International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

The Combination Effect Analysis of *Catharanthus roseus*, *Abelmoschus manihot* and *Dysphania ambrosioides* on *Rattus norvegicus* Blood Triglyceride Content

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Abstract: ***In the Pandemic Covid-19 era, the search for herbal medicines for community use became the hope of researchers. Triglycerides in the blood are needed by the body at certain levels, but it is a problem at excessive levels or called hypertriglyceridemia. The study aims to analyse the combination effect of the Tapak dara (Catharanthus roseus), Gedi (Abelmoschus manihot) and Pasote (Dysphania ambrosioides) on the blood triglyceride levels of white rats (Rattus norvegicus). The research method used is experimental. This method is carried out gradually starting with the maintenance of rats and administering fats, making extracts, administering treatment, and blood trafficking mice. The research uses samples of Tapak dara, Gedi and Pasote. The dried sample is then extracted by brewing simplisia in the form of tea using boiling water 100 mL, then administered to rats by way of Sonde/Cekok for 30 days. Rat blood after treatment is taken from the heart and then analyzed its triglyceride levels with an enzymatic spectrometer. Results of analysis of the influence of the combination of Tapak dara, Gedi and Pasote show distinct results. After further testing with Duncan's test that the Gemfibrozil treatment resulted in 17.2 mg/dL triglyceride levels and the highest was the third combination treatment of 112.7 mg/dL. The visible effect is the decrease and increase in triglyceride levels. The conclusion is that there are different influences from the combination administration of Tapak dara, Gedi and Pasote against the blood triglyceride levels of white rats with uncombined treatment. Triglyceride levels given the combination of the third leaf extract (PTG) have increased, while in Gedi treatment only and the combination of Tapak dara Pasote is decreased. The combination of several medicinal plants produces a new effect which is the interaction of the effect of a single active compound.***

Keywords: Triglycerides, *Catharanthus roseus*, *Abelmoschus manihot*, *Dysphania ambrosioides*.

1. Introduction

In the Pandemic Covid-19 era, the search for herbal medicines for community use became the hope of researchers. No one has stated clearly that there is one that can treat Covid 19. Herbal remedies that singly sometimes also have side effects that are expected to be overcome by combining them with other medicinal plants. How the effect of the combination treatment of several medicinal plants with a single administration in white mice is the aim of this study.

In the days of globalization as now, the changing of living patterns in developed countries and in developing countries greatly affects the diet and habits of one's daily activities. This change carries the impact of increasing tendency to consume food fatty (junk food) which can lead to the onset of fat metabolism disorder in the blood that affects the increase in lipid profile levels in the blood (hyperlipidemia state), cardiovascular disease, diabetes mellitus and others, causing an increase in mortality (mortality) [1]. One of the most researched and efficacious medicinal plants lowers high cholesterol levels in the blood, namely, Tapak Dara plant (*Catharanthus roseus)* [2].

Tapak dara is one of the many plants that have been utilized as a medication that can lower the total cholesterol levels in the blood and can also be used as cancer drugs [3].
Traditionally Tapak dara have been used for the treatment of hypertension and cholesterol diseases [4]. According to Aydogan (2015) [5], the results of research that has been done on sheep given Tapak dara extract showed the presence of necrosis in the kidneys and liver. Unlike the Hartati (2015) [6], which reported that the administration of the extract combination of Tapak dara with other plants does not give a toxicity effect to renal function in test animals. The use of combination extracts may be helpful in the process of solving proteins in the blood, so the process of crexetion of creatinine (protein) in the kidneys can run constantly. Besides Tapak dara, Gedi plant (*Abelmoschus manihot*) has also been used as a medicine. Mamahit and Soekamto (2010) [7] report that the Gedi leaves are boiled without salt used to treat kidney ache, gastritis and lower cholesterol in the blood. Similarly, the leaves of Pasote (*Dysphania ambrosioides*) have been traditionally utilized, which can cure diabetes and cholesterol which is the cause of cardiovascular [8]. According to Pandiangan et al. (2017) [9], Pasote can be used as an ingredient for herbal medicine for people with degenrative disease.

