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STUDY OF EFFECTIVENESS OF TURMERIC ETHANOL EXTRACT (CURCUMA LONGA) AS AN ANTI-DYSLIPIDEMIA AGENT IN MALE WISTAR RATS EXPOSED TO PROPYLTHIOURACIL

Ling Jinmei[1], Wienaldi[2]
[1]*Master of Clinical Medicine, Department of Clinical Medicine, Faculty of Medicine, Dentistry, Health Sciences, Universitas Prima Indonesia*
[2]*Department of Clinical Medicine, Faculty of Medicine, Dentistry, Health Sciences, Universitas Prima Indonesia*

ABSTRACT
Turmeric, a type of Curcuma domestica, is a traditional medicinal plant in Indonesia. It contains curcuminoids, essential oils, starches, and various minerals. Turmeric has pharmacological benefits like improving blood circulation, reducing inflammation, and acting as an antibacterial agent. Dyslipidemia, a lipid metabolism disorder, can be evaluated in vivo and in vitro. This study aims to investigate the effectiveness of the ethanol extract of turmeric (Curcuma longa) as an anti-dyslipidemia agent in male Wistar rats treated with Propylthiouracil. The study was experimental with Pre-test and Post-test group-only control designs, using male Wistar rats as subjects. It was implemented in January 2024 with samples determined using the Federer formula. The calculations indicate that a minimum of 4 male Wistar rats (Rattus norvegicus) is required per treatment group. The rats should weigh between 180 and 200 grams and be between 2 and 4 months old. Turmeric ethanol extract (Curcuma Longa) significantly improved lipid profiles, especially at higher doses, reducing total Cholesterol, triglycerides, and LDL while increasing HDL in groups II and III. This anti-dyslipidemia effect is attributed to turmeric phytochemicals, particularly polyphenolic compounds, regulating pro-inflammatory cell signaling and interacting with gut microbiota. The extract, rich in curcumin antioxidants, shows promise as a non-pharmacological therapy for dyslipidemia and antiatherosclerosis. Moreover, it reduced SGOT and SGPT levels significantly, suggesting protection against Non-Alcoholic Fatty Liver Disease (NAFLD). However, potential mild NAFLD in the turmeric extract group requires further investigation.

KEYWORDS: *Turmeric, Curcuminoids, Anti-dyslipidemia, Wistar rats, NAFLD protection*

BACKGROUND

Turmeric, which is a variety of Curcuma domestica, is a traditional medicinal plant used in herbal medicine in Indonesia. The main component of turmeric is curcuminoids, a type of dye that is present in about 2.5 to 6% of the plant. Turmeric rhizomes contain other chemical elements, such as essential oils, starches, bitter substances, resins, cellulose, and minerals. Turmeric has various beneficial pharmacological effects, such as improving blood circulation and vitality, reducing blockages in the menstrual cycle, having anti-inflammatory (anti-inflammatory) properties, facilitating childbirth, effective as an antibacterial, increasing bile flow (chole gum), reducing farting (carminative), and moisturizing the body (El-Sayed *et al.*, 2011): (Manarin *et al.*, 2019); (Rezzani, Franco and Rodella, 2019); (Sabale, Modi and Sabale, 2013). Dyslipidemia is a lipid metabolism disorder characterized by an increase or decrease in the lipid fraction in plasma. There are several ways to screen or evaluate anti-dissipidemia activity, namely in vivo and in vitro methods (Jijith and Jayakumari, 2018; Untari and Pramukantoro, 2020).

