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ORIGINAL ARTICLE

Hypolipidemic effect of lime (*Citrus aurantifolia*) fruit ethanol extract on total cholesterol levels in rats

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ABSTRACT

High cholesterol levels are a significant risk factor for cardiovascular disease. Statins are commonly used to reduce total cholesterol levels; however, long-term use can lead to adverse effects such as myalgia, an increased risk of type 2 diabetes, and liver damage. As a result, there is a growing need for research into natural medicines for cholesterol management. Lime (*Citrus aurantifolia*) is rich in flavonoids, which have been shown to reduce cholesterol by inhibiting the HMG-CoA reductase enzyme, a mechanism similar to that of statins. This study aimed to evaluate the effect of lime fruit ethanol extract on total cholesterol levels in male Wistar strain rats induced with a high-fat diet and PTU. This study was conducted as a laboratory experiment using a post-test control group design. Male Wistar rats were divided into six groups: a normal control group, a negative control group (high-fat diet + PTU), a positive control group (simvastatin), and three test groups (lime fruit ethanol extract 0.875, 1.75, and 3.5 g/kg BW). The high-fat diet and PTU were induced for 14 days, and total cholesterol levels were determined using the enzymatic colorimetric method (CHOD/PAP) at the end. The administration of lime fruit ethanol extract at a dose of 0.875 g/kgBW significantly reduced total cholesterol compared to the negative control group (p=0.013), with no significant difference from the normal group (p=0.965). However, higher doses of 1.75 and 3.5 g/kg BW caused mortality in the test groups. These findings suggest that lime fruit ethanol extract effectively reduces total cholesterol levels at a dose of 0.875 g/kg BW. Further research is needed to determine the optimal dose and toxicity profile of lime fruit ethanol extract for hyperlipidemia therapy.

Keyword: *Citrus aurantifolia*, flavonoids, hypercholesterolemia, lime extract, total cholesterol

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INTRODUCTION

Total cholesterol refers to the overall amount of cholesterol circulating in the blood and bound to LDL, HDL, or other lipoproteins such as VLDL and IDL.[1,2] Cholesterol serves as a precursor to various compounds in the human body, including steroid hormones, bile acids, and vitamin D. Impaired cholesterol production can trigger hemorrhagic strokes, mental disorders, and cancer. Meanwhile, excessive cholesterol levels can also trigger the onset of cardiovascular diseases such as coronary heart disease and stroke.[3,4] In a day, the body requires 160-200 mg/dl of cholesterol.[3] Therefore, a total cholesterol level considered normal is less than 200 mg/dl. A total cholesterol level between 200-239 mg/dl is considered borderline high, while a level of ≥ 240 mg/dl is considered high risk and requires immediate intervention.[5] Risk factors associated with elevated total cholesterol levels include the consumption of foods high in saturated fats, low intake of fruits and vegetables, obesity, low physical activity, hypertension, stress, smoking, and alcohol use.[6]

Hypercholesterolemia, which is high cholesterol, is one of the main causes of atherosclerosis and ischemic heart disease, according to the World Health Organization (WHO).[7] Based on data from the American Heart Association from 2017 to 2020, the prevalence of total cholesterol levels ≥ 200 mg/dl in the world among adults was 32.8% in men and 36.2% in women.[8] Meanwhile, in Indonesia, data from the Basic Health Research (Riskesdas) in 2018 revealed that 28.8% of the population aged ≥15 years had total cholesterol levels exceeding the normal limit, with a higher prevalence in women and urban residents.[9] The management of hypercholesterolemia involves both pharmacological and non-pharmacological approaches. Lifestyle modifications that can help lower total cholesterol levels include avoiding trans-fat diets, reducing saturated fat diets, increasing fiber-rich food consumption, losing weight, and increasing physical activity.[5] Meanwhile, the treatment of hypercholesterolemia currently often uses statins, lipid-lowering drugs that effectively reduce total cholesterol levels by inhibiting the HMG-CoA reductase enzyme. However, long-term use of statins can cause several side effects, such as myalgia, an increased risk of type 2 diabetes mellitus, and liver dysfunction.[10] Therefore, it is necessary to consider the use of herbal medicines based on natural ingredients that have similar effects but with a better safety profile.