Based on the above statement, there has been no related research on the influence of the combination of Tapak dara, Gedi and Pasote on decreasing triglyceride levels, so it needs to be conducted research on the combination of Tapak dara, Gedi and Pasote against rat triglyceride-induced high fat feed levels. Based on the background, research will be conducted to analyze the effect of giving a combination of water extract of Tapak dara with Gedi and Pasote against the blood triglyceride levels of white rats (*Rattus norvegicus*).

2. Research Methods

Research Plan
This research is experimental with complete random draft (RAL) using 27 white rat tails with the following divisions:
Treatment I : Administration of Pasote water extracts
Treatment II: Tapak dara water extract administration
Treatment III: Gedi water extract
Treatment IV: Supply of Pasote + Tapak dara water extracts
Treatment V: Tapak dara + Gedi water extract
Treatment VI: Administration of Gedi + Pasote water extract
Treatment VII: Water extracts for Pasote + Tapak dara + Gedi
Positive control (K+): Gemfibrozil Water extract
Negative control (K-): not given treatment but replaced with aquades

Rats Maintenance
The study used a white mouse test animal (*Rattus norvegicus*) weighing 150-200 g and aged ± 10 months. The rats were kept individually in a 25 cm × 40 cm plastic enclosure with a wire cover that had been equipped with bottled water and a dining area. White rats were adapted to laboratory conditions for two weeks by feeding and drinking adlibitum [10].

Making of Combination of Dara, Gedi, and Pasote Water Extracts
This procedure follows the modified Pandiangan et al. (2018) [3]. The leaves of Tapak dara, Gedi and Pasote taken as much as 1000 g are then cleaned. Next, the leaves are washed clean, then dried anginkan. The leaves are then dried in the sun until the weight is constant. The dried leaves are then smoothed using a blender to become a powder. The powder is a total of 100 g of dry weight combined and blended evenly. The leaf powder is then taken and weighed as much as 1 g per tea bag according to the specified label, with a weight distribution system of 1 g that will suit many types of leaves to be combined. Then the powders were inserted into the bag of tea bags. The tea is then brewed in boiling water as much as 100 ml, and then the extract of water is awaited until lukewarm and ready to be treated at the next stage [3].

Treatment of White Rats
Giving treatment according to the analysis design is made. After 14 days of adapting, then given the extract in the rat by sonde/cekok in accordance with the treatment group is prescribed and given as much as 1, 5 mL/tail given on the morning at 08.00 WITA and the evening at 18.00 WITA.

White Mouse Blood Screening
The examination of white rat blood is done in two stages namely, the first stage of sampling the initial blood and the second stage of the final blood sampling. The first phase of blood screening was carried out after the adaptation period and blood samples were taken to analyze early triglyceride levels using the Pro Lipid tool. The way of taking blood through a mouse tail that is cut then blood is affixed to a special Stik Pro Lipid tool and will be read by the Pro Lipid tool.

The second stage of blood screening is performed after 31 days of treatment of white rats and blood sampling will be carried back to analyze blood lipid profile (triglycerides) from the treatment that has been administered using a spectrophotometer (BioSystems). A white mouse blood sampling, firstly the white mouse was drugged using chloroform for 5 minutes then the white mouse was placed onto the surgical board where the four members of the movement pointed upwards. Then the white mouse will be surgized its abdomenous cavity that contains the heart after it is removed from the heart using a 3 mL syringe. His blood was then moved into the tube clot activator (Red Cap of GP Care) to continue on blood screening [3].

A rat blood screening procedure was conducted by taking rat blood serum. The rat blood Serum was separated by other blood components by centrifugation with Centrifuge MPW-56 (MPW MED Instrument) for 10 minutes at a speed of 5000 rpm. The blood Serum is at the top of the clear coloured tubes. The Serum is subsequently processed to determine its total blood lipid content [3].

