

Effect of *Carica papaya* L. Stem Bark Extracts on Cholesterol Concentration in Rats Induced with Streptozozin

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Abstract. This study was designed to determine the effect of *Carica papaya* L. stem bark extracts on cholesterol concentration in rats induced with glibenclamide. A completely randomized design was used for the experiment which consisted of 6 treatment groups, each group consisted of four rats, as follows: 1) KN (negative control, non-diabetic rats); KP, diabetic rats given glibenclamide 10 mg/kg body weight; EP 1, diabetic rats given 0 mg/kg body weight/day extract; EP2, diabetic rats given 100 mg/kg body weight/day extract; and EP3, diabetic rats given 200 mg/kg body weight/day extract, EP4, diabetic rats given 300 mg/kg body weight/day extract for 28 day. The results showed that *C. papaya* L. stem bark extract decreased ($p < 0.05$) cholesterol levels in diabetic rats. It was concluded that *C. papaya* L. stem bark extract had potential as anti-hypercholesterolemic in diabetic rats.

Keywords: cholesterol levels, glibenclamide, diabetic rat, *C. papaya* L.

1 Introduction

One type of fat in the blood is cholesterol. Cholesterol is a normal fat which is an excessive state that can cause atherosclerosis and eventually will cause acute disease, namely coronary heart disease [1]. Increased levels of total cholesterol and Low Density Lipoprotein (LDL) in the blood are also called Hypercholesterolemia [2]. Blood cholesterol can decrease if cholesterol is excreted through bile. So that bile is bound by pectin and causes at least bile acids to return to the liver [3].

Treatment of hypercholesterolemia and hyperglycemia requires a relatively long time. So the medical costs incurred are also relatively high, as well as causing side effects from the use of synthetic drugs in the long term. Side effects caused include nausea, itching, headaches, psychological disorders, hair loss (reversible), tachycardia, hyperuricemia and even impaired liver function/hepatitis [4]. This can be minimized, by overcoming the reduction in cholesterol levels in the blood using traditional ingredients namely stem bark extract *Carica papaya* L. The material is used naturally to reduce the use of synthetic drugs and minimize side effects.

Papaya has very useful substances including saponins, tannins, flavonoids, and the enzyme papain [5]. According to Dalimartha [6], Papaya contains papain. Flavonoids can reduce blood cholesterol levels by increasing the excretion of bile acids and reducing blood viscosity, thereby reducing the occurrence of deposition of fat in blood vessels [7]. According to Haris [8], flavonoids are compounds consisting of 15 carbon atoms

which are useful as cholesterol-lowering and as an antibiotic. Saponins can taste bitter, sweet and frothy. Saponin is useful as an exterminator of shellfish, hypoglycemic, and hypocholesterol effects [9].

Based on the benefits of the content of substances possessed by papaya, as well as the side effects of the use of synthetic drugs, this study needs to be done to determine the effect of giving papaya bark extract (*C. papaya* L) to reduce cholesterol levels in rat blood.

2 Materials and Methods

2.1 Place and time of research

This research was conducted at the Biology Education and Chemistry Education Research Laboratory of FKIP Universitas Syiah Kuala, Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala. The study period starts from March to September 30, 2019

2.2 Research types and design

This type of research is quantitative research. The research was an experimental study with a completely randomized design consisting of six treatments and five replications. KN (negative control, non diabetic rats), KP (positive control; Glibenclamide), EP1 (diabetic rats given papaya stem bark extract 0 mg / 200 g BW), EP2 (diabetic rats given papaya stem bark extract 200 mg / 200 g BW), EP3 (diabetic rats given papaya stem bark extract

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300 mg / 200 g BW), EP4 (diabetic rats given papaya stem bark extract 400 mg / 200 g BW).

2.3 Procedure for making papaya stem bark extract

The making of papaya stem bark ethanol extract refers to modified [10,11]. Dry leaves were dried for 3 days. The dried leaves were then turned into simplicia using a blender. Simplicia as much as 50 grams was put into a conical flask and stirred in 250 mL of 70% ethanol at room temperature. The mixture was then stirred using magnetic stirred for 1 hour and stored in the dark for 24 hours. The resulting extract was then filtered using filter paper and evaporated to separate the solvent. Evaporation was carried out using a rotary evaporator at 60 °C for 4 hours until the solvent has completely evaporated so that a thick papaya extract was obtained.