One of the natural ingredients that has the potential to lower total cholesterol levels is lime (*Citrus aurantifolia*), which is rich in active compounds such as flavonoids.[11] Flavonoids are known to have the ability to inhibit the HMG-CoA reductase enzyme, thereby helping to lower cholesterol levels in the blood.[12] Lime is one of the plants that has the potential to lower total cholesterol levels.[11] One of the main flavonoids contained in lime is hesperidin. In 100 g of lime, there are 64 mg of hesperidin, which is higher than that found in sweet oranges and lemons.[13] To date, research on the effects of lime fruit extract in lowering cholesterol levels has been limited to leaf and peel extracts. A study by Lin et al. (2019) demonstrated that lime leaf extract was able to lower total serum cholesterol, serum triglycerides, and LDL, but did not show a significant increase in HDL levels in mice with high cholesterol levels.[14] Another study by Cyndi et al. (2016) using ethanol extract from lime leaves at various doses in mice with high cholesterol levels also reported a significant decrease in total serum cholesterol at a dose of 3.5 g/kgBW.[15] This effect may be attributed to the presence of sugar-bound flavonoids, which increase the solubility of flavonoids in water. Meanwhile, the effect of lime fruit extract on total cholesterol levels, particularly in animal models induced by a high-fat diet, has not been extensively studied. This study aims to determine the effect of administering ethanol extract of lime fruit on the total cholesterol levels of Wistar strain rats induced with a high-fat diet and propylthiouracil (PTU). The rats were induced using quail egg yolks, as they contain the highest cholesterol content among egg yolks, with 2139.17 mg per 100 grams, making them effective for elevating cholesterol levels. The quail egg yolks were diluted in used cooking oil, which had been reused multiple times at temperatures above 100°C, leading to the oxidation of unsaturated fatty acids into saturated fatty acids, which are known to increase blood cholesterol levels. Saturated fatty acids in the body will be converted into cholesterol in the liver, intestines, and other tissues, which can lead to an increase in blood cholesterol levels. PTU is an antithyroid drug that damages the thyroid gland and inhibits the production of thyroid hormones, which functions to lower blood cholesterol levels.[16,17] This research can provide a herbal-based alternative for the management of hypercholesterolemia, particularly in Indonesia.

METHODS AND SUBJECT

The study was conducted from September to October 2024. This research was a laboratory experimental study using a posttest-only control group design.

Experimental Animal

The experimental animals used in this study were male Wistar strain white rats (*Rattus norvegicus*), which were divided into six groups: a normal group, a negative control group (high-fat diet + PTU), a positive control group (simvastatin), and three test groups receiving lime fruit extract at doses of 0.875, 1.75, and 3.5 g/kg BW. The sample size for each group was determined using the Federer formula: (n-1) (t-1) ≥ 15, where *t* represents the number of groups and *n* represents the number of samples in each group.[18] Based on this formula, the calculation is as follows:

$$(n-1)(t-1) \geq 15$$
$$(n-1)(6-1) \geq 15$$
$$5(n-1) \geq 15$$
$$5n-5 \geq 15$$
$$5n \geq 20$$
$$n \geq 4$$

Thus, a total of 24 experimental animals were obtained, with a minimum sample size of four rats per group. The experimental animals were obtained from the Cytohistotechnology Laboratory at the Faculty of Technology and Health Sciences, Universitas Jenderal Achmad Yani. The inclusion criteria for the rats in this study were a body weight of 150–250 grams, an age of 2–3 months, and good health, characterized by active movement, clean fur, no wounds, and a good appetite for food and water. The exclusion criteria included Wistar strain rats that experienced a weight loss of more than 10% after the adaptation period. Additionally, a dropout criterion was applied for rats that died during the treatment.

This study used induction with a high-fat diet and PTU. The high-fat diet was prepared by mixing quail egg yolks with used cooking oil. It was administered to the rats via a gastric tube for 14 days at a dose of 2 ml/200 g BW. Meanwhile, PTU (0.01%) was mixed into drinking water and provided *ad libitum*.[19]

Plant Material

The material used in the research was lime fruit (*Citrus aurantifolia*), including its peel. The limes were obtained from the Manoko Experimental Garden, Lembang, West Java. The fruit samples underwent a determination test at the Herbarium Bandungense SITH ITB to ensure that the samples belonged to the species *Citrus aurantifolia*.

