

# The Potential of Cashew Apple Juice as Anti Hypercholesterol Agent on Whistar Rats (*Rattus norvegicus* Berkenhout, 1769)

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**Abstract.** Cashew (*Anacardium occidentale* L.) apple are rich in nutritional value, especially vitamin C and bioactive compounds such as tannins that are very beneficial to support health. This research aims to find out the effects of cashew apple juice on lipid profile and its potential as an anti-Hypercholesterol agent. In this study used experimental animals in the form of male Whistar Rats Type (*Rattus norvegicus* Berkenhout, 1769) as much as 21 rats are divided into three groups and each group consists of seven rats were fed with AIN '76 diet for 28 d, given the treatment of cashew apple fruit juice 2 mL, two times a day by were force-fed and as control treatment is given only drinking aquabidest. The results showed that cashew apple juice was influential for the lipid profile of the whistar rats experiment, potentially as an anti-Hypercholesterol agent by lowering total cholesterol levels 6.37 % to 7.16 %, Triglyceride (9.71 % to 10.76 %) LDL-Cholesterol (11.19 % to 13.37 %) and increased cholesterol levels of HDL (6.10 % to 6.46 %).

**Keywords:** *Anacardium occidentale* L., increase HDL cholesterol, reduce total cholesterol, tannin, waste to herbal

## 1 Introduction

Cashew plants (*Anacardium occidentale* L.) is a commodity that has an essential position because almost all of its parts can be utilized as a variety of products with high economic value and health support. This plant produces fruit consisting of two parts, namely cashew nuts as true fruit-producing cashew nuts and cashew apple as pseudo fruit. The existence of cashew nuts is only 10 % of the total weight of cashews, but the economic value is very high [1]. When compared with cashew fruit (Cashew) has an abundant potential (90 %) of the total weight of the fruit, but has not been maximally utilized, this is because (80 % to 90 %) the product is disposed of in the form of waste and animal feed. Only a small portion can be consumed directly as fresh fruit and other processed products.

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Cashew apple has considerable potential to be developed into food and beverage products when viewed from its chemical composition and nutritional value. One that has a bright prospect is cashew apple juice. This is because the cashew apple is juicy with a moisture content of 86.3 g 100 g<sup>-1</sup> [2] and rich in vitamin C which is 180 mg 100 g<sup>-1</sup> [2] and 126 mg 100 mL<sup>-1</sup> to 372 mg 100 mL<sup>-1</sup> [3] three to six times more vitamin C than orange juice [4], and as a very good source of antioxidants [5].

The processing of cashew apples into food and beverage products has significant constraints, such as the taste of the astringency and itching (acid). The taste of the mouth and throat, which is the characteristic of both flavors, is not so preferred by consumers, so it needs to be reduced. The presence of soluble tannins mainly causes the astringency taste in cashew apple. It is one component of polyphenols with levels of 0.35 % [6] and 147 mg 100 mL<sup>-1</sup> to 164 mg 100 mL<sup>-1</sup> [7] depending on variety and level of maturity.

The presence of tannin in food and drinks can have a positive and negative effect on those who consume it. The negative effects are, i) can reduce the nutritional value of macromolecules (protein, amino acids, carbohydrates, sugars), micromolecules (vitamins, minerals) and their bioavailability [5, 8]. ii) negative effect to digestive [9] by forming insoluble complexes, thereby increasing levels of nitrogenous stool, which can inhibit the growth of experimental animals including rats. The positive effect on tannin is that it can reduce total cholesterol concentration, and increase HDL cholesterol in the blood so that it can strengthen the transport back of cholesterol and inhibit absorption in the intestine, which results in the amount of cholesterol being excreted with stool. Therefore, cashew apple can be used as an anti-hypercholesterolemia [10].

Based on the description above, a research has been conducted on the potential of cashew apple juice as an anti-hyper cholesterol agent in Wistar white rats (*Rattus Norvegicus* Berkenhout, 1769). This study aims to determine the effect of giving cashew juice to the lipid profile of experimental rats and its potential as an anti-hyper cholesterol agent.

## 2 Material and methods

### 2.1 Materials

The materials used in this study are, i) Yellow-orange cashew apple obtained from Lombok Utara NTB as raw material for making cashew juice, ii) Experimental animals namely Wistar male white rats (*R. Nervegicus*) who were 6 wk old with an average body weight of 104 g ± 8 g obtained from the Experimental Animal Development Unit (UPHP) of Universitas Gadjah Mada, Yogyakarta, Indonesia who had the qualification standards for research, iii) Experimental rats feed during the study referred to the standard feed set by the American Institute of Nutrition 1976 (AIN 76) made in the nutritional laboratory of the Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia, iv) Other materials in the form of kits for the analysis of lipid profiles of experimental rats are kit no. 1.14992.0250, 1.14210.0001 and 1.14366.0001. obtained from chemical stores in Yogyakarta, Indonesia.