Triglyceride Analysis
The white mouse blood Serum that has been given treatment will be analyzed for triglyceride levels using a spectrophotometer (*BioSystems*) tool. The first stage, each of the blood serum samples was moved into the test tube by 10 µL. Similarly for the standard solution and akuades (for blanks) is taken 10 µL on a separate tube and labeled as in Table 1. Furthermore, a triglyceride reagent (*BioSystems*) was added on each tube as much as 1000 µL. It is then mixed and incubated for 20 minutes at a temperature of 25 ° C or 10 minutes at a temperature of 37 °c. Then the absorbance of the sample mixture is measured/read at a wavelength of 500 nm. This analysis uses the "GPO-PAP" method, which is an enzymatic photometric test [11].

Table 1: Levels of Blanko, Standard (*BioSystem*) and samples used in the examination of blood triglyceride levels (mg/dL)

	Blanko	Standar	Sampel
Trigliserida	-	10 µL	-
Sampel	-	-	10 µL
Reagen	1.0 mL	1.0 mL	1.0 mL

Statistical Analysis
Data triglyceride levels (mg/dL) have a normal distributed pattern ($p > 0.05$) and have a homogeneous variant ($P > 0.05$) [12]. Furthermore, all data was tested using One Way Anova which will be followed by Duncan's test on a 95% confidence level when there is a difference. The significance value of less than 0.05 indicates a meaningful difference.

3. Results and Discussion

3.1 Measurement of rat body weight before and after given treatment

The results of weight measurement of white rats (Table 2) showed that there was an increase in the weight of white rats. The most high weight gain seen in white rats given the

treatment of Virgin Tread (T) with a final weight of 150 g of the initial weight of 75.33 g. This increase can also be seen morphologically. The white mouse with a high weight gain has a larger body.

This increase can allow the occurrence of elevated triglyceride levels in the blood. The higher the fat consumption The higher the synthesis of triacylglycerol in Hepar which causes increased triglyceride levels in the blood [13]. This is followed by an increase in the reverse cholesterol transport activity, starting to be unable to compensate and begin to decrease. This situation is characterized by increased triglyceride levels [14]. Therefore, there will be a measurement of triglyceride levels to see whether or not the influence of weight gain on the increase in triglycerides.

Table 2. The results of weight measurement of white rats (g) before and after treatment is administered with a brewing water pasote (P); Tapak dara (T); Gedi (G); Pasote and Tapak dara (PT); Gedi and Tapak dara (TG); Gedi and Pasote (GP); and combination of Pasote, Tapak dara and Gedi (PTG); The drug triglycerides Gemfibrozil 300 mg in 100 mL of a solution of NaCl 0.9% (K +); and without treatment (K-).
Note: SD = Standard deviation

Sample Code (Treatment)	*Average initial body weight (g)±SD*	*Average final body weight (g)±SD*	*Weight increase (g)*
P	95,00 ±20,42	143,67 ±37,85	48,67
T	75,33 ±20,50	150,00 ±24,88	74,67
G	87,67 ±31,02	147,67 ±24,58	60,00
PT	94,67 ±22,19	142,00 ±40,15	47,33
TG	78,67 ±7,02	145,33 ±22,30	66,67
GP	96,67 ±8,62	137,00 ±21,93	40,33
PTG	94,33 ±20,65	138,67 ±18,82	44,33
K+	74,00 ±16,37	114,33 ±4,04	40,33
K-	98,67 ±9,02	163,00 ±18,36	64,33

3.2 Measuring triglycerides levels of white rats before administered treatment

The results of triglyceride measurements before the treatment showed a difference in the triglyceride levels of white rats in each group. Data of white rat blood triglycerides levels prior to treatment in each group were analyzed in statistics including test normality, homogeneity test and one-way test of ANOVA. Test normality at the level of triglycerides of white rats before administered treatment showed F count > Fα (0.05). Test results Homogenity of the ninth group showed Fcount = 0,197 (Fcount > Fα (0.05), so that it can be concluded that the results of normal distributed data and data variants between groups homogeneous. Further analysis is conducted statistically, using the one-way ANOVA test using the SPSS application. The result is the value of F-count = 0,460 (P > 0.05), which means there is no difference between the significant triglyceride levels of the group, so that it can be given treatment to continue the research.