Plant Extraction

To prepare the ethanol extract from lime fruit, 2 kg of limes were first washed under running water and drained to remove any dirt or foreign particles. The limes were then thinly sliced, including the peel, and dried in an oven at 60°C for two days to produce dry simplicia. The dried simplicia was then ground into a fine powder. A total of 300 grams of lime powder was divided into three Erlenmeyer flasks, each containing 100 grams. The powder was macerated in 3 L of 90% ethanol and left to soak for four days at room temperature, with occasional stirring. After maceration, the filtrate was collected and filtered through filter paper. The filtered extract was then concentrated using a rotary evaporator.[20]

Preparation of 0.5% Na CMC Solution

The Na CMC solution was prepared by dissolving 0.5 grams of Na CMC in 10 ml of hot distilled water, stirring until homogeneous, and then adding distilled water to a final volume of 100 ml. This solution was used as a suspending agent in simvastatin and lime fruit ethanol extract suspensions.

Formulation of Simvastatin Suspension

Simvastatin is one of the lipid-lowering medicines. The dose of simvastatin for human adults is 10-20 mg/day; however, for a 200-gram rat, the dose must be adjusted using a conversion factor of 0.018, which is based on a standard human weight of 70 kg. Thus, the dose of simvastatin for adult rats weighing 200 grams is 10 mg x 0.018 = 0.18 mg/200 gr = 0.9 mg/kg BW.[21]

Phytochemical Screening

1. Flavonoid Test

   One gram of lime fruit ethanol extract was heated with water in a water bath and then filtered. 5 mL of the filtrate were placed into a test tube, followed by the addition of magnesium powder and 1 mL of 2N hydrochloric acid. The mixture was heated in a water bath and filtered again. The resulting orange-colored filtrate was placed into a test tube, and 5 ml of amyl alcohol was added. The mixture was shaken vigorously and allowed to separate. A reddish-brown color in the amyl alcohol layer indicated the presence of flavonoids.
2. Alkaloid Test

   One gram of lime fruit ethanol extract was basified with 5 ml of dilute ammonia and ground in a mortar. Then, 20 ml of chloroform was added while continuing to grind. After the grinding process was complete, the mixture was filtered, and the filtrate was placed into a test tube. 5 mL of 2N hydrochloric acid were added, and the mixture was shaken until two layers formed. The acid layer was separated and divided into three parts. The first part served as a blank. The second part was given 2-3 drops of Mayer's reagent and examined. The absence of a white precipitate indicated a negative result. The third part was given 2-3 drops of Dragendorff's reagent. The appearance of an orange-brown or brick-red precipitate confirmed the presence of alkaloids in the extract.
3. Polyphenol and Tannin Test

   One gram of lime fruit ethanol extract was heated with water in a water bath, then filtered while hot. The filtrate was divided into two equal parts. The first part was treated with 2-3 drops of iron (III) chloride reagent solution until a green-black color formed, indicating the presence of natural polyphenols. The second part was tested by adding 5 drops of 1% gelatin solution. The formation of a white precipitate confirmed the presence of tannins.
4. Saponin Test

   The lime fruit ethanol extract was stored in a test tube and heated in a water bath with hot water, then filtered. After cooling, the filtrate was shaken vigorously for 30 seconds. The formation of a stable foam (at least 1 cm in height) that persisted for several minutes indicated the presence of saponins.

5. Quinone Test

   One gram of lime fruit ethanol extract was heated with water in a water bath and then filtered. The resulting filtrate was added with 2-3 drops of KOH solution. The appearance of a deep red color confirmed the presence of quinone compounds.[22]

Experimental Animal Treatment

Before the treatment, 24 Wistar strain rats were adapted for one week in the laboratory with a standard diet of 20 g/head/day and *ad libitum* drinking water to prevent stress. After adaptation, the rats were weighed to ensure none met the exclusion criteria. They were then divided into six treatment groups:

1. Normal group: Received standard feed (20 g/head/day) with water provided *ad libitum* for 21 days.
2. Negative control group: Received standard feed (20 g/head/day) with water provided *ad libitum* for seven days, followed by additional high-fat diet feed and PTU in drinking water for 14 days.
3. Positive control group: Received standard feed (20 g/head/day) with water provided *ad libitum* for seven days, followed by high-fat diet feed, PTU in drinking water, and simvastatin (0.9 mg/kg BW) for 14 days.
4. Test group 1: Received standard feed (20 g/head/day) with water provided *ad libitum* for seven days, followed by a high-fat diet, PTU in drinking water, and lime fruit ethanol extract (0.875 g/kg BW) for 14 days.
5. Test group 2: Received standard feed (20 g/head/day) with water provided *ad libitum* for seven days, followed by high-fat diet feed, PTU in drinking water, and lime fruit ethanol extract (1.75 g/kg BW) for 14 days.
6. Test group 3: Received standard feed (20g/head/day) with water provided *ad libitum* for seven days, followed by high-fat diet feed, PTU in drinking water, and lime fruit ethanol extract (3.5 g/kg BW) for 14 days.

