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Effect of Specific Formulated Feed to Alter the Glucose, Triglycerides and Total Cholesterol Level in Rat

(EFEK FORMULASI PAKAN TIKUS TERHADAP PERUBAHAN KADAR GLUKOSA, TRIGLISERIDA DAN KOLESTEROL TOTAL)

Jihan Mudrika Rahmi[1], Ronny Lesmana[2,3,7*], Hanna Goenawan[2,3], Setiawan[2,3], Vita Murniati Tarawan[2,3], Okta Wismandanu[4,5], Unang Supratman[3,6]

[1]Faculty of Medicine, UniversitasPadjadjaran, Jalan Raya Bandung-Sumedang km.21, Jatinangor, Sumedang
West Java, Indonesia45363, Email: Jihan15002@mail.unpad.ac.id
[2]Physiology Division, Departement of Basic Science,
Faculty of Medicine, UniversitasPadjadjaran,
Jalan Raya Bandung-Sumedangkm.21, Jatinangor, Sumedang
West java Indonesia. 45363, Email: Ronny@unpad.ac.id
[3]Physiology Molecular Laboratory, Biological Activity Division,
Central Laboratory, UniversitasPadjadjaran
Jalan Raya Bandung-Sumedangkm.21,Jatinangor,
Sumedang, West Java, Indonesia45363,
[4]Veterinary Study Program, Faculty of Medicine, UniversitasPadjadjaran,
Jalan Raya Bandung-Sumedangkm.21,Jatinangor, Sumedang
West Java, Indonesia45363,
[5]Department of Public Health, Faculty of Medicine,
UniversitasPadjadjaran, Jalan Raya Bandung-Sumedangkm.21,Jatinangor,
Sumedang, West Java, Indonesia45363,
[6]Department of Chemistry, Faculty of Mathematics and Natural Sciences
,UniversitasPadjadjaran. Jalan Raya Bandung-Sumedangkm.21,Jatinangor,
Sumedang,West Java, Indonesia45363,
[7]Center of Excellent in Higher Education for
Pharmaceutical Care Innovation, UniversitasPadjadjaran.
Jalan Raya Bandung-Sumedangkm.21,Jatinangor, Sumedang
West Java, Indonesia, 45363,

ABSTRACT

Understanding the molecular mechanism of type 2 diabetes mellitus using human sample is very difficult, therefore utilization of animal model can be the solution for studying diabetes mellitus. However, there is still limited option of the animal model that can describe the process of type 2 diabetes mellitus. The aim of this study is to develop a formulated feed with increased glucose, triglycerides, and total cholesterol as characteristics of type 2 diabetes mellitus. This study was done using a descriptive method and experimental study design for 8 weeks. Total 9 male and 12 female *Wistar* rats were randomly divided into 3 groups: 1 control group and 2 treatment group (formula 1: high carbohydrate, formula 2: high fat). The increase of delta mean body weight was higher in the group given formula 1 for both male and female rats (f: 52g, m: 71.67g). The glucose level was increase from 4th- 8th week in both gender of rats and higher in the group given formula 1 of female rats (4W: 221.13 mg/dl; 8W: 249.83 mg/dl). The triglycerides level was increased from 4th to 8th week and higher in group given formula 2 either male (4W: 37.42 mg/dl; 8W: 58.92 mg/dl) but not in female rats (4W: 76.78 mg/dl; 8W: 71.22 mg/dl). The cholesterol total level also increased in male (4W: 93.05 mg/dl; 8W: 282.5 mg/dl) and female (4W: 101.58 mg/dl; 8W: 227.17 mg/dl) rats and higher in the group given formula 1. In conclussio, either high carbohydrate or high fat pellet showed potential capability to increase the glucose, triglyceride and total cholesterol level especially in female rats.

Keywords: **g**lucose; specific formulated feed; total cholesterol; triglycerides.

