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Research Article

Potential of Lime Juice with Dregs on Lipid Profiles in Mice Using Depo Medroxy Progesterone Acetate (DMPA)

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ABSTRACT

Depo Medroxy Progesterone Acetate (DMPA) is an injectable method for contraception, containing progesteron, with high pregnancy protection (99,9%). However, the use of DMPA may result in imbalance of estrogen, in turn, can decrease High Density Lipoprotein (HDL) and increase Low Density Lipoprotein (LDL). A study on the use of DMPA showed a decrease in HDL cholesterol while an increase in LDL cholesterol and total cholesterol. Flavonoids from citrus fruits, in the form of hesperidin, can increase HDL cholesterol levels in hypercholesterolemic human subjects. Another study also stated that giving lime juice increased HDL cholesterol levels. The purpose of this study was to determine the effectiveness of lime juice with pulp in increasing HDL cholesterol levels in mice using Depo Medroxy Progesterone Acetate (DMPA). Method: The method in this study is an experimental study with a pretest-posttest control group design. Results: Potential of Lime Juice with Dregs on Livid Profile in Mice using DMPA. The data were analyzed by using the Paired t-test parametric test to compare between treatment groups. There is an effect of giving lime juice without pulp on day 35 and giving lime juice with pulp on day 14 and day 35 on HDL levels of mice given DMPA injection. There was no effect of giving lime juice without pulp on day 14 and day 35 as well as giving lime juice with pulp on day 14 and day 35 on LDL levels of mice given DMPA injection. There was an effect of giving lime juice without pulp on day 35 and giving lime juice with pulp on day 14 and day 35 on the triglyceride levels of mice given DMPA injection. There is an effect of giving lime juice with dregs on day 14 and day 35 on total cholesterol levels in mice given DMPA injection. Conclusions: Giving lime juice with pulp can be recommended as a product that can be consumed to lower cholesterol levels.

Keywords: Depo Medroxy Progesterone Acetate, Lime Juice with Dregs, Lipid Profiles

Introduction

Depo Medroxy Progesterone Acetate (DMPA) is an injectable method for contraception, containing progesteron, with high pregnancy protection (99,9%). However, the use of DMPA may result in imbalance of estrogen, in turn, can decrease High Density Lipoprotein (HDL) and increase Low Density Lipoprotein (LDL). A study on the use of DMPA showed a decrease in HDL cholesterol while an increase in LDL cholesterol and total cholesterol. Flavonoids from citrus fruits, in the form of hesperidin, can increase HDL cholesterol levels in hypercholesterolemic human subjects. Another study also stated that giving lime juice increased HDL cholesterol levels. The purpose of this study was to determine the effectiveness of lime juice with pulp in increasing HDL cholesterol levels in mice using Depo Medroxy Progesterone Acetate (DMPA). Several previous studies conducted by Yadav et al., (2011)

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regarding the effect of long-term use of DMPA on lipid metabolism, in 60 women in Nepal who had used it for more than 36 months showed that triglyceride, total cholesterol and LDL levels were higher than non-acceptors, while HDL levels decreased. The results of research in Tomohon City have examined the effect of Depo Medroxy Progesterone Acetate injections with lipid profiles in 250 women, it was found that the average total cholesterol in the use of DMPA injections at 1 month (174.42 m/dl) decreased significantly (p < 0 0.05) compared to baseline (188.31 mg/dL), but there was an increase in the average total cholesterol level again after 3 months (182.35 mg/dL), 6 months (184.08 mg/dL), 9 months (182.50 mg/dL) to 12 months (184.30) (Sanger, Loho and Wirasti, 2008). Of this study, DMPA injections can cause increased levels of triglycerides, total cholesterol and LDL which can increase the risk of cardiovascular disease.