2.4 Procedure for Making Papaya Stems with Nanoemulsion Technique

First, the oil phase is carried out 100 ml of Papaya stem bark extract added with 1000 ml of 98% ethanol. Then a liquid phase is carried out which is a solution of Phosphate Buffer (PH 7) and mixing with Tween 80 10% (v / v) and maltodextrin 1: 1 (w / v) with oil. Then the 30% (v / v) oil phase was mixed in the aqueous phase, then a homogenizer was carried out at a speed of 22,000 rpm and a stirring time of 20 minutes to obtain a papaya stem bark nanoemulsion extract [12].

2.5 In Vitro Animal Test Model

Animal models used were diabetic male white rats given Streptozozin (STZ) at a dose of 150 mg/kg body weight. Rats were adapted with a fork cage for 1 week, then diabetes was induced. Rats were divided into their respective treatments. These treatments are KN (negative control), KP (Glibenclamide positive control), EP1 (diabetic rats and and given papaya stem extract 0 mg / 200 g BW), EP2 (diabetic rats and given papaya stem extract 200 mg / 200 g BB) , EP3 (diabetic rats and given papaya stem extract 300 mg / 200 g BW), EP4 (diabetic rats and given papaya stem extract 400 mg / 200 g BW). Routine measurements are done once a week, namely measurement of blood cholesterol levels

3 Results and Discussion

Before researching the reduction in rat cholesterol levels, it must be known in advance the normal cholesterol levels of rats. After that, the rats must be injected first using streptozozin. Initial cholesterol levels and cholesterol levels of rats after streptozozin injection can be seen in Table 1.

Table 1. Rats Cholesterol Levels Early and After Streptozozin (STZ) Injection

Treatment	Initial Cholesterol Levels (mg / dL)	Cholesterol Levels After Injection (mg / dL)
KN	106	111,25
KP	88,25	238
EP1	105,75	332,25
EP2	86,75	185,75
EP3	89,5	194,25
EP4	97	192
Amount	573,25	1253,5
Average	95,54167	208,9167

The treatment that has been done for 45 days with 4 times of data collection (once a week) shows that there are variations in the average difference of cholesterol levels in rats that have been given Carica papaya L bark extract in the first week of data collection. These variations can be seen in Figure 1.

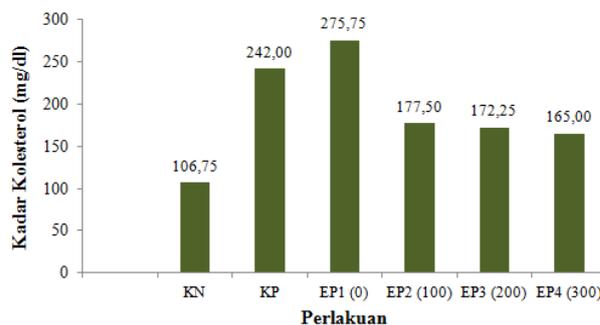


Figure 1. Average graph of decrease in cholesterol levels after *C. papaya* L stem bark extract on Week 1.

ANOVA results indicate that the value of F-count (26.721) > F-table value (2.77) at the test level of 5% (0.05). This means that there is an effect of a decrease in rat cholesterol levels after being given an extract of Carica papaya L bark in the first week. To find out the difference between each treatment, a BNT Test of 5% was performed. BNT Test Results of 5% can be seen in Table 2.

Table 2. Results of 5% LSD Test for Decrease in Cholesterol Levels after Carica papaya L Bark Extract Week 1

Treatment	Average(X)	BNT 5%	X + BNT 5%	Code
KN	106,75	34,566	141,316	A
EP4 (300)	165,00		199,566	B
EP3 (200)	172,25		206,816	Bc
EP2 (100)	177,50		212,066	Bcd
KP	242,00		276,566	E
EP1 (0)	275,75		310,316	F

Table 2 shows that the administration of 300 mg papaya stem bark extract decreased cholesterol levels better than positive controls and other treatments, approaching cholesterol levels in rats without diabetes (KN).

