

## **The Effect of Rambutan Seed (*Nephelium lappaceum* L.) Infusion on Blood Glucose and Pancreas Histology of Mice Induced with Alloxan**

### **(Pengaruh Air Seduhan Biji Rambutan (*Nephelium lappaceum* L.) terhadap Glukosa Darah dan Histologi Pankreas Mencit yang Diinduksi Aloksan)**

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**Abstract:** Rambutan (*Nephelium lappaceum* L.) is a plant which can be used to treat various diseases, including diabetes mellitus. This research was conducted to investigate the effect of rambutan seed infusion on the mice blood glucose levels and body weight. In this research, 30 mice (*Mus musculus*) were divided into 6 groups, each group consists of 5 mice. The 6 groups consists of a normal control group (distilled water), negative control group (alloxan tetrahydrate 250 mg/kg bw + distilled water), three experimental groups (induced with alloxan 250 mg/kg bw) were given rambutan seed infusion of 1.56 gram/kg bw, 2.34 gram/kg bw and 3.12 gram/kg bw and a positive control group (alloxan tetrahydrate 250 mg/kg bw + treated with glibenclamide 0.65 mg/kg bw). After given treatment for 16 days, it was observed that high dose of rambutan seed infusion has significant effects in reducing the blood glucose and body weight of mice. Histology of the pancreas were checked and the ammount of live pancreatic beta cells found in high dose of rambutan seed infusion were almost the same as the positive control group. It can be concluded that the effect of high dose of rambutan seed infusion (3.12 gram/kg bw) was not significantly different from glibenclamide (0,65 mg/kg bw).

**Key words:** losartan, chitosan, nanoparticle, factorial design.

**Abstrak:** Rambutan (*Nephelium lappaceum* L.) merupakan salah satu tanaman yang dapat digunakan untuk mengobati berbagai macam penyakit, salah satunya diabetes melitus. Penelitian ini dilakukan untuk menguji efek seduhan biji rambutan terhadap penurunan kadar glukosa darah dan berat badan mencit. Dalam penelitian ini 30 mencit (*Mus musculus*) dibagi menjadi 6 kelompok perlakuan, masing-masing kelompok terdiri dari 5 ekor. Keenam kelompok tersebut adalah kontrol normal (air suling), kontrol negatif (aloksan 250 mg/kg bb + air suling), tiga kelompok uji (diinduksi dengan aloksan tetrahidrat 250 mg/kg bb) kemudian masing-masing kelompok diberi air seduhan biji rambutan dosis 1,56 g/kg bb, 2,34 g/kg bb dan 3,12 gram/kg bb) dan kontrol positif (diinduksi aloksan tetrahidrat 250 mg/kg bb + glibenklamida 0,65 mg/kg bb). Setelah diberikan perlakuan selama 16 hari diperoleh hasil bahwa pemberian air seduhan biji rambutan pada dosis tinggi mampu menurunkan kadar glukosa darah dan berat badan secara signifikan. Pemeriksaan histologi pankreas mencit dilakukan terhadap kelompok perlakuan dan diketahui bahwa jumlah sel beta yang hidup pada kelompok air seduhan biji rambutan dosis tinggi hampir mendekati kelompok kontrol positif. Dapat disimpulkan bahwa khasiat air seduhan biji rambutan dosis 3,12 gram/kg bb tidak berbeda secara signifikan dengan glibenklamida 0,65 mg/kg bb.

**Kata kunci:** biji rambutan, *Nephelium lappaceum* L , antidiabetes, aloksan, mencit.

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## INTRODUCTION

CHANGES in the community's lifestyle can lead to a shift in the disease pattern from infection to long term/chronic degenerative diseases. Among the chronic diseases associated with metabolism and are likely to increase every year is diabetes mellitus. The main cause of this disease is the reduction of insulin hormone produced by the beta cells of the pancreas which plays an important role in glucose metabolism in body cells<sup>(1)</sup>.

Along with the development of pharmaceutical science and technology, the use of medicinal plants in curing various diseases are increasing rapidly. This is because the drugs price in markets are relatively high. Therefore, people are trying to find alternatives in treating diseases by using natural products/remedies. In addition, these products are readily available, relatively inexpensive and have fewer side effects compared to synthetic drugs<sup>(2)</sup>.

Rambutan (*Nephelium lappaceum* L.) is a plant which produces fruits that is known to be delicious. Nowadays, this plant is thought to have other benefits besides producing fruits. It is suspected that the seed of this plant has an effect in lowering blood sugar levels. Previous studies conducted by other researcher have proven that methanol extract of the rambutan seeds has a hypoglycemic activity compared to the negative controlled group<sup>(3)</sup>.