In this study, the high-fat diet was administered at 8 a.m., followed by simvastatin and lime fruit ethanol extract at 5 p.m. The time interval between injections was greater than one hour to align with the gastric emptying time in rats.[20] In addition, simvastatin and lime fruit ethanol extract were administered in the evening because cholesterol synthesis increases at night.[23] Lime fruit ethanol extract was administered at the same time as simvastatin because both were part of the treatment groups.

Measurement of Total Cholesterol Levels

After 21 days of treatment, blood samples were collected from the test animals. The rats were fasted for 12 hours before the blood collection. Blood samples were taken on the 22nd day through cardiac puncture after anesthetizing the rats with CO2 inhalation. A total of 3 ml of blood was taken and then stored in a red-capped vacutainer tube. The blood samples were then centrifuged to obtain serum. A volume of 50 microliters of serum was used to measure the total cholesterol levels using the enzymatic colorimetric method (CHOD/PAP).

The reagent blank, standard, and sample were prepared before proceeding. The ingredients were then combined until homogenous, followed by incubation for 10 minutes at 37°C. The absorbance of the standard and sample was measured against the reagent blank using a photometer with a wavelength of 500 nm (492-546 nm). This reaction produced a stable red color, and absorbance was measured for 30 minutes.[24]

Statistical Analysis

The total cholesterol level data obtained from this study were tested for normality using the Shapiro-Wilk test, as the sample size was fewer than 50. If the data were normally distributed and the variance was homogeneous ($p > 0.05$), differences between all groups were analyzed using the parametric statistical test ANOVA. Conversely, if the data were not normally distributed or the variance was not homogeneous ($p < 0.05$), differences between groups were analyzed using the non-parametric statistical test Kruskal-Wallis. If significant differences between groups were found ($p < 0.05$), a post hoc analysis was conducted as the next step. Meanwhile, weight data before and after the intervention period were analyzed using a paired T-test. Significant differences in weight before and after the intervention period were indicated by $p<0.05$ in the paired t-test results.

Ethical Aspect

This research was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, and received ethical approval on September 24, 2024, with the letter number 041/UH2.09/2024. There are ethical rules in place for doing animal research, including the 3R principle: replacement, reduction, and refinement. Replacement refers to attempts to replace or avoid the use of animal subjects, whereas reduction refers to reducing the number of animal subjects employed. The goal of refinement is to reduce the amount of pain, suffering, distress, or danger that animal subjects may feel. In this study, the rats were housed in cages containing four rats each, allowing them to engage in regular behaviors.[25] The cages were lined with bedding, which was replaced every two days. The storage area for the cages had adequate ventilation, proper air circulation, lamp lighting, and natural sunlight entering through shuttered windows. The room temperature was maintained between 26°C and 28°C.

RESULTS AND DISCUSSION

Results

Before treatment was administered to the experimental animals, the lime fruit ethanol extract underwent phytochemical screening as a preliminary test to identify the secondary metabolites contained within it. The results of the phytochemical screening on the lime fruit ethanol extract are listed in Table 1. These results confirmed that the lime fruit ethanol extract contained several secondary metabolites, namely flavonoids, alkaloids, tannins, polyphenols, saponins, and quinones.