Dyslipidemia can develop due to a daily diet that raises cholesterol levels, particularly LDL cholesterol. In dyslipidemia, there is typically a reduction in HDL cholesterol levels, which play a crucial role as natural antioxidants. This decrease in HDL levels can lead to endothelial dysfunction in blood vessels, causing increased permeability in the endothelial lining. This heightened permeability allows high LDL cholesterol levels in the blood to penetrate the endothelium. Dyslipidemia is classified as a lipid metabolism disorder characterized by abnormal plasma lipid levels, either an increase or decrease. It is a significant risk factor for various diseases, including coronary heart disease (CHD), which remains a significant health concern in Indonesia (Irmadoly *et al.*, 2014). Significant abnormalities in the lipid fraction usually involve increased levels of total Cholesterol, LDL (Low-Density Lipoprotein) cholesterol, and triglycerides, as well as a decrease in HDL (High-Density Lipoprotein) cholesterol (Dipiro J *et al.*, 2015) (Ardhani *et al.*, 2017). The disease is also one of the risk factors for atherosclerosis that can cause coronary heart disease (CHD) (D'Agostino *et al.*, 2008). According to American Heart Association data from 2013 to 2016, 92.8 million people, or 38.2% of adults in the United States, have total Cholesterol over 200 mg/dl (Aparicio *et al.*, 2021). Cardiovascular disease is the most common non-communicable disease in the world and was the cause of 17.8 million deaths in 2017 (Wulansari, 2020).

The use of drugs derived from natural ingredients is generally considered safer than chemical drugs, as stated in the Hariana

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study in 2007. One natural plant often used is turmeric, which contains a main compound called curcumin, according to research conducted by Ariani in 2017 and Winarto in 2004. Curcumin, in addition to acting as an antioxidant, can also help lower cholesterol levels by inhibiting the reabsorption of Cholesterol from outside sources (exogenous). In addition, curcumin can also increase the activity of HMG-CoA reductase inhibitor enzymes, which play an essential role in the regulation of fat synthesis. Thus, turmeric or curcumin supplements can help keep cholesterol levels within a healthy range. However, remember that natural medicines should also pay attention to the proper dosage and consult a healthcare professional (Komang and Laksmi, 2014); (Yunarto *et al.*, 2019). The efficacy of curcumin was demonstrated in a study involving dyslipidemia patients in the Sawotratap village area of Sidoarjo Regency who were administered turmeric extract for 12 days. Cholesterol levels were measured before and after the administration of turmeric rhizome extract. The research results, analyzed using the Paired t-test, indicated a significant difference in blood lipid levels among the research participants (Gustomi Rima, 2015). This study aims to investigate the effectiveness of the ethanol extract of turmeric (Curcuma longa) as an anti-dyslipidemia agent in male Wistar rats treated with Propylthiouracil.

RESEARCH METHODS

This study adopts an experimental design with a Pre-test and Post-test group-only control design approach, utilizing male Wistar rats as the experimental animals. The study was conducted in January 2024. The sample size for this study was determined using Federer's formula:

$$(r-1)\,(t-1) \geq 15$$

Information:
r: Number of samples in each treatment group
q: Number of treatment groups

$$5(r-1) \geq 15$$
$$r-1 \geq 15/5$$
$$r \geq 3+1$$
$$r \geq 4$$

Based on the results of these calculations, it can be concluded that at least four male Wistar rats (Rattus norvegicus) are needed in each treatment group. The rats weigh 180-200 grams and age between 2-4 months.

Table 1. Overview of the treatment of each group

No	Test Group	Treatment
1.	Normal	Test animals were not given any particular treatment and were only fed and drank ad libitium.
2.	Control	Test animals were given 1 ml of 0.5% Na CMC suspension daily for 14 days. Food and drink are provided ad libitum.
3.	Standard (25 mg/kgBB)	Test animals were given an oral suspension of simvastatin 5 ml/kgBB daily for 14 days. Food and drink are provided ad libitum.
4.	Turmeric Extract (Curcuma Longa) - I (300 mg / kgBB)	Test animals were given a Turmeric Extract (Curcuma Longa) dose of 2.5 ml/kg body weight once a day for 14 days. Food and drink are provided ad libitum.
5.	Turmeric Extract (Curcuma Longa) - II (600 mg/kgBB)	Test animals were given a Turmeric Extract (Curcuma Longa) dose of 5 ml/kg body weight once a day for 14 days. Food and drink are provided ad libitum.
6.	Turmeric Extract (Curcuma Longa) - III (1200 mg/kgBB)	Test animals were given a Turmeric Extract (Curcuma Longa) dose of 10 ml/kg body weight once a day for 14 days. Food and drink are provided ad libitum.