ABSTRAK

Pemahaman mengenai mekanisme molekuler diabetes melitus tipe 2 menggunakan sampel manusia sangat sulit, oleh karena itu adanya model hewan dapat menjadi solusi untuk mempelajari diabetes melitus. Namun, hingga saat ini pilihan hewan model yang ada masih terbatas untuk menggambarkan proses diabetes melitus tipe 2. Penelitian ini bertujuan untuk mengembangkan pakan yang diformulasikan yang dapat meningkatkan glukosa, trigliserida, dan kolesterol total sebagai karakteristik diabetes melitus tipe 2. Penelitian ini menggunakan metode deskriptif dan desain penelitian eksperimental selama delapan minggu. Total sembilan jantan dan 12 betina tikus Wistar dibagi secara acak menjadi tiga kelompok: 1 kelompok kontrol dan 2 kelompok perlakuan (formula 1: karbohidrat tinggi, formula 2: lemak tinggi). Peningkatan delta berarti bobot badan lebih tinggi pada kelompok yang diberi formula 1 untuk tikus jantan dan betina (f: 52 g; m: 71,67 g). Glukosa meningkat dari minggu ke-8 pada kedua jenis kelamin tikus dan lebih tinggi pada kelompok diberikan formula 1 dari tikus betina (4W: 221,13 mg/dL; 8W: 249,83 mg/dL). Trigliserida meningkat dari minggu ke-4 hingga ke-8 dan lebih tinggi pada kelompok yang diberi formula 2 pada jantan (4W: 37,42 mg/dL; 8W: 58,92 mg/dL) tetapi tidak pada tikus betina (4W: 76,78 mg/dL; 8W: 71,22 mg/dL). Kolesterol total juga meningkat pada tikus jantan (4W: 93,05 mg/dL; 8W: 282,5 mg/dL) dan betina (4W: 101,58 mg/dL; 8W: 227,17 mg/dL) dan lebih tinggi pada kelompok yang diberi formula 1. Simpulannya, baik pada formula pakan bentuk pelet dengan kandungan carbohydrate tinggi atau lemak tinggi berpotensi meningkatkan kadar glukosa, triglyceride dan total cholesterol, khususnya pada tikus-tikus betina.

Kata-kata kunci**:** glukosa; kolesterol total; pakan formulasi spesifik; trigliserida

INTRODUCTION

Type 2 diabetes mellitus is a complex metabolic disorder that is influenced by environmental factors (such as obesity, age) and genetics (Chatzigeorgiou *et al.,* 2009). It is characterized by insulin resistance, impaired insulin secretion, and increased blood glucose (King *et al.,* 2012). The prevalence over the past three decades from 1980-2014 continued to increase from 4.5% to 8.5%, and it still a burden and causes of death in most countries (World Health Organization, 2016).

Understanding on pathophysiology of type 2 diabetes is important due to the high number of patient with type 2 diabetes mellitus. But understanding the molecular mechanism of type 2 diabetes mellitus using human sample is very difficult, therefore studies on diabetes mellitus can be obtained with animal models. In recent years animal model development has been carried out with pancreatectomy, genetic manipulation, use of diabetogenic drugs (alloxan, streptozotocin, ditizona, ferric nitrioltriacetate), and the administration of glucose per oral (Etuk, 2010).

Rodents are the most commonly used as animal model to mimic type 2 diabetes mellitus due to their small size, can be used in large quantities at same time, easy to modify its diet, allowing control of confounding factors, and in biomedical research are used to see pathological process spontaneously or induced because the conditions that occur in the animal model resemble human. Various type of studies use animal model to learn about type 2 diabetes mellitus had reported that rat became obese, hyperglycemia, dyslipidemia, by giving streptozotocin and/or modification of the diet (Adeyi *et al.,* 2012; Chatzigeorgiou *et al*., 2009; King., 2010; McMullen *et al.*, 2006; Srinivasan *et al.,* 2005). However, there has been no natural model induced using formulated feed that can describe the process of type 2 diabetes mellitus. Therefore, finding suitable animal models of type 2 diabetes mellitus naturally becomes one of the best solutions to study the pathophysiology mechanism naturally in type 2 diabetes mellitus. Based on the known baseline information, the aim of this study is to develop a formulated feed with increased glucose, triglycerides, and total cholesterol of rat blood as characteristics of type 2 diabetes mellitus and for clinical use, it can

help us to prevent type 2 diabetes mellitus by reflect on the type of feed and amount of the nutrient, that could increase possibility type 2 diabetes mellitus.