Data in week 2 also showed a variation in the average decrease in cholesterol levels of rats after being given extracts of *C. papaya* L. stem bark. These differences can be seen in Figure 2

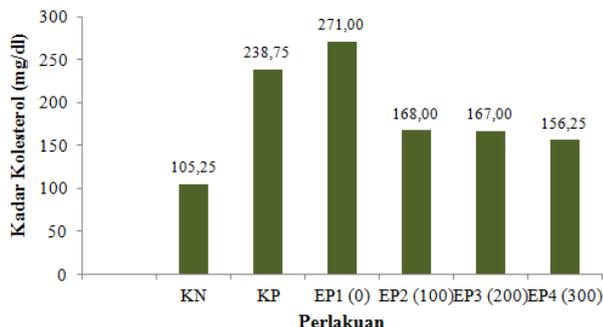


Figure 2. Graph of Average of Decrease in Cholesterol Levels after Extracting *C. papaya* L. Stem Bark Extract on Week 2

ANOVA results show that the value of F-count (25.693) > F-table value (2.77) at the test level of 5% (0.05). This means that there is an effect of decreasing the cholesterol level of rats after being given Carica papaya L bark extract in week 2. To find out the differences in each treatment, a BNT Test of 5% was performed. BNT Test Results of 5% is provided in Table 3.

Table 3. Results of a 5% LSD Test for Decreased Cholesterol Levels after being given a *C. papaya* L Stem Bark Extract Week 2.

Treatment	Average(X)	BNT 5%	X + BNT 5%	Code
KN	105,25	35,250	140,500	A
EP4 (300)	156,25		191,500	B
EP3 (200)	167,00		202,250	Bc
EP2 (100)	168,00		203,250	bcd
KP	238,75		274,000	E
EP1 (0)	271,00		306,250	Ef

Table 3. showed that there were significant differences in the reduction in cholesterol levels in rats. The decrease in rat cholesterol levels is best demonstrated by the EP4 treatment.

Furthermore, data in week 3 also showed a variation in the average decrease in cholesterol levels in mice after being given extracts of *C. papaya* L. stem bark. These differences can be seen in Figure 3

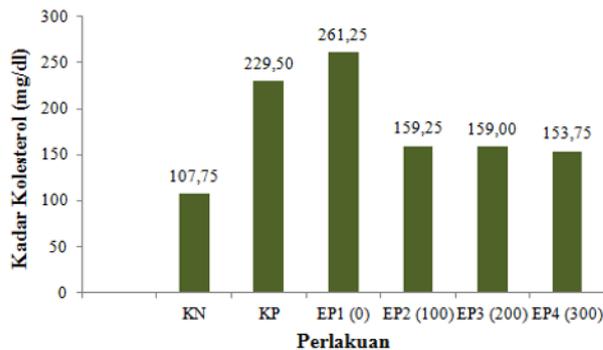


Figure 3. Graph of Average of Decrease in Cholesterol Levels after *C. papaya* L. Stem Bark Extract Extract on Week 3.

ANOVA results that the calculated F-count (25.883) > F-table value (2.77) at the test level of 5% (0.05). This means that there is an effect of a decrease in rat cholesterol levels after being given an extract of *C. papaya* L. stem bark in the third week of observation. To find out the difference between each treatment, a BNT Test of 5% was performed. BNT Test Results of 5% can be seen in Table 4.

Table 4. Results of a 5% LSD Test for Decreased Cholesterol Levels after Carica papaya L Bark Extract Week 3.

Treatment	Average (X)	BNT 5%	X + BNT 5%	Code
KN	107,75	32,847	140,597	A
EP4 (300)	153,75		186,597	B
EP3 (200)	159,00		191,847	Bc
EP2 (100)	159,25		192,097	bcd
KP	229,50		262,347	E
EP1 (0)	261,25		294,097	Ef

In Table 4 it is known that there is a very significant difference in the reduction in rat cholesterol levels. The decrease in rat cholesterol levels is best demonstrated by the EP4 treatment.

Furthermore, the data at week 4 also showed a variation in the average decrease in cholesterol levels in mice after being given extracts of Carica papaya bark L. These differences can be seen in Figure 4.