a) Measurement of Lipid Profile Parameters

Before the blood draw, the rats were satisfied at least 8 hours before the blood draw. Blood collection is done by direct withdrawal from the heart of mice as much as 1 ml. Put into a microtube and let stand ± 20 minutes. Then, the blood was centrifuged at a rate of 3000 rpm for 15 minutes to obtain the blood serum of the rats. The determination of lipid profiles is determined by the colorimetric method. Lipid profile examination is conducted at the Health Laboratory, North Sumatra Provincial Health Office.

b) Measurement of Biochemical Parameters of SGOT and SGPT

Blood collection was performed by direct withdrawal from the rat's heart, yielding approximately 1 ml of blood, which was then transferred into a microtube and allowed to stand for approximately 20 minutes. Subsequently, the blood was centrifuged at 3000 rpm for 15 minutes to obtain rat serum. The determination of SGOT and SGPT levels was based on enzymatic reactions using the Dyasis® reagent kit. The procedure for determining the catalytic activities of SGOT and SGPT followed the working procedure outlined by Dyasis®. SGOT and SGPT examinations were conducted at the Health Laboratory, North Sumatra Provincial Health Office.

The research data were analyzed using SPSS 25 software. Descriptive analysis was conducted on the research data, focusing on central tendency and dispersion, including lipid profile (LDL, HDL, Total Cholesterol, and Triglycerides), color, texture, and weight. Subsequently, the lipid profile data were analyzed using One-Way ANOVA if the data were normally distributed, followed by a post hoc Tukey HSD test to determine significant differences between treatments. However, the Kruskal-Wallis test was used as an alternative for non-normally distributed data.

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RESEARCH RESULTS

a. **Extract Characteristics:** After extraction using the maceration method on the ethanol turmeric (Curcuma longa) sample, the extract was found to have the following characteristics.

Table 2. Characteristics of Turmeric Ethanol Extract (Curcuma Longa)

Characteristic	Value
Fresh Simplisia Weight (gr)	500 gr
Dry Simplisia Powder Weight (gr)	214 gr
Solvent Volume (ml)	2120 ml
Extract weight (gr)	15,19 gr
Yield (%)	7.20%

The table data above shows that from 500 grams of turmeric (Curcuma Longa), an extract of 15.19 grams was found. Thus, the yield obtained from turmeric ethanol extract (Curcuma Longa) is 7.20%.

b. Phytochemical Screening

The results of phytochemical screening on turmeric ethanol extract samples (Curcuma Longa) can be seen in the following table.

Table 3. Phytochemical Screening Results of Turmeric Ethanol Extract (Curcuma Longa)

Phytochemical	Reagent	Results
Alkaloids	Bouchardart	+
Saponins	Mayer	+
Flavonoids	Dragondroff	-
	Wagner	+
	Aquadest + Alcohol 96%	-
Tannins	FeCl3 5%	+
Alkaloids	Mg(s) + HCl(p)	-
	NaOH 10%	-
	H2SO4 (p)	-
Saponins	FeCl3 1%	+
Flavonoids	Salkowsky	-
	Liberman Bouchard	+

The table data above shows that turmeric ethanol extract (Curcuma Longa) contains several phytochemical compounds including Alkaloids, Saponins, Flavonoids, Tannins, and Steroids and Terpenoids.

c. Evaluation of Anti-Dyslipidemia Effects

All parameters evaluated in this study, including body weight, total Cholesterol, lipid profile, SGOT levels, and SGPT, were analyzed for normality data using the Shapiro-Wilk test. The results of the normality analysis can be seen in the table below.