RESEARCH METHODS

Pellet Making

The formulated pellet was made in Laboratorium Sentral Universitas Padjadjaran. The first formula contains 48% carbohydrate, 22% fat, 20% protein; the second formula contains 22% carbohydrate, 48% fat, 20% protein. All cattle fat were used as the source of fat in the formula were melted with stove, the granulated sugar as the source of carbohydrate was smoothed with blender, and the normal pellet as the source of protein was smashed with mortar. All the ingredients were mixed in super mixer then place it into the baking sheet and put it in the oven for 12 hours to dry it. The first formula was added talcum and magnesium stearate as adhesive and formed into a pellet using rotatory tablet press. The second formula cannot be made into pellet form due to higher fat content.

Animals

Male and female *Wistar* rats from Biofarma Laboratory, Bandung, Indonesia at the age of 10-12 weeks with body weight 200-250 g were used in this study. A total of nine male rats and 12 female rats were housed in a cage with the size 50cm x 47cm x 45cm (3-4 rats/cage) and maintained under controlled room temperature with 12:12 hour light and dark cycle. The rats were acclimatized for two weeks were given the normal pellet and water *ad libitum*, prior to dietary manipulation. All experiment procedures were approved by Health Research Ethic Committee, Faculty of Medicine Universitas Padjadjaran, No. 875/UN6.KEP/EC/2018.

Experimental Set-up

Twenty-one rats were grouped into three groups consist of three male rats and four female rats in each group given food and water *ad libitum* for eight week. The first group as control was given normal animal pellet, the second group was given the first formula, and the third group was given the second formula. The rats were weighed weekly and record as mean weight each group per gender. Rats were fasted for 16 hours prior to blood collection. The fourth-week blood was collected from the lateral vein in the tail and the eighth-week at the end of the experimental all rat groups were sacrificed under inhaled isoflurane anesthesia and blood was collected from each rat by cardiac puncture. The blood was centrifuge to get the serum and we measure the fasting blood glucose, triglyceride, total cholesterol level using colorimetric diagnostic kits (Randox Laboratories Ltd., Crumlim, United Kingdom) according to the instruction. Data was measured and presented as mean value ± standard error mean (SEM).

RESULTS AND DISCUSSION

In the present study we compared two types of feeding formula. The first formula (formula 1) contains higher carbohydrate than other macronutrients in normal feed of rats. The second formula (formula 2) contains higher fat and the composition of protein in each formulated feed were the same (Table 1).

The increase of delta mean body weight of female rats given formula 1 was the highest (control: 32.25 g; formula 1: 52 g; formula 2: 45.67 g). The same also occurs in male rats given formula 1, which have higher increase in delta mean body weight compared to other group (control: 45.67g; formula 1: 71.67 g; formula 2: 10.5 g). Delta mean body weight increase higher on male rats compared to female rats in control and formula 1 groups, while in formula 2 the mean body weight of female rats was higher compared to the male rats (Table 2, Fig. 2).

The mean glucose level in all groups of female rats was increased at 4^{th} week and 8^{th} week. Group given formula 1 has higher glucose level compared to other groups among female rats. The glucose level of male rats in control group and group given formula 2 were also increased from the fourth to eighth week but not in the group given formula 1 at the eight week. Among male rats, the control group has higher glucose level compared to the other groups as shown in (Table 3, Fig 3).

The triglycerides level of control and formula 1 of female rats were increased from the fourth week to the eighth week but the group given formula 2 was decreased from the fourth week to the eighth week as described in Table 2. The triglycerides level of group given formula 2 was higher compared to other groups among female rats as shown in Figure 4. The mean triglycerides level in all groups of male rats were increased at fourth week and eighth week, and the group given formula 2 have higher

Table 1. Composition of each formula

Type of Feed	Composition	Source (Per 3 kg pellet)
Formula 1	48 % Carbohydrate 20 % Protein 22 % Fat	Granulated sugar (1.85 kg) Normal pellet (0.84 kg) Cattle fat (0.31 kg)
Formula 2	22% Carbohydrate 20% Protein 48% Fat	Granulated sugar (1.05 kg) Normal pellet (1.03 kg) Cattle fat (0.93 kg)

Table 2. The increase of delta mean body weight each group per gender at 8th week and 4th week

Group	Average Mean ± SEM	
	Female	Male
Control	32.25 ± 2.87	45.67 ± 5.84
Formula 1	52 ± 6.43	71.67 ± 5.24
Formula 2	45.67 ± 14.97	10.5 ± 7.81

Figure 1. Formulated diet with different formula, Formula 1 (high carbohydrate) on the left and Formula 2 (high fat) on the right