This is due to the high content of carbohydrates, protein, fat and polyphenols found in the ripend rambutan seeds. Other constituents are chemical compounds such as fatty acids and phenolic compounds of the flavonoid class<sup>(3)</sup>.

This study aims to determine the effect of Rapih rambutan (*Nephelium lappaceum* L.) seed infusion in decreasing blood glucose levels and its effect on the repair of pancreatic beta cells in diabetic mice induced by alloxan. The purpose of the research is to provide information to the community about the benefit and efficacy of consuming rambutan seed infusion.

## MATERIALS AND METHODS

**MATERIALS.** Rambutan seeds (*Nephelium lappaceum* L.) are obtained from a rambutan tree in Jl. O 3, RT 10 RW 3, Srengseng Sawah, Jagakarsa, South Jakarta and has been verified by the Center for Biology-LIPI, Bogor; thirty male adult mice (*Mus musculus*) ddY strain aged 2-3 months with weight 20-35 g, obtained from the Faculty of Animal Science, Bogor Agricultural University; alloxan tetrahydrate 1% (hyperglycemic inducer), glibenclamide (standard antidiabetic medicine for positive control group).

Instruments used in this research were oral sonde and Life Scan Glucometer Strip Sure.

**METHODS.** The study was conducted in the Biochemistry Laboratory and the Pharmacology Laboratory of the Faculty of Pharmacy, Pancasila University, Srengseng Sawah, Jagakarsa, South Jakarta.

**Preparation of rambutan seed dry powder.** The flesh of the ripe rambutan fruit was separated from the seed. Then the seed was chopped into small parts and dried. This dried chopped seeds were then milled into powder and ready for use.

**Preparation of rambutan seed infusion.** Twelve grams of rambutan seed dry powder were brewed (steeped) with boiling water until 100 mL volume was collected. Stir for about ten minutes, then let it cool.

Thirty male ddY strain mice (*Mus musculus*), age of 2-3 months and weight of 20-35 g were used in this research. All mice were adapted (acclimatized) for one week to adjust to the new environment and food uniformity and also to achieve weight target<sup>(4)</sup>.

There were six experimental groups consists of 5 mice in each group. Group I were normal control mice given standard food, distilled water, and received no treatment; Group II were negative control mice given standard food, distilled water and alloxan; Group III were mice given standard food, distilled water, alloxan and given low dose (1.56 g/kg bw) of water rambutan seed infusion; Group IV were mice given standard food, distilled water, alloxan and medium dose of rambutan seed infusion (2.34 g/kg bw); and Group V were mice given standard food, distilled water, alloxan and high dose of rambutan seed infusion (3.12 g/kg bw); and Group VI were positive control mice given standard food, distilled water, alloxan and oral antidiabetic suspension of glibenclamide (0.65 mg/kg bw).

The mice in the groups above were given alloxan tetrahydrate to achieve hyperglycemic stage. This compound works by destroying the pancreatic  $\beta$  cells. The dose used was 250 mg/kg bw by daily intraperitoneal injection for 10 days. This dose is effective in increasing blood glucose levels of mice based on the initial test conducted previously<sup>(5)</sup>.

Before being treated, blood are drawn on the tenth day from the mice that have been induced by alloxan tetrahydrate. This is done to determine the hyperglycemic condition of the mice that have been fasted for 16 hours. These mice were then treated with rambutan seed infusion for 16 days. Blood were drawn from the tail vein and measurement of blood glucose were performed on day 10<sup>th</sup>, 14<sup>th</sup>, 18<sup>th</sup>, 22<sup>nd</sup> and 26<sup>th</sup> by using glucometer. The weight of these mice were also monitored once every 2 days (after injection of

alloxane) on day 0, 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup>, 16<sup>th</sup>, 18<sup>th</sup>, 20<sup>th</sup>, 22<sup>nd</sup>, 24<sup>th</sup> and 26<sup>th</sup>(4).

Data of blood glucose levels and body weight were then analyzed using SPSS 13.0 program, one-way ANOVA ( $p < 0.05$ ) and then followed by Least Significant Difference test (LSD)(6).

After measurement of blood glucose level and body weight, pancreas of four mice were taken on day 26<sup>th</sup> from each experimental group and histology of pancreas were inspected. Staining were carried out to observe specific characters of pancreatic beta cells and the number of pancreatic beta cells calculated.