## 2.2 Methods

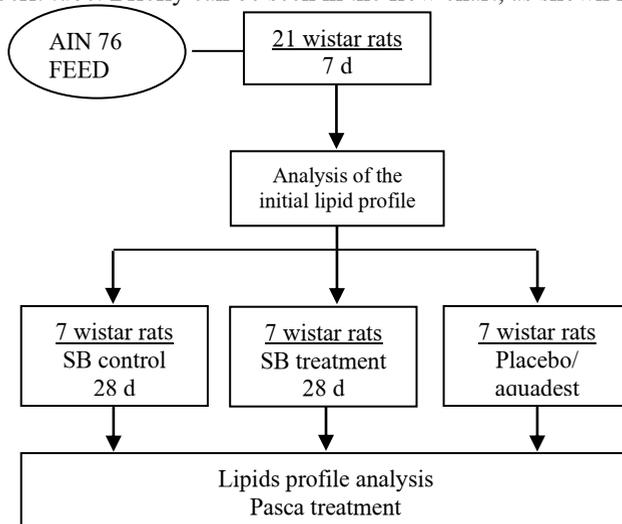
### 2.2.1 The processing of cashew apple juice

Cashew apple juice used in this study was obtained by the following method: i) Cashew apple that have been sorted, then blanched for 1 min to soften the texture to facilitate the extraction process to produce juice, the resulting juice is called cashew apple juice control, then tannin content are analyzed (0.29 % DB), ii) The same treatment with the control of cashew apple Juice, but first soaked in a calcium hydroxide solution ( $\text{Ca}(\text{OH})_2$  3 % for 15 min, then extracted to produce juice treatment for tannin content analysis (0.14 % DB)

### 2.2.2 Research procedur

The research procedure has been approved by the Ethical Commission of the Faculty of Medicine, Al-Azhar Islamic University Mataram, West Nusa Tenggara, Indonesia (Number 29/EC/FK-06/UNIZAR/VIII/2020). The stages are as follows :

- i. 21 male white rats (*R.norvegicus*) were adapted for 7 d, and during the adaptation, the experimental rats were fed AIN 76 and drinking distilled water. After adjustment, all blood rats were taken to analyze the presence of lipid profiles (total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol).
- ii. Experimental rats were divided into three groups, and each group consisted of seven mice.
- iii. Experimental rats were treated in the form
  - Group 1 was given control juice
  - Group 2 was given drinking cashew apple juice treatment
  - Group 3 was only given aquadest (placebo group).
  - giving of drinking cashew apple juice in experimental rats and aquadest in the placebo group were given forcibly (forced feeds) each 4 mL d<sup>-1</sup> given two times (morning and evening) and were still fed during the study with a standard feed from AIN 76 This treatment lasted for 28 d.
- iv. At the end of the study (28 d), all rats from each group were taken blood from their eyes (Retroorbital plexus) and their hearts to analyze their lipid profile using a microhematocrit tube. Briefly can be seen in the flow chart, as shown in the Figure 1.



**Fig. 1.** Scheme of the research

### 2.2.3 Measurement of research parameters

- i. The tannins content in Cashew apple juice were analyzed the burn method (1963)
- ii. Lipid Profile of experimental rats namely total cholesterol by the CHOD–PAP method [11], TG by the GPO–PAP method [12] LDL cholesterol by the CHOD–PAP method [13] and HDL cholesterol by the CHOD–PAP method.

### 2.2.4 Data analysis (statistical analysis)

This research was conducted using an experimental method. The data design using a completely randomized design. Data obtained by analysis of variance, differences among samples would be tested using Least Significant Different (LSD) with the significance level set at  $\alpha = 0.05$  [14].

## 3 Result and discussions

The results showed that the giving of cashew apple juice significantly affected the blood serum lipid protein on the experimental group of rats compared to the placebo group. These results can be seen in Table 1 below.