Table 3. Glucose, triglycerides, total cholesterol level between female an male rat groups

Gender	Week	Groups	Mean ± SEM		
			Glucose	Triglycerides	Cholesterol Total
Female	4th Week	Control	159.58 ± 14.67	18.33 ± 11.20	75.31 ± 20.1
		Formula 1	221.13 ± 39.47	18 ± 5.7	93.05 ± 14.24
		Formula 2	160.56 ± 24.71	76.78 ± 5.28	96.42 ± 20.58
	8th Week	Control	185.04 ± 27.78	32.58 ± 8.7	100.71 ± 15.02
		Formula 1	249.83 ± 32.59	40.91 ± 4.8	282.5 ± 45.41
		Formula 2	203.56 ± 30.87	71.22 ± 8.6	184.42 ± 45.97
Male	4th Week	Control	163.33 ± 19.48	3.28 ± 6.75	123.42 ± 20.69
		Formula 1	146.22 ± 10.2	13.14 ± 8.24	101.58 ± 26.05
		Formula 2	139.37 ± 5.28	37.42 ± 15.2	117.68 ± 5.23
	8th Week	Control	170.44 ± 3.62	20.44 ± 6.03	125.25 ± 22.88
		Formula 1	136.17 ± 28.41	24.33 ± 9.95	227.17 ± 43.29
		Formula 2	164.08 ± 39.19	58.92 ± 14.04	206.81 ± 51.53

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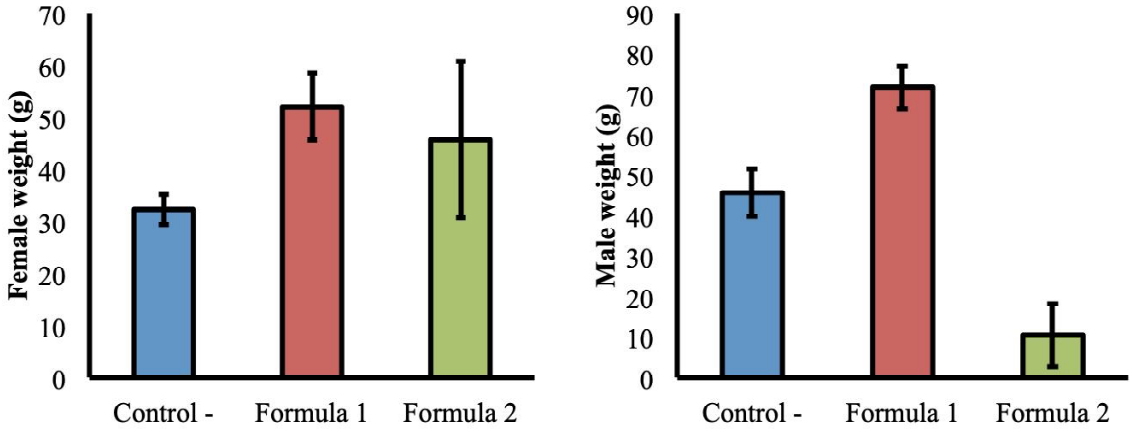