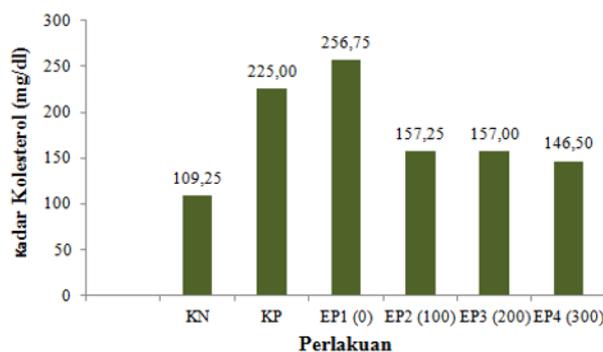


Figure 4. Average Graph of Decreased Cholesterol Levels after Carica papaya L Bark Extract Extract on Week 4

ANOVA results show that the value of F-count (23.443) > F-table value (2.77) at the test level of 5% (0.05). This means that there is an effect of a decrease in rat cholesterol levels after being given an extract of *C. papaya* L stem bark at the 4th week of observation or data collection. BNT Test Results of 5% can be seen in Table 5.

Table 5. Results of a 5% LSD Test for Decrease in Cholesterol Levels after Carica papaya L Bark Extract Week 4.

Treatment	Average (X)	BNT 5%	X + BNT 5%	Code
KN	109,25	33,562	142,812	A
EP4 (300)	146,50		180,062	B
EP3 (200)	157,00		190,562	Bc
EP2 (100)	157,25		190,812	Bcd
KP	225,00		258,562	E
EP1 (0)	256,75		290,312	Ef

Table 5 showed that there is a very significant difference in the reduction of cholesterol levels in mice that have been injected with STZ. The decrease in rat cholesterol levels is best demonstrated by the treatment of EP4 approaching rat without diabetes.

This research was conducted on rats as test animals to see a decrease in cholesterol levels in their blood after being given an extract of *C. papaya* L. stem bark. Initially, the average rat cholesterol level was 95.5 mg / dL, after STZ injection, rat cholesterol levels increased to reach an average of 208.9 mg / dL.

From the results of the analysis data, it can be seen that there is a significant influence on the level of 5% test for the reduction in cholesterol levels of rat that have been injected with STZ after given stem bark extract *C. papaya* L. The average cholesterol level decreases with a range of 50 mg/dl to 70 mg/dl after administration of *C. papaya* L. stem bark extract at week 4 of observation. The decrease in cholesterol levels was due to the influence of substances contained in the *C. papaya* L. stem bark. According to Septiningsih [13] the leaves, roots, and bark of *C. papaya* L. contain alkaloids, saponins, and flavonoids.

Previous research conducted by Pramesti [14] regarding the utilization of bay leaves containing flavonoid compounds in the form of quercetin was proven to reduce blood cholesterol levels in male rats. This quercetin can inhibit LDL (Low Density Lipoprotein) oxidation. According to Prahastatuti, *et al* [15], flavonoid compounds work by inhibiting the enzyme HMG-CoA Reductase which causes cholesterol levels in the blood to decrease.

Previous research by Endrinalsi and Asterina [16] stated that papaya extract showed a significant increase in serum HDL (High Density Lipoprotein) levels in rats. The increase in HDL will cause the absorption of excess free

cholesterol in the body, then it will be brought back to the liver [17].

If cholesterol in the blood increases more than the normal threshold, there will be some deadly diseases. Some diseases such as heart disease and stroke are caused by hypercholesterolemia which is a condition of elevated levels of cholesterol in excess conditions in the blood [18]. When the HDL content in the blood increases, the excess cholesterol in the blood will be captured by HDL and brought back to the liver to be broken down. After being elaborated it will be circulated back to all body tissues by LDL. While the remainder of the breakdown of cholesterol in the liver is channeled into the gallbladder as bile.

4 Conclusion

The administration of *C. papaya* L. stem bark extract can reduce rat blood cholesterol levels. *C. papaya* L. stem bark extract had potential as anti-hypercholesterolemic in diabetic rats.

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