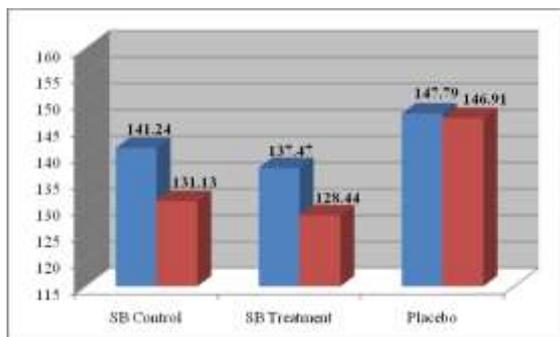
**Table 1.** The changes in blood serum lipid profile of rats experiments on various treatments given by cashew apple juice

Treatment	Cholesterol total	Triglyceride	LDL Col	HDL Col
SB control	-7.16 a	-10.76 a	-13.37 a	+6.46 a
SB treatment	-6.37 a	-9.71 a	-11.19 a	+6.10 a
Placebo	-0.60 b	-2.63 b	-0.07 b	+0.86 b

Noted: Numbers followed by the same letter in the same column are not significantly different at 5 % level. (- is down) (+ is up)

### 3.1 Cholesterol total

The results of an analysis of total cholesterol concentration on blood serum of the experimental rats group showed that after given of cashew apple juice was decreased. The decrease occurred respectively by 7.16 % (SB control) and 6.37 % (SB treatment). The results were not significantly different between treatments. When compared to the placebo group, there was a decrease of only 0.60 %, and it was significantly different from the other two groups. These results can be seen in the following Figure 2.



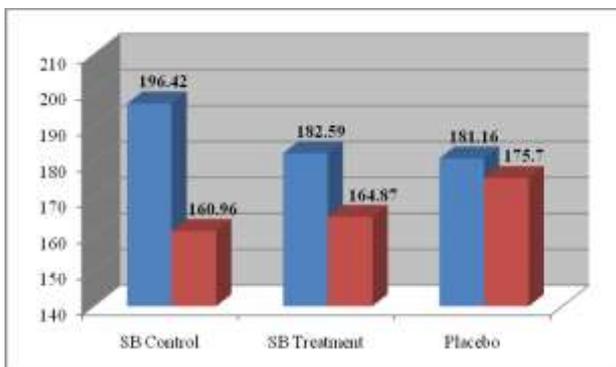
**Fig. 2.** Total cholesterol concentration of blood serum rats experiments on various treatments given by Cashew apple juice

In Figure 2, it can be seen that there was a significant decrease in total cholesterol concentration ( $p < 0.05$ ) after being given a cashew apple juice in rats. There was a decrease from  $141.24 \text{ mg dL}^{-1}$  after adaptation (before treatment) to  $131.13 \text{ mg dL}^{-1}$  (7.16 %) on SB control group. While in the group SB treatment, that is equal to 6.37 %, from  $137.47 \text{ mg dL}^{-1}$  to  $128.44 \text{ mg dL}^{-1}$ . As for the placebo group (only given aquadest) not significant decrease occurred, from  $147.79 \text{ mg dL}^{-1}$  to  $146.91 \text{ mg dL}^{-1}$  (0.60 %).

A decrease in the total cholesterol content in the group of rats that had been given cashew juice by 7.16 and 6.37 %. This is most likely due to the tannin content in the juice, the tannin content in SB control and SB treatment were 0.29 % db and 0.14 % db, respectively. The role of tannins in inhibiting or reducing the absorption of cholesterol in the intestine, namely by forming complexes with cholesterol, resulting in the deposition of cholesterol in the intestinal lumen resulting in the amount of cholesterol that is excreted with feces. This phenomenon is consistent with the results reported by [12].

### 3.2 Triglyceride

The results of triglyceride analysis showed that giving of cashew fruit juice in experimental rats could significantly reduce triglyceride concentrations in rat blood serum ( $p < 0.05$ ) when compared to the placebo group as shown in Figure 3.



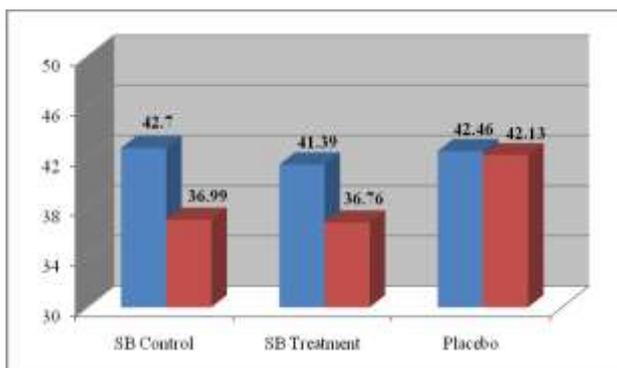
**Fig. 3.** Triglyceride concentration of blood serum rats experiments on various treatments given by Cashew apple juice

In Figure 3 above, it can be seen that the administration of cashew juice can decrease triglyceride concentrations from  $196.42 \text{ mg dL}^{-1}$  to  $160.96 \text{ mg dL}^{-1}$  in the SB control group (a reduction of 10.76 %). Whereas the SB treatment group could decrease the concentration from  $182.59 \text{ mg dL}^{-1}$  to  $164.87 \text{ mg dL}^{-1}$  (a reduction of 9.71 %). However, there was no significant difference between treatments compared to the placebo group which could only reduce from  $181.16 \text{ mg dL}^{-1}$  to  $175.70 \text{ mg dL}^{-1}$  (2.63 %).