Table 4. Results of Data Normality Test with Shapiro-Wilk Test on All Research Parameters

Parameter		P Value	Data Distribution
Weight		0.399	Usual
Total Cholesterol before induction		< 0.05	Abnormal
Total Cholesterol after induction		< 0.05	Abnormal
Lipid Profile After Treatment	Total Cholesterol	0.445	Usual
	Triglycerides	0.004	Abnormal
	HDL levels	< 0.05	Abnormal
	LDL levels	0.143	Usual
SGOT levels		< 0.05	Abnormal
SGPT Rate		0.056	Usual

The data table above shows that body weight, total Cholesterol, LDL levels from the lipid profile after treatment, and SGPT levels have a standard data distribution. In contrast, the other parameters, including total Cholesterol before and after induction, triglyceride levels, HDL levels, and SGOT levels, have a non-normal distribution. Based on these data distributions, parametric statistical analysis was performed on data with a normal distribution, while non-parametric statistical analysis was performed on data with a non-normal distribution.

d. Total Cholesterol

To evaluate the anti-dyslipidemia effects of ethanol turmeric (Curcuma longa), a high-fat diet was given to the control group, standard group, ethanol turmeric extract (Curcuma longa)-I, II, and III groups. Before and after the administration of PTU, total Cholesterol in all rats was measured, and all total cholesterol data were analyzed using non-parametric statistics. The results of the analysis can be seen in the following table.

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Table 5. Comparison of Total Cholesterol Before and After PTU (Propylthiouracil) Administration in All Treatment Groups

Treatment Group	Total Cholesterol (mg/dL)	
	Before Induction	After Induction
Normal	118.00 (110-112)	118.73 (113-165)b
Standard	114.00 (110-117)	211.00 (209-211)a
Kontrol	115.50 (110-116)	210.00 (210-214)b
Ekstrak Ethanol Kunyit *(Curcuma Longa)* -I	116.00 (110-115)	210.50 (218-223)b
Ekstrak Ethanol Kunyit *(Curcuma Longa)*-II	113.50 (100-112)	210.00 (210-214)b
Ekstrak Ethanol Kunyit *(Curcuma Longa)*-III	117.00 (117-125)	209.50 (212-214)b
Nilai P	**0.846**	**0.012**

The data is presented as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column indicate significant differences.

From the data table above, it can be observed that before being given a high-fat diet, the total Cholesterol of rats in all treatment groups did not show significant differences (P value = 0.846). This indicates that the total cholesterol data of rats before the high-fat diet was consistent. However, the total cholesterol levels in all rat groups after the high-fat diet showed a different distribution, with only the control group, standard group, and Turmeric extract (Curcuma Longa)-I, II, and III groups showing consistent total cholesterol levels.

e. Lipid Profile

At the end of the study, all mice were terminated for blood collection and analysis of lipid profile and liver function (SGOT/SGPT). A comparison of lipid profiles in the entire rat treatment group can be seen in the table below.

Table 6.Comparison of Lipid Profiles in the Entire Mouse Treatment Group

Treatment Group	Lipid Profile			
	Total Cholesterol*	Triglycerides**	LDL*	HDL**
Usual	155.00 ± 2.40a	97.50 (97-100)a	55.50 ± 1.64a	61.50 (61-64)a
Standard	147.50 ± 0.58b	105.00 (101-105)b	65.00 ±1.27b	60.50 (60-65)a
Control	178.50 ± 6.05c	167.00 (162-179)c	107.20 ±5.60c	28.50 (57-45)b
Turmeric Ethanol Extract I	168.25 ± 1.50d	155.50 (155-155)d	85.75 ±2.62d	57.50 (56-59)b
Turmeric Ethanol Extract II	165.25 ± 2.22e	120.50 (119-122)e	77.50 ± 1.29e	61.50 (61-65)a
Turmeric Ethanol Extract III	151.70 ± 0.95e	110.00 (109-112)f	68.50 ±1.28f	61.00 (60-62)a
P Value	**< 0.05**	**0.012**	**< 0.05**	**0.012**

*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; **Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

Table 6. illustrates the comparison of lipid profiles among treatment groups in rats. The Normal group shows total Cholesterol of 155.00 ± 2.40, triglycerides of 97.50 (97-100), LDL of 55.50 ± 1.64, and HDL of 61.50 (61-64). Meanwhile, the Standard group has total Cholesterol of 147.50 ± 0.58, triglycerides of 105.00 (101-105), LDL of 65.00 ± 1.27, and HDL of 60.50 (60-65).