## RESULTS AND DISCUSSION

Male mice were used in this experiment to minimize the hormonal influence which can affect the blood glucose levels. Administration of the right dose of alloxan into mice will selectively destroys the  $\beta$  cells of Langerhans islands(7) causing the animal's insulin production to decrease. The hyperglycemic effect usually will occur after 2-3 days. When mice are treated with alloxan, the cells in the islands of Langerhans show changes such as atrophy, hypertrophy and hyperplasia, depending on the length of the treatment, which results in the disruption of pancreatic  $\beta$  cells to produce insulin and may lead to elevated blood glucose levels of mice(8). The dose used is based on the empirical dose of one tablespoon (12 g) per day for diabetic patients.

Results from this experiment showed that the hyperglycemic mice for groups II, III, V and VI lost weight on the 10<sup>th</sup> day of observation and re-gained their weight on day 14<sup>th</sup>. This condition was caused by the depletion of fat cells and proteins in order to meet energy needs that can not be fulfilled by glucose metabolism(9). On the last day of experiment (day

26<sup>th</sup>), the average weight of all mice have increased. This was due to the supply of energy can be fulfil by existing glucose metabolism, so there would be no cell depletion of fat and protein.

The data used for this analysis is obtained by calculating the differences in body weight of mice before given treatments on day 10<sup>th</sup> with their body weight after given treatments on day 26<sup>th</sup>. Complete data can be seen in Table 1.

Table 1 and Figure 1 showed that the highest differences in the body weight reduction are found in group III (the low dose group), especially in mice number 5. One-way analysis of variance (ANOVA) was tested and showed significant result with value of 0.015 ( $p < 0.050$ ), indicating a significant differences in the body weight before and after treatment between group II, III, IV, V and VI.

The following Least Significant Difference (LSD) test for the difference in body weight of mice before and after treatment are shown in Table 2. This table shows that there is a noticeable difference in the weight between group III and VI with group II. This indicates that there is a significant difference in the body weight reduction between groups given low

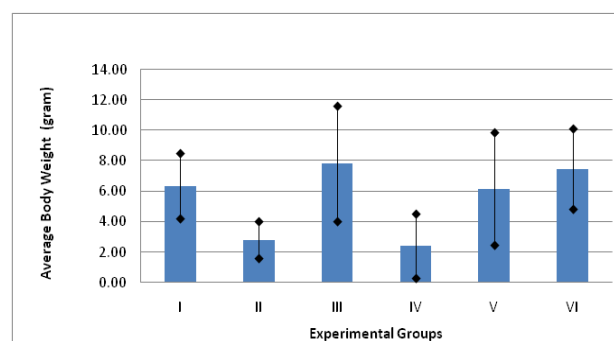


Figure 1. Average of body weight differences before and after treatment.

Table 1. Data differences in body weight of mice before and after treatment.

Mice number	Mice body weight (gram)					
	I	II	III	IV	V	VI
1	6.10	0.90	11.80	4.50	10.30	8.50
2	6.00	2.70	8.10	2.40	9.30	6.60
3	3.40	2.70	1.70	1.90	4.00	11.30
4	6.90	4.10	9.80	4.10	5.90	4.20
5	9.40	3.60	7.80	0.80	1.40	6.80
x	6.36	2.80	7.84	2.74	6.18	7.48
SD	2.15	1.22	3.78	1.54	3.69	2.63

Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

**Table 2. LSD test of differences in body weight of mice before and after treatment.**

Group	Mean	Mean differences				
		II	III	IV	V	VI
II	2.80	-				
III	7.84	5.04*	-			
IV	2.74	0.06	5.10*	-		
V	6.18	3.38	1.66	3.44	-	
VI	7.48	4.68*	0.36	4.74*	1.30	-

\*significant difference

Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

dose of rambutan seed infusion and glibenclamide with the negative control group. It can be concluded that the experimental groups have different respond with the control group. Significant results are also seen between group VI with group III and IV. This means that the groups treated with low dose and medium dose of rambutan seed infusion cannot give the same respond in reducing the body weight as the positive group.

There is no real difference in weight between the group IV and V with group II; group V and VI with group III; group V with group IV; and group VI with group V. This means that low dose and high dose of rambutan seed infusion gives the same respond as the positive control group (glibenclamide) in reducing the body weight of mice.