3.3 Measurement of triglyceride levels of white rats after administered treatment

Based on triglyceride data before and after treatment (table 3), two treatments have decreased triglyceride levels. The results of the measurement decreased in the treatment of Gedi (G) and Pasote + Tread (PT) which obtained the results of 2.17 mg/dL and 6.00 mg/dL respectively. The results of these two treatments also have lower triglyceride levels compared to the control results (K-) which is 84.00 mg/dL.

The result of determining the blood triglyceride levels of white rats after treatment showed that in a group given Gemfibrozil (K +) decreased triglycerides levels by 47.16 mg/dL (table 3) initial triglyceride levels of 64.33 mg/dL after 31 days were administered a Gemfibrozil triglyceride drug decreased to 17.17 mg/dL. These results showed a much greater decrease in triglyceride levels compared to other groups as well as negative (K-) controls. This suggests that the research and engineering methods of the Gemfibrozil drug can lower the level of blood triglycerides in white rats significantly compared to groups that are not given the drug is the proof of the use of the method is appropriate.

Table 3: Triglycerides (mg/dL) Blood of white rats (Rattus novergicus) have been given treatment administered by Pasote Water (P); Tread (T); Gedi (G); Pasote and the site of Dara (PT); Gedi and Gedi sites (TG); Gedi and Pasote (GP); and Gedi (PTG); Triglyceride drugs Gemfibrozil 300 mg in 100 mL of a solution of NaCl 0.9% (K +); and without treatment (K-).

Sample Code (Treatment)	Early triglyceride average (mg/dL)	Treatment triglyceride average	Decreased triglycerides	Increased triglycerides
K-	70.00^{a}	84.00^{b}	-	14.00
P	69.67^{a}	86.50^{b}	-	16.83
T	65.00^{a}	80.00^{ab}	-	15.00
G	68.67^{a}	66.50^{ab}	2.17	-
PT	72.67^{a}	66.67^{ab}	6.00	-
TG	64.67^{a}	93.17^{b}	-	28.50
GP	73.33^{a}	86.33^{b}	-	13.00
PTG	74.00^{a}	112.67^{b}	-	38.67
K+	64.33^{a}	17.17^{a}	47.16	-

Note: Different letters in the numbers in one column show significant differences in the blood triglyceride levels of rats with a 95% confidence level

The results of the test one-way ANOVA were obtained indicating Fcount > Ftable0.05. This indicates that there is a combination of virgin leaf extract, Gedi and pasote against a very significant (real) white rat blood triglyceride level. The Data of the ANOVA results are presented in table 4. Duncan's advanced test results are presented in table 3 with different letters on numbers in a single column indicating a noticeable difference in the blood triglyceride levels of rats.

Table 4: A test result of ANOVA one-way on blood triglycerides levels of white rats (mg/dL) which has been administered treatment. Significancy α = 0.05

ANOVA					
Kadar Trigliserida	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	16697.667	8	2087.208	1.700	0.166
Within Groups	22095.333	18	1227.519		
Total	38793.000	26			

Late triglyceride levels have increased in some treatment when compared to early triglyceride levels. This can be caused by the use of a combination of leaf leaves, Gedi and pasote are more effective in decreasing levels of other lipid profiles, such as total cholesterol, LDL and HDL. According to Pandiangan et al. (2020 [3], the leaf extract of the site has potential as Anticholesterol. Just like the previous statement, according to Dharma et al. (2013) [15], the leaf ethanol extract can lower the total cholesterol level in the blood of the male white mice. This makes it possible for the site to be more potent in lowering cholesterol levels in the blood compared to its ability to lower blood triglyceride levels. Gani (2013) [16] reported a standard feed containing 36% of red gedi leaf paste (*Abelmoschus manihot* L.) may lower total cholesterol levels, LDL cholesterol and animal blood plasma triglycerides test that suffer from hypercholesterolemia. Similarly, Pasote is widely utilized by the Minahasa community as an anticholesterol drug that can be consumed as a companion food in the pulp of Manado (Tinutuan).