The Control group shows a significant change with total Cholesterol of 178.50 ± 6.05, triglycerides of 167.00 (162-179), LDL of 107.20 ± 5.60, and HDL of 28.50 (57-45). Furthermore, the treatment groups with Ethanol Turmeric Extract (Curcuma Longa) show a decrease in total Cholesterol, triglycerides, and LDL successively for Ethanol Turmeric Extract (Curcuma Longa)-I (168.25 ± 1.50, 155.50, 85.75 ± 2.62, and 57.50), Ethanol Turmeric Extract (Curcuma Longa)-II (163.25 ± 2.22, 120.50, 77.50 ± 1.29, and 61.50), and Ethanol Turmeric Extract (Curcuma Longa)-III (151.70 ± 0.95, 110.00, 68.50 ± 1.28, and 61.00). A P value less than 0.05 indicates significant differences among treatment groups, particularly in total Cholesterol and LDL. This suggests that administering ethanol turmeric extract in rats can potentially improve lipid profiles, primarily by reducing total Cholesterol and LDL and increasing HDL in specific treatments.

Table 7.Comparison of SGOT and SGPT Levels in All Treatment Groups

Treatment Group	SGOT (U/L)	SGPT Rate (U/L)
Usual	37.50 (36-30)a	47.50 ± 1.50a
Standard	110.00 (106-110)b	171.00 ± 1.34b
Control	167.50 (163-170)c	97.35 ± 1.50c
Turmeric Ethanol Extract (Curcuma longa)-I	117.50 (116-130)d	100.75 ± 3.56d
Turmeric Ethanol Extract (Curcuma Longa)-II	131.00 (130-134)e	115.00 ± 4.50e
Turmeric Ethanol Extract (Curcuma Longa)-III	139.50 (134-130)f	143.00 ± 3.04b
P-Value	**0.012**	**< 0.05**

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*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; **Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

Table 7 provides an overview of the comparison of SGOT and SGPT levels among treatment groups in rats. The Normal group shows SGOT levels of 37.50 (36-30) and SGPT levels of 47.50 ± 1.50. On the other hand, the Standard group shows a significant increase, with SGOT levels reaching 110.00 (106-110) and SGPT levels of 171.00 ± 1.34. The Control group shows a substantial change with SGOT levels of 167.50 (163-170) and SGPT levels of 97.35 ± 1.50. Furthermore, the treatment groups with Ethanol Turmeric Extract (Curcuma Longa) show a decrease in SGOT and SGPT levels successively for Ethanol Turmeric Extract (Curcuma Longa)-I (117.50, 100.75 ± 3.56), Ethanol Turmeric Extract (Curcuma Longa)-II (131.00, 115.00 ± 4.50), and Ethanol Turmeric Extract (Curcuma Longa)-III (139.50, 143.00 ± 3.04). A P value less than 0.05 indicates significant differences among treatment groups, particularly in SGOT and SGPT levels. These results suggest that administering ethanol turmeric extract in rats can decrease SGOT and SGPT levels, indicating a possible improvement in liver function using ethanol turmeric extract in specific treatments.

DISCUSSION

The results of this study indicate that ethanol turmeric (Curcuma Longa) extract shows significant improvement in lipid profile at the end of the study. The highest dose of ethanol turmeric (Curcuma Longa) exhibited the most optimal improvement in lipid profile. This can be seen from the reduction in total Cholesterol, triglycerides, and LDL levels, as well as the increase in HDL levels in the Turmeric Extract (Curcuma Longa)-II and III groups. However, the lipid profile improvement in the Turmeric Extract (Curcuma Longa)-III group did not surpass the improvement shown in the standard group.