#### Antidiabetic testing in mice induced by alloxan.

On day-0, intraperitoneal induction with alloxan tetrahydrate 250 mg/kg bw were carried out to all groups, except group I. After 10 days of treatment with alloxan tetrahydrate to groups II, III, IV, V and VI, there were an increased in blood glucose levels. The value of the blood glucose in these groups ranges from 200 to 281 mg/dL. This showed that alloxan has the effect of inducing hyperglycemia on experimental animals. Literature stated that mice is hyperglycemic when their fasting blood glucose level are between 62.8 to 176 mg / dL<sup>(10)</sup>.

On day 14<sup>th</sup>, the blood glucose levels of mice treated with low dose (1.56 g/kg bw, Group III) of rambutan seed infusion decreased to 144-179 mg/dL. The blood glucose level measured here is still classified to be hyperglycemic. The range for the normal blood glucose levels in mice is 94.40 to 110 mg/dL. This value is achieved from the normal control group in this experiment.

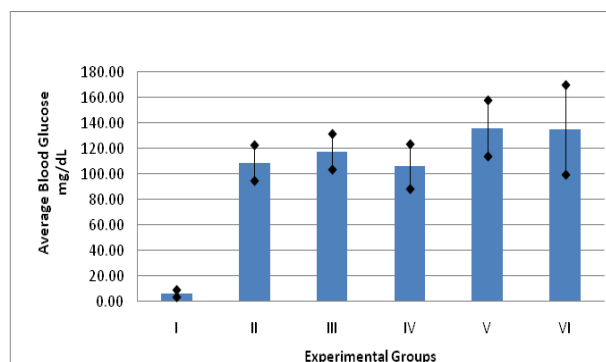
On day 18<sup>th</sup>, the blood glucose levels decreased in group treated with medium dose (2.34 g/kg) and high dose (3.12 g/kg BW) of rambutan seed infusion

and also positive control group (glibenclamide, 0.65 mg/kg bw). But the level in group treated with low dose of rambutan seed infusion (1.56 g/kg bw) have returned to normal.

On day 22<sup>nd</sup>, the blood glucose levels decreased in the experimental groups given low, medium and high dose of rambutan seed infusion and the control positive group. In the negative control group (not given any treatment) also undergo a decreased in the blood glucose level, but this group is still categorized to be in hyperglycemic conditions because the average blood glucose were 171-235 mg/dL.

On day 26<sup>th</sup>, the blood glucose levels in all experimental groups has decreased to 80-134 mg/dL and it can be stated that these mice has reached a normal blood glucose level.

Analysis of the difference in blood glucose levels in mice before and after administration of alloxan is obtained by calculating the difference in blood glucose levels prior to alloxan administration (day 0) and blood glucose levels after alloxan administration (day 10<sup>th</sup>). Data of differences in blood glucose levels of mice can be seen in Table 3. Graphic which shows the average value of the differences in blood glucose level and its standard deviation (after administration of alloxan) can be seen as Figure 2.



**Figure 2. Average of blood glucose differences before and after induction with alloxan.**

**Table 3. Data of differences in blood glucose levels before and after alloxan tetrahydrate administration.**

Mice number	Mice blood glucose level (mg/dL)					
	I	II	III	IV	V	VI
1	9.00	118.00	111.00	98.00	133.00	190.00
2	7.00	127.00	109.00	114.00	114.00	147.00
3	6.00	93.00	112.00	80.00	150.00	104.00
4	2.00	110.00	115.00	117.00	166.00	129.00
5	8.00	97.00	142.00	123.00	117.00	107.00
X	6.40	109.00	117.80	106.40	136.00	135.40
SD	2.70	14.20	13.70	17.42	22.08	35.18

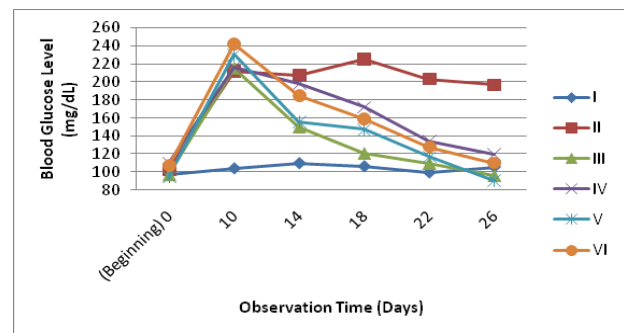
Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

Based on one-way analysis of variance (ANOVA) on the data found in Table 3, a significance of 0.123 ( $p > 0.050$ ) were obtained. This showed that the differences of blood glucose before and after alloxan administered to group II, III, IV, V and VI are not significant. Meaning that the blood glucose among these groups are the same.