Figure 2. The increase of delta mean body weight of rats at the 8th and 4th week

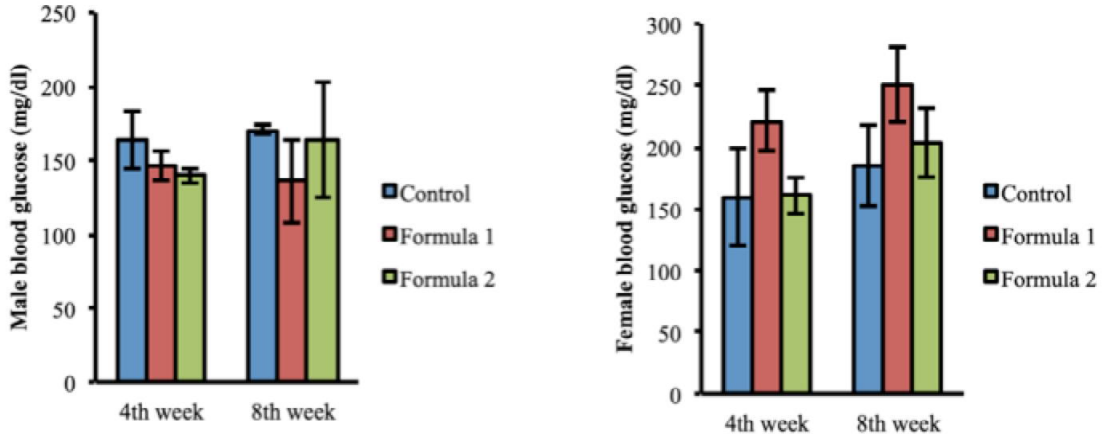

Figure 3. Mean blood glucose level of rats between group in the 4th and 8th week

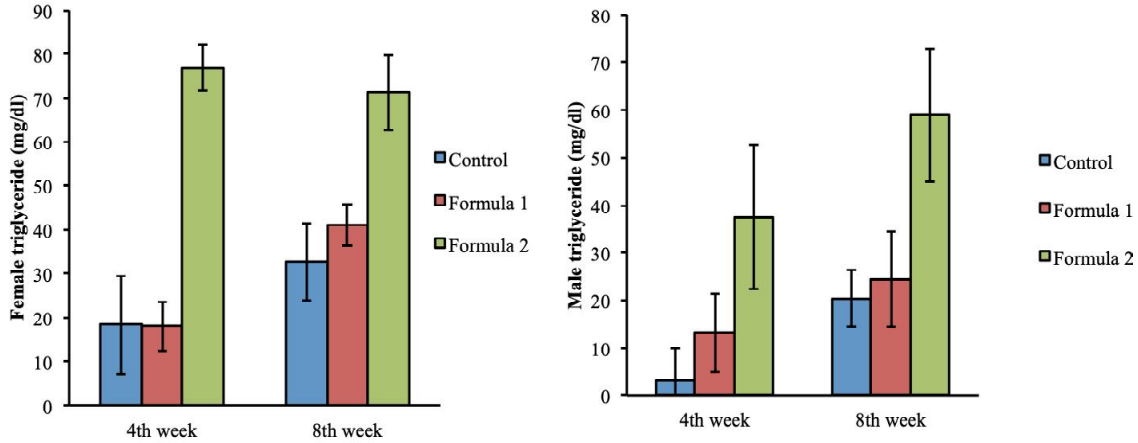

Figure 4. Triglycerides levels of rats between groups in the 4th and 8th week

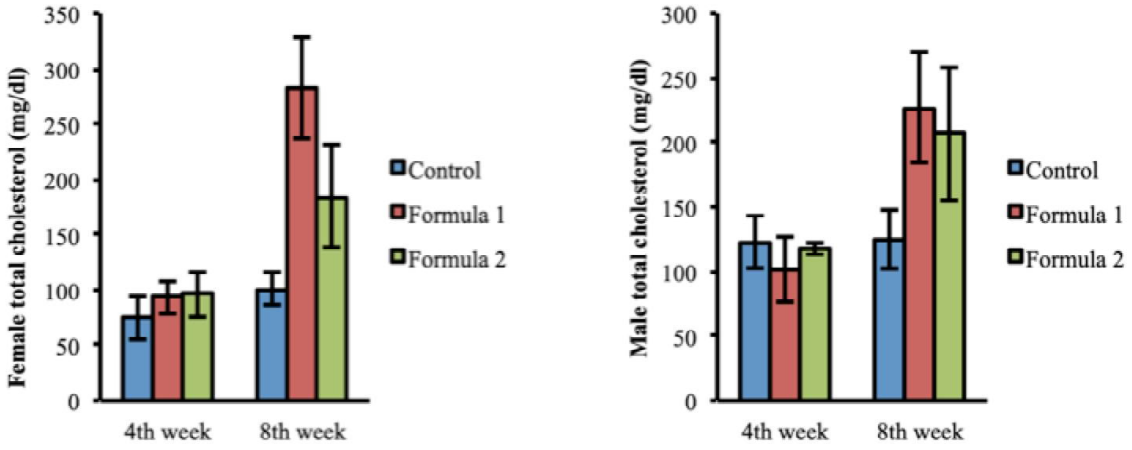

Figure 5. Total cholesterol levels of rats between groups in the 4th and 8th week (*p<0,05)

triglycerides level compared to other groups among male rats as shown in (Table 3, Fig. 4).

The mean total cholesterol level in all groups of female rats and male rats was increased at fourth week and eighth week and also group given formula 1 has higher total cholesterol level compared to other group either in female rats or male rats (Fig.5).