The anti-dyslipidemia effect of ethanol turmeric (Curcuma Longa) extract may be related to various phytochemicals in turmeric rhizomes. Some studies have demonstrated the potential of phytochemicals as anti-dyslipidemia agents. Polyphenol content can lead to down-regulation of pro-inflammatory cell signaling pathways such as nuclear factor-κB, activated protein-1, and mitogen-activated protein kinase by inhibiting the arachidonic acid cascade and eicosanoid derivatives. Another possible mechanism for the anti-dyslipidemia effect of polyphenolic compounds is the regulation of gut microbiota. Polyphenolic compounds in the gut interact with gut microbiota, leading to increased production of beneficial metabolites such as short-chain fatty acids. Additionally, gut microbiota such as Akkermansia municiphilia sp. can restore intestinal inflammation and improve gut permeability and insulin sensitivity. Furthermore, improvement in gut microbiota protects the gut-liver axis, thus reducing the lipid profile in the body. (Sun, Wang, and Qin, 2018; Feldman et al., 2021)

Other studies focusing on the anti-dyslipidemia effects of ethanol turmeric are limited. However, the survey by Ardhani (2017), titled "Effectiveness of Turmeric Extract (Curcuma domestica) as Non-Pharmacological Therapy for Dyslipidemia and Anti-Atherosclerosis," stated that turmeric extract administration can be a non-pharmacological therapy for dyslipidemia and as an anti-atherosclerotic agent. Turmeric extract contains curcumin compounds, which are antioxidants. Curcumin can reduce LDL oxidation, which plays a role in foam cell formation, suppresses blood vessels' inflammation, and protects blood vessel endothelium from free radicals (Ardhani et al., 2017). Besides its antioxidant properties, curcumin can lower cholesterol levels by inhibiting the reabsorption of exogenous Cholesterol and increasing the activity of Hmg-CoA reductase inhibitors, thus promoting proper fat synthesis (Komang and Laksmi, 2014). Treatment and prevention of diseases with curcumin are considered as effective as pharmacological approaches (Shishodia et al., 2005).

Additionally, ethanol turmeric extract significantly reduced SGOT and SGPT levels compared to the control group. The decrease in SGOT and SGPT levels is related to the improvement of Non-Alcoholic Fatty Liver Disease (NAFLD). Several studies have shown that NAFLD is a risk factor for atherosclerosis. This is because NAFLD causes dysfunction of blood vessel endothelium. Thong and Quynh (2021) reported that SGOT and SGPT correlate with NAFLD occurrence, but using SGOT and SGPT separately can lead to errors in confirming mild NAFLD. In cases of severe NAFLD, SGOT will increase slightly, and in milder cases, SGOT levels may be found within the normal range. Therefore, using SGOT and SGPT separately may lead to errors in confirming mild NAFLD degrees (Thong and Quynh, 2021).

In this study, SGOT and SGPT levels in the rat groups receiving ethanol turmeric extract (Curcuma Longa) were lower than SGOT and SGPT levels in the control group. This indicates that ethanol turmeric extract (Curcuma Longa) can protect liver tissue from NAFLD compared to the group not receiving ethanol turmeric extract (Curcuma Longa). However, the possibility of mild NAFLD in the rat group receiving ethanol turmeric (Curcuma Longa) extract cannot be ruled out.

CONCLUSION

Turmeric ethanol extract (Curcuma Longa) provided significant lipid profile improvements, especially at the highest doses, with reductions in total Cholesterol, triglycerides, and LDL, as well as increased HDL in groups II and III, although not yet exceeding the standard group. Anti-dyslipidemia effects are linked to turmeric phytochemicals, especially polyphenolic compounds, which lower lipid profiles by regulating pro-inflammatory cell signaling and interaction with gut microbiota. Turmeric extract, with curcumin compounds as antioxidants, has potential as a non-pharmacological therapy for dyslipidemia and antiatherosclerosis. In addition, turmeric ethanol extract showed a significant reduction in SGOT and SGPT levels, showing the potential to protect against Non-Alcoholic Fatty Liver Disease (NAFLD). However, the

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possibility of mild NAFLD in the group of rats receiving turmeric extract needs further investigation.

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