Analysis of the differences in blood glucose levels before and after treatment is obtained by calculating the differences in blood glucose levels before given treatment on day 10<sup>th</sup> with blood glucose levels after given treatment on day 26<sup>th</sup>.

The measurement of blood glucose in mice during treatment from day 0 to day 26<sup>th</sup> can be seen in Figure 3. From the figure, it can be understood that the blood glucose reaches the highest value (peak) on day 10<sup>th</sup> for experimental group III, IV, V and VI. After that the blood glucose will fall until day 26<sup>th</sup>. The blood glucose in group I will reach its peak on day 14<sup>th</sup> and group II on day 18<sup>th</sup>. The data of differences in blood glucose levels can be seen in Table 4.

**Figure 3. Measurement of blood glucose during treatment from day 0 to day 26<sup>th</sup>.**

From the data in Table 4, it can be seen that the highest reduction of blood glucose are found in group V. The value of this reduction is 140 mg/dL. High dose of rambutan seed infusion has the best potential in reducing the blood glucose.

In the one way ANOVA analysis, a significance value of 0.000 ( $p < 0.050$ ) was obtained. This represents a significant differences in blood glucose levels before and after treatment between groups II, III, IV, V and

**Table 4. Data of differences in blood glucose levels before and after treatment.**

Mice number	Mice blood glucose level (mg/dL)					
	I	II	III	IV	V	VI
1	6.00	10.00	106.00	86.00	95.00	159.00
2	9.00	39.00	122.00	105.00	116.00	140.00
3	3.00	13.00	111.00	92.00	183.00	101.00
4	20.00	13.00	112.00	79.00	163.00	146.00
5	5.00	21.00	143.00	117.00	143.00	115.00
x	8.60	19.20	118.80	95.80	140.00	132.20
SD	6.73	11.80	14.72	15.22	35.31	23.66

Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

Table 5. LSD test of differences in mice blood glucose before and after treatment.

Group	Mean	Mean differences				
		II	III	IV	V	VI
II	23.40	-				
III	118.80	95.40*	-			
IV	95.80	72.40*	23.00	-		
V	140.00	116.60*	21.20	44.20*	-	
VI	132.20	108.80*	13.40	36.40*	7.80	-

\*significant difference

Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

Table 6. Ammount of pancreatic  $\beta$  cells in Langerhans island.

Mice number	Groups (ammount of pancreatic $\beta$ cells)					
	I	II	III	IV	V	VI
1	138.50	27.90	64.90	79.98	105.11	114.13
2	131.17	38.40	67.00	84.17	98.37	116.30
3	134.80	34.81	56.87	78.67	107.00	102.70
4	142.15	24.30	59.33	82.86	96.50	100.54
X	136.66	31.35	63.17	81.42	101.75	108.42
SD	4.73	6.41	5.42	2.53	5.09	7.95

Note:

I: Normal control group; II: negative control group; III: low dose group; IV: medium dose group; V: high dose group; VI: positive control group.

VI. The Least Significant Difference Test (LSD) are conducted and the result can be seen in Table 5.

Based on the above data it can be seen that on day 26<sup>th</sup> (last day) all experimental groups have shown significant differences in blood glucose levels. This is due on day 26<sup>th</sup>, all experimental groups showed normal blood glucose levels in all treated mice.

From the LSD analysis, there is a noticeable difference in the blood glucose level between group III, IV, V and VI with group II. This means that the blood glucose level in the negative control group are different with others and the treatment given to mice in other groups are giving good response in reducing blood glucose. Significant difference are also seen between group V and VI with group IV. This means that the effect given by group V and VI are different than group IV.