Table 1. Results of Phytochemical Screening of Lime Fruit Ethanol Extract

Identification	Result	Description
Flavonoids	+	Formation of reddish-brown layer
Alkaloids	+	Formation of brick-red precipitate
Polyphenols	+	Formation of green-black color
Tannins	+	Formation of white precipitate
Saponins	+	Formation of 1 cm stable foam lasting ± 10 minutes
Quinones	+	Formation of deep red color

All male Wistar rat samples were kept in cages, grouped according to their treatment to facilitate feed intake control throughout the study. Cage maintenance and cleaning were performed daily by the researchers to minimize the risk of disease transmission from the surrounding environment. During the study, the rats in the normal group, negative control group, and Test 1 group (lime fruit ethanol extract 0.875 g/kg BW) remained generally healthy. However, two rats died in the positive control group that was given simvastatin. Meanwhile, three rats died in the Test 2 and 3 groups that were given lime fruit ethanol extract at doses of 1.75 and 3.5 g/kg BW, most likely as a result of the high dose of lime fruit ethanol extract, which caused more than half of the rats in those groups to die during the study. The first rat to drop out was from the Test 3 group (lime fruit ethanol extract 3.5 g/kg BW) on day 9, followed by a rat from the Test 2 group (lime fruit ethanol extract 1.75 g/kg BW) on day 10. Another rat from the Test 3 group died on day 12, and finally, a rat from the Test 2 group died on day 15. Although the mortality intervals were close, the first rat to die was in the group receiving the higher dose (3.5 g/kg BW). As a result, only data from the normal group, negative control group, and Test 1 group (lime fruit ethanol extract 0.875 g/kg BW) were included. All rats were treated equally, with the only difference being the type and dose of treatment, making it difficult to rule out other potential causes of death beyond toxicity.

The researchers weighed the rats weekly. Table 2 shows that all groups exhibited a significant increase in body weight after the intervention period compared to the acclimatization period. This was confirmed by paired T-test analysis with a p-value < 0.05 in each analyzed group.

Table 2. Rats' Body Weight

Group	Body Weight (g) Acclimatization (Mean ± SD)	Intervention (Mean ± SD)	Difference (Mean ± SD)	p-value
Normal	201.25 ± 19.46	218.00 ± 12.83	16.75 ± 6.84	0.016*
Negative control	184.00 ± 7.83	208.00 ± 9.20	24.00 ± 6.97	0.006*
Lime fruit ethanol extract 0.0875 g/kg BW	182.00 ± 6.05	212.00 ± 6.48	30.00 ± 12.13	0.016*

*) p<0.05

Following the intervention period, blood samples were collected from all groups, each consisting of more than three rats. The normal total cholesterol level in rats ranges from 10-54 mg/dL. Table 3 shows the total cholesterol measurement findings in the three examined groups. The negative control group, which received a high-fat diet and PTU without lime fruit ethanol extract, had the highest average total cholesterol level (66.25 ± 3.77 mg/dL). The group that received a high-fat diet, PTU, and lime fruit ethanol extract at 0.875 g/kg BW exhibited the lowest total cholesterol level (56.30 ± 4.5 mg/dl). Although the total cholesterol levels in all groups were higher than normal, the normal group and the lime fruit ethanol extract 0.875 g/kg BW group had levels closest to the normal range. ANOVA analysis revealed a significant difference in total cholesterol levels across the three groups, with p=0.007 (p<0.05).

Table 3. Total Cholesterol Levels of All Analyzed Groups

Group	Mean Total Cholesterol Level (mg/dl) ± SD	p-value
Normal	57.00 ± 1.82	0.007*
Negative control	66.25 ± 3.77	
Lime fruit ethanol extract 0.0875 g/kgBW	56.33 ± 4.50	

*) p<0.05

To determine significant differences in each group, a post-hoc Tukey test was conducted as three groups were analyzed. The post-hoc Tukey test results in Table 4 demonstrate a significant difference between the normal group and negative control group (p=0.012), showing that the induction of a high-fat diet and PTU successfully elevated the experimental animals' total cholesterol levels. However, no significant difference was found between the normal group and the group treated with lime fruit ethanol extract at 0.875 g/kg BW (p=0.965), suggesting that the total cholesterol levels of rats induced with a high-fat diet and PTU but treated with lime fruit ethanol extract remained close to the total cholesterol levels of rats not induced with a high-fat diet and PTU. The group treated with lime fruit ethanol extract at 0.875 g/kg BW also showed a significant difference when compared to the negative control group (p=0.013). This indicates that the administration of lime fruit ethanol extract at 0.875 g/kg BW can lower total cholesterol levels even though the rats were induced with a high-fat diet and PTU.