The result of the graph (Fig. 1) indicates that the occurrence of the pattern is almost identical in increasing the final triglyceride levels with increased rat weight. However, there are a number of groups that do not show the continuity of the pattern, such as the site treatment group (T) and Pasote + Dara + Gedi (PTG). At the site treatment (T) The final weight gain was obtained very high compared to other groups, and for the final triglyceride levels gained is quite low when compared to negative control (K-). Conversely, at the treatment of the Pasote + Tread + gedi (PTG) increased final weight gained is fairly low compared to other groups, and for the final triglyceride levels gained is very high compared to negative control (K-). Therefore, the graph cannot be used as a strong reason for a statement that says weight gain may indicate elevated triglyceride levels in the blood.

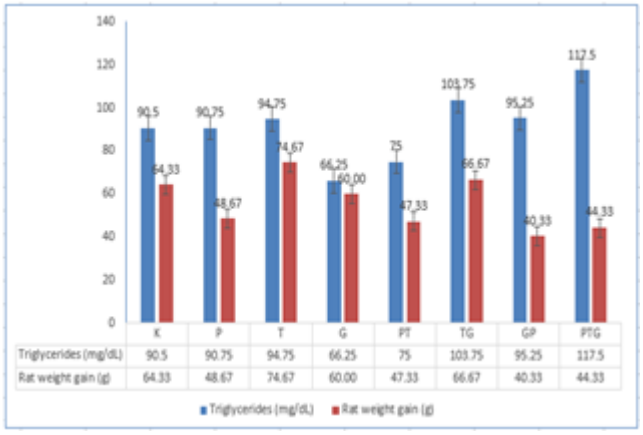

Figure 1: Comparison of triglyceride blood levels of white rats (mg/dL) after the treatment of Pasote (P) brewing water; tread (T); Gedi (G); Pasote and T.Dara (PT); tread and Gedi (TG); Gedi and Pasote (GP); Pasote, tread and Gedi (PTG); the drug triglyceride Gemfibrozil (K+); without treatment (K-), and increased body weight of white mice (g).

4. Conclusions

Based on the results and the discussion of the research can be concluded that there is an influence on the combination of extract leaves that differ between the combination of virgin leaf extract (*Catharanthus roseus*), Gedi (*Abelmoschus manihot*) and Pasote (*Dysphania ambrosioides*) on blood triglyceride levels of white rats (*Rattus norvegicus*). The effect combination treatment of leaf extract Tapak dara, Gedi and Pasote (PTG treatment) there is an increase ± 38.67 mg/dL and there is a decline in the treatment of Gedi (G) and PT (Pasote & Tapak dara) with triglyceride levels respectively at 2.17 mg/dL and 6.00 mg/dL, in line with Gemfibrozil drugs which also decreased by 47.16 mg/dL. The combination of several medicinal plants produces a new effect which is the interaction of the effect of a single active compound.

5. Acknowledgment

Thank you for the research grant from Ministry of Research, Technology and Higher Education based on Agreement / Contract no 06/SP2H/LT/DRPM/2019 with the Research and Service Institute (LPPM) of Sam Ratulangi University (UNSRAT) on March 2019 and the contract between the Chairperson of LPPM UNSRAT with Researcher Prof. Dr. Dingse Pandiangan, MSi Number 132/UN12.13/LT/2019 on April 2019. We thank you for funding this research from the Indonesian get a Research Budget management this research.

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