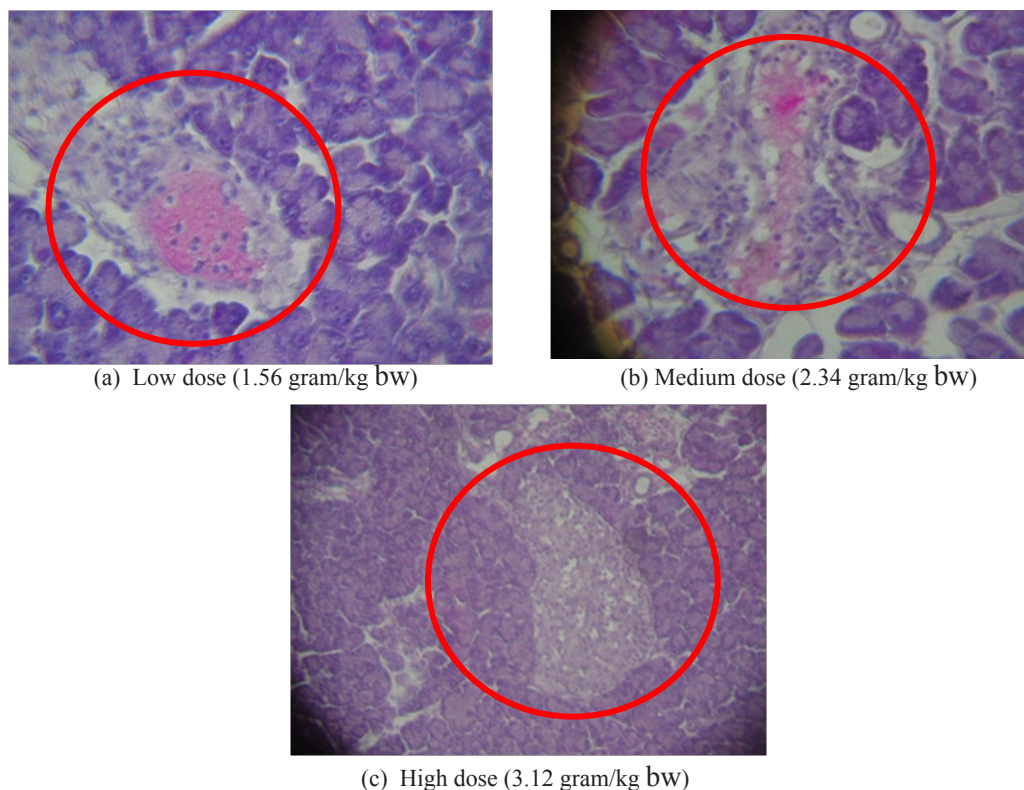
There is no real difference in blood glucose level between the group IV, V and VI with group III; and also between the group VI with V. This means that the effect in blood glucose reduction given by high dose rambutan seed infusion are the same as the positive control (glibenclamide). So it can be concluded that high dose of rambutan seed infusion has the same

potential as glibenclamide in reducing blood glucose.

**Histology of pancreas.** Effect of alloxan induction on the pancreatic cells is reducing the number of intact  $\beta$  cells in the Langerhans island. The number of  $\beta$  cells in Langerhans island was calculated by this formula:  $X = \Sigma$  (ammount of pancreatic  $\beta$  cells)  $\div$  ammount of Langerhans islands.

In this study, only 4 mice were taken from each group to be examined for their pancreas histopathology. This examination only provide a descriptive information. The number of  $\beta$  cells counted are taken from mice that experienced the largest margin decreased in glucose levels in each group. The results of the calculations are shown in Table 6. Observations are made to the ammount of pancreatic  $\beta$  cells.

The most ammount of pancreatic  $\beta$  cells are found in the normal group (group I). Comparison are made to this group and it can be seen that other groups do not have the same ammount of cells. In the experimental groups, it showed that the highest dose of rambutan seed infusion (group V) can protect/repair the pancreatic  $\beta$  cells from damages or destructions caused by alloxan tetrahydrate. The destruction of pancreatic



**Figure 4. Illustration of the pancreatic  $\beta$  cells in mice given rambutan seed infusion.**

$\beta$  cells by alloxan tetrahydrate is irreversible and this can be observed in group II where the amount of pancreatic  $\beta$  cells are the least found.

One of the important purpose in this research is to find the dosage of rambutan seed infusion that has the same or closest effect towards the pancreatic  $\beta$  cells as the positive control group. That is why comparison are also made to positive control group (group VI). It can be seen that high dose of rambutan seed infusion (group V) has the amount of pancreatic  $\beta$  cells closest to this group.

The illustration of living pancreatic  $\beta$  cells and dead cells caused by alloxan tetrahydrate can be seen in Figure 4. In the low and medium dose of rambutan seed infusion it can be observed that most of the pancreatic  $\beta$  cells are destructed and seems dissolved, however in the high dose the cells seems to be in normal forms.

## CONCLUSION

It can be concluded that the rambutan seed infusion has an effect in reducing the blood glucose level and body weight of mice induced with alloxan tetrahydrate. The 3.12 g/kg bw dose of rambutan seed infusion given to mice achieved similar reduction in blood glucose level and body weight with the positive control treated daily with glibenclamide 0.65 mg/kg bw.

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