The high decrease in triglycerides in SB control and SB treatment is due to the presence of tannin in cashew apple juice, which can inhibit the absorption of triglycerides so that absorption is slower or inhibited. This inhibition, according to [15], is thought to result in slower chylomicrons and remnant chylomicrons (the remaining kilo microns) formation. Besides, this inhibition also related to the chance of the lipoprotein lipase enzyme to catalyze more micron kilos to triglycerides before reaching the liver. Then triglycerides are sent to the network and cause blood triglyceride levels to decrease.

### 3.3 LDL (Low Density Lipoprotein)

The results showed that there was a significant decrease in LDL cholesterol as a result of giving cashew juice to rats. But the placebo group did not have a significant effect.

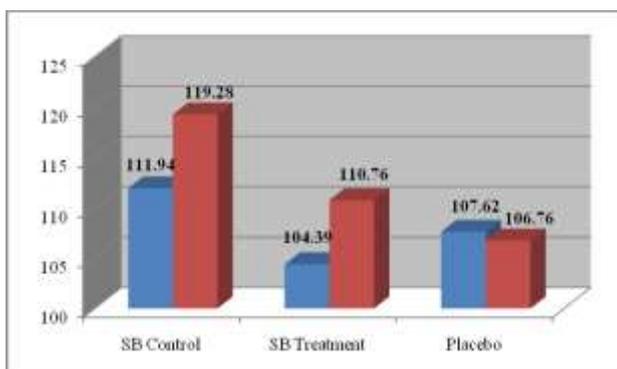


**Fig. 4.** Blood LDL cholesterol concentration of rats experiments on various treatments given by Cashew apple juice.

Figure 4 showed that there was a significant decrease in LDL cholesterol ( $p < 0.05$ ) of 13.37 % in the control SB group, from 42.70 mg dL<sup>-1</sup> to 36.99 mg dL<sup>-1</sup> (13.37 %). In SB treatment, there was a decrease from 41.39 mg dL<sup>-1</sup> to 36.76 mg dL<sup>-1</sup>. They decreased LDL cholesterol along with a decrease in triglycerides. This phenomena is because LDL cholesterol is a natural result of the VLDL catabolism, which contains a lot of triglycerides. Subsequently, by VLDL, triglycerides are lifted from the liver to other tissues, especially to adipose tissue so that triglycerides in the blood are reduced [15].

### 3.4 HDL (High Density Lipoprotein)

The results showed that there was a significant increase ( $p < 0.05$ ) of HDL cholesterol in the blood of experimental rats due to the administration of cashew apple juice compared with the placebo group, while the SB control and SB treatments showed did not significantly different.



**Fig. 5.** Blood HDL cholesterol concentration of rats experiments on various treatments given by Cashew apple juice.

In Figure 5, it can be seen that there was an increase in HDL cholesterol in SB control and SB treatment, respectively, from 104.39 mg dL<sup>-1</sup> to 110.76 mg dL<sup>-1</sup> and from

111.94 mg dL<sup>-1</sup> to 119.28 mg dL<sup>-1</sup>. Whereas in the placebo group, there was a tendency to decrease from 107.62 mg dL<sup>-1</sup> to 106.76 mg dL<sup>-1</sup>.

The increasing in HDL cholesterol level in a group of rats given cashew apple juice, probably due to the presence of tannin in the juice. These results are in according to the research [12] that tannin polymer of grape seed can increase HDL cholesterol concentration and decrease plasma VLDL.

Low VLDL was causing low triglycerides in the blood of experimental rats, where a transfer of triglycerides to the liver produces more HDL cholesterol because the remaining protein and triglyceride release will cause HDL cholesterol formation [16].

## 4. Conclusion

- i. The administration of cashew apple juice in experimental rats affected the blood serum lipid profile of the experimental rats' group compared to the placebo group.
- ii. The administration of cashew apple juice with levels of 0.29 % (SB Control) and 0.14 % (SB treatment) has the same ability to reduce levels of total cholesterol, triglycerides, and LDL cholesterol and increase HDL cholesterol concentrations.
- iii. Cashew apple juice has the potential as an anti-hyper cholesterol agent in rats (*R. norvegicus*).

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