The glucose level from formula 1 and formula 2 of female rats were higher compared to the formula 1, and formula 2 of male rats from the fourth to the eighth week. As shown in Table 3 and Figure 4, the triglycerides level of control, formula 1 and group given formula 2 of female rats were higher compared to the male rats of all group from the fourth week to the eighth week. The cholesterol level of control group and formula 1 group in male rats were higher compared to the control and formula 1 group of female rats at the fourth week and conversely at the eight week. But the cholesterol level of group given formula 2 from male rat at eight weeks was higher compared to formula 2 group of female rat (Table 3, Fig. 5).

The objective of this study was to develop a formulated feed with increased glucose, triglycerides, and total cholesterol of rat blood as characteristics of type 2 diabetes mellitus without any chemical or drug induction that would reflect on natural process of diabetes mellitus type 2 in human due to lifestyle. Furthermore, the development of formulated feed can help further research in understanding molecular process of type 2 diabetes mellitus.

Group given the formula 1 in this study have higher mean delta body weight and blood glucose compared to control group and group given formula 2. It is probably de novo lipogenesis started to happen. Carbohydrate overfeeding would make the glycogen storage were saturated and lead to increase lipogenic gene that contribute to *de novo* lipogenesis (Acheson *et al.,* 1988; Minehira *et al*., 2003). High intake of carbohydrate, then the glucose will be transported into liver and adipose tissue and converted into glucose 6 phosphates to form fatty acid, and then it will be stored in the form of triacylglycerol in adipocyte (Bender *et al*., 2009; Clark *et al.,* 2012). Increased storage in adipose cells will reduce insulin function through an imbalance adipokine hormone production, inflammation in hypertrophic adipose cells which resulting in glucose being unable to enter the cell and circulating in the blood then it was indicated by an increase in glucose levels blood by the group given high carbohydrate feed (formula 1) and also increase body weight due to deposition of various body fat pad (Rabe *et al.,* 2008; Srinivasan *et al.,* 2005; Tilg *et al.,* 2006). In addition, the increase in carbohydrate consumption for a long time will increase the level of insulin 2-3 times in the circulation resulting in the insulin receptor affinity decreases trough down-regulation process and finally decreases the effect of insulin (Clark *et al.,* 2012; Gardener *et al.,* 2011; Steiner *et al.,* 1990).

In previous study, it has been shown that in human there was an association between percentage of calorie intake from carbohydrate and the level of total cholesterol and low-density lipoprotein cholesterol (LDL-C). The result shows that higher total carbohydrate intake decrease the level of high-density lipoprotein cholesterol

(HDL-C), increase the level of triacylglycerol, low-density lipoprotein cholesterol (LDL-C), and total cholesterol. In Ma *et al.* (2006), study also has similar result with our study, which the increase of total cholesterol in the group given formula 1. Down-regulation of insulin receptor lead to hyperinsulinemia, this condition enhances the production of SREBP-1c in the liver, leading to activation of the lipogenic gene. This gene would activate enzymatic process conversion glucose into fatty acid, production of VLDL and lead to the production of cholesterol from liver (Browning *et al.,* 2004; Gylling *et al., 2*010). The presence of high level of triglycerides in the group was given formula 2 due to excess intake of fat from their feed, which could increase the formation of fatty acid and increase storage of fat in adipose tissue in the form of triglycerides. This result is similar to the previous study they develop a model for type 2 diabetes mellitus using a combination of high-fat diet and low dose streptozotocin (Srinivasan *et al.,* 2005).

In this study, we found that there were changes in each variable (blood glucose, triglycerides, and total cholesterol) at week four due to during these period several things started to happen, namely glucose intolerance, increased body fat percentage. Hence, this period of time mimic prediabetes condition in human (Skovsø., 2014).

CONCLUSSION

Either high carbohydrate or high fat pellet showed potential capability to increase the glucose, triglyceride and total cholesterol level especially in female rats group not in male rats group.

SUGGESTION

On the other hand, our study also has several limitations. The number of sample in this study was small therefore it was difficult to find a significant relationship between the groups, due to the limitation of time; this study had not been able to get a significant result. Even though this study had not been able to get a statistically significant result, but in this study we found a tendency in our sample that gender differences produce different result in each parameters. Therefore, further study is needed to do it longer time, use larger number of sample, and test the molecular of each gender.

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