Table 4. Comparison of Total Cholesterol Levels Between Groups

Group		Post Hoc Tukey Test p-value
Normal	Negative control	0.012*
	Lime fruit ethanol extract 0.875 g/kg BW	0.965
Negative control	Lime fruit ethanol extract 0.875 g/kg BW	0.013*
	Normal	0.012*
Lime fruit ethanol extract 0.0875 g/kg BW	Normal	0.965
	Negative control	0.013*

*) p<0.05

Discussion

The increase in body weight of the rats observed during the study, from highest to lowest, was noted in the group receiving a high-fat diet with lime fruit ethanol extract, followed by the high-fat diet only group, and finally, the standard feed group. This trend can be attributed to the high-fat diet and interventions that increase energy consumption, leading fat to accumulate in the body. In the group that received a high-fat diet, sensitivity to leptin, a hormone that regulates appetite and hunger, was reduced, resulting in an increase in appetite.[26]

Induction of a high-fat diet with propylthiouracil (PTU) for 14 days has been shown to raise blood cholesterol levels. The standard feed group had an average total cholesterol of 57 ± 1.82 mg/dl, while the high-fat diet induction group had an average total cholesterol of 66.25 ± 3.77 mg/dl ($p<0.05$). This shows a considerable increase in the group treated with a high-fat diet with PTU. Quail egg yolks contain more cholesterol than other egg yolks, and cooking oil can exacerbate hyperlipidemia due to its saturated fatty acid concentration.[19] PTU raises total cholesterol levels by inhibiting the production of thyroid hormones (T3, T4). Thyroid hormones can stimulate the production of LDL in the liver, lowering blood cholesterol levels. When thyroid hormone levels fall, cholesterol catabolism declines, resulting in an increase in blood cholesterol levels.[17,27]

The treatment of lime fruit ethanol extract at 0.875 g/kg BW for 14 days effectively lowered total cholesterol levels. This finding is consistent with previous research by Cyndi, who demonstrated that ethanol extracts of lime leaves reduced overall blood cholesterol levels in rats.[15] Although the mechanism is unknown, it is most likely owing to the phytochemical content in the ethanol extract of lime fruit, which has hypolipidemic effects.

The phytochemical screening results revealed that the ethanol extract of lime fruit contains a variety of secondary metabolites capable of lowering blood cholesterol levels. Flavonoids reduce total cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which reduces cholesterol synthesis and the activity of the enzyme acyl-CoA cholesterol acyltransferase, resulting in decreased cholesterol absorption in the digestive tract.[28] Polyphenols can lower cholesterol levels by inhibiting Apo B (Apolipoprotein B) secretion and reducing cholesterol absorption by binding to cholesterol carriers as they pass through the small intestine's brush border membrane. Meanwhile, tannins limit the function of HMG-CoA reductase, which reduces cholesterol synthesis in the liver. Saponins bind to bile acids and form giant micelles, inhibiting cholesterol absorption on the surface of the small intestine epithelium.[29] Alkaloids can reduce the activity of pancreatic lipase enzymes, which increases fat outflow and inhibits the liver’s absorption of fat, thus preventing its conversion to cholesterol.[30]

Although this study can contribute to a better understanding of the effects of lime fruit ethanol extract on total cholesterol levels, there are limitations. The optimal concentration of lime fruit ethanol extract for lowering total blood cholesterol levels remains unknown. This is because administering high doses of the extract, especially at 1.75 g/kg BW and 3.5 g/kg BW, resulted in the deaths of more than half of the rat population. This suggests that increasing the dosage does not necessarily result in increased therapeutic efficacy.

Recently, there has been no research on the lethal dose of lime fruit (*Citrus aurantifolia*) in mg or gram units. However, previous studies determined that lime (*Citrus aurantifolia*) had an LD50 of 54.8 ml/kg BW.[31] In another study, Shchérazade found that an aqueous extract of lime leaves did not cause mortality at a dose of 2000 mg/kg BW. As a result, according to OECD guideline 423, the lethal dose for lime extract ranges between 2000 and 5000 mg/kg BW. However, further research is needed to determine the lethal dose of lime extract, whether derived from the leaves, peel, or fruit.[32] Additionally, further evaluation of the optimum dose of lime fruit ethanol extract, as well as toxicological investigations, is required for future study to ensure the safety of lime fruit ethanol extract as a hyperlipidemia therapy in humans.

CONCLUSION

The research findings indicate that lime fruit ethanol extract at a dose of 0.875 g/kg BW exhibits a hypolipidemic effect.

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DECLARATION OF INTERESTS

The author hereby declares that there are no conflicts of interest in this publication.

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