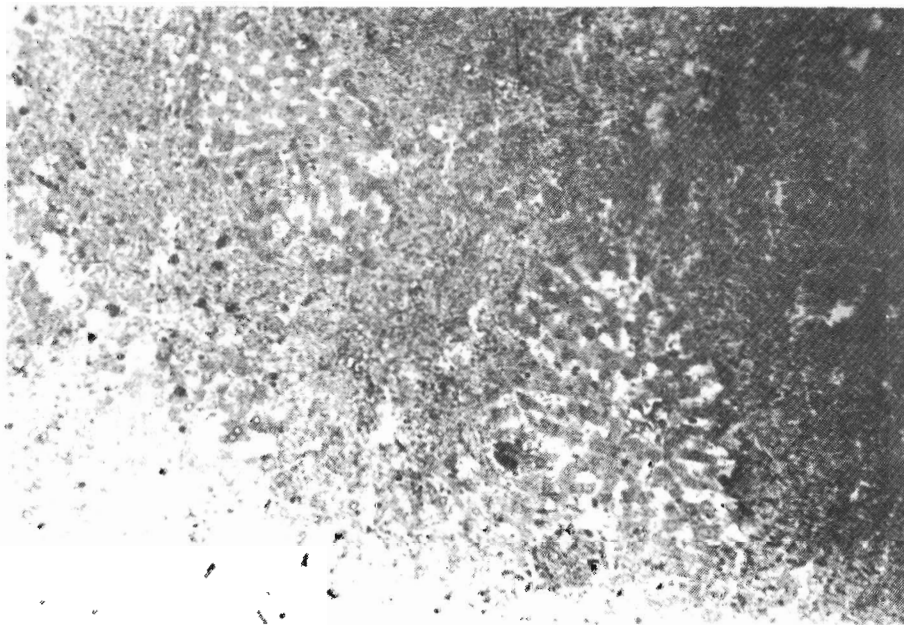
c. HISTOLOGICAL FINDINGS

Group A : All control animals had no microscopic change in their visceral organs and musculatures.

In GG treated rats, besides of the above mentioned findings in the clinical signs and gross findings obvious microscopic lesions were mainly found in 2 animals which were fed on 10% GG powder (Group B). These changes consisted of moderate haemorrhages, hepatocytes degeneration and perilobular hepatic cell necrosis. Inflammatory reaction was not apparent and the bile ducts were not affected (Table 1 and Figure 3).

Two rats in Group C were not examined due to severe post mortum autolysis.

Figure 3. Liver of rat fed 10% *Gnetum gnemon*. Note difuse perilobular coagulative necrosis of hepatic cells. H & E, 100x.



DISCUSSION

All rats which were treated with *G. gnemon* (GG) were either unthrifty or stunted in growth. They showed also brownish discoloration and partial alopecia of their hair coats, with addition of posterior weakness and emaciation. All of these signs seemed to indicate that GG contained toxic substances. At present, the only known toxic agent contained in GG is hydrocyanic acid (HA). Acute HA poisoning in animals, however, usually will cause dizziness, hypernea, cyanosis and aphecial convulsions preceding to their death, without any gross lesion in the organs at necropsy.

In this study it was observed that the toxic substances had affected more severely the younger rats, by the evidence that 2 rats died within 2 weeks of feeding period. However, among the survivors which grew older, although clinically, they appeared to be less susceptible. but on macroscopic examination, their livers showed streaks and patches of haemorrhage and necrosis.

Histologically, the liver showed perilobular necrosis, which means that the toxic substances were brought to the lobules by blood stream without any impairment of the blood circulation and oxygenation of the cells. In this case, peripheral hepatic cells received the most toxic blood and this resulted in the most serious effects.

If this was solely due to HA poisoning, the clinical signs and pathological changes in these study animals would not

fit the description, since in chronic HA poisoning with repeated minute doses over a long period of time have produced only multiple necrotic foci of the brain, without affecting the liver⁵. Thus it is suspected that GG may contain other unknown toxic substance (s) in addition to the well known HA. In our analysis, the GG used in this study happened to contain 60 ppm HA.

The possibility that GG powder used in this study may contain other hepatotoxic substance(s) beside HA could not be neglected. A further study to elucidate the incidence of hepatic damage because of GG consumption is warranted

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