The use of Depo Medroxy Progesterone Asetate found temporary changes in blood lipids and began to appear within a few weeks after injection, so it is recommended to measure blood fat levels in long-term use (Adam, 2009). Efforts to provide family planning and reproductive health services, namely providing pre-selection counseling for contraceptive methods, before giving birth and after delivery or changing ways that have no side effects/complications by increasing the use of long-term contraceptive methods (WHO, 2020). The use of injectable contraception which is in great demand by women of childbearing age cannot be prohibited because it is a person's right to make his/her choice. In addition, the target for the use of family planning must be in line with government programs in achieving national health targets. However, the impact of using it must be suppressed. One of the effects is an increase in blood cholesterol levels. The longer the use of DMPA, the more the impact on increasing blood cholesterol levels.

The results of the study by Suratiah, et al (2021), stated that there was a significant difference in HDL levels between before and after administration of DMPA injection on day 14 and day 35. There was a relationship between the duration of administration of DMPA injections with HDL levels in mice. There was no difference in LDL levels between before and after administration of DMPA injection on day 14 and day 35. There is no relationship between the duration of administration of DMPA injections with LDL levels in mice. There was no difference in triglyceride levels before and after administration of DMPA injection on day 14, but on day 35 there was a significant difference but very little. There is no relationship between the duration of administration of DMPA injections with triglyceride levels in mice. There was a difference in total cholesterol levels between before and after administration of DMPA injection on day 14 and day 35. There was a relationship between the duration of administration of DMPA injections with HDL levels in mice.

To inhibit the increase the level of cholesterol is lime. Lime is very easy to get, easy to grow and very cheap so it is very affordable. Lime in Indonesia is commonly used as a food ingredient to add aroma and taste to food. There are already many lime juice drinks in the community, but it is necessary to see the direct impact on HDL cholesterol empirically.

Lime (Citrus aurantifolia) is a fruit that contains vitamin C and the main flavonoid substance in the form of hesperidin. The hesperidin content of lime is greater than that of other oranges, which is 15.64 mg/100g. Previous studies have shown that a flavonoid from citrus fruits, in the form of hesperidin, can increase HDL cholesterol levels in hypercholesterolemic human subjects. Another study using hypercholesterolemic wistar rats given two types of oranges (lemon and lime) at a dose of 1 ml/day for 7 days showed a significant decrease in cholesterol levels in both types of oranges, but a greater decrease occurred in limes than oranges. lemons. Another study also stated that giving lime juice increased HDL cholesterol levels by 116.4% (p = 0.000). (Muthia Nada, 2014) This study used lime because it has antilipidemic potential.

The results of research by Suratiah, et al (2021), stated that the most flavonoid compounds were found in lime which was blended with the pulp and squeezed with a value of 20.83 Mg/100g QE. This shows that the flavonoid content is abundant in the skin of the fruit. Flavonoids are secondary metabolites in lime which have the highest concentration in the peel. Flavonoids are one of the polyphenolic compounds that have antioxidant properties. Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that these free radicals can be suppressed and do not damage body cells. (Sayuti and Yenrina, 2015). Based on research conducted by Elon

(2015), lime has been shown to have an effect in lowering cholesterol levels in the blood, where lime juice therapy, which can be accompanied by exercise or not, has the same results in reducing cholesterol levels. The content of flavonoids in lime can reduce blood cholesterol, triglyceride and LDL-cholesterol levels (Elon, 2015).

In the study of Suratiah, et al (2021), it was also found how to use lime fruit with its pulp to get the maximum flavonoid compounds for the body's needs. In line with the research of Hindun et al. (2017), orange peel extract contains flavonoids, with a total flavonoid of 0.667%.

This study will see how the potential of lime juice with pulp and without pulp on HDL cholesterol and lipid profile of mice given DMPA injection?

Methods

Experimental research with pretest-posttest control group design (Pocock, 2008). The research subjects were female mice with the Sprague dawley strain. During the initial 2 weeks, the mice were collected, then pre-test blood was taken for examination of the lipid profile through the orbital vein as much as 0.5 cc, then examined in the laboratory using the spectrophotometric method. After that, the mice in the treatment group were given DMPA injection of 38 mg every day and orange juice with and without pulp each as much as 0.3 cc every day for 14 and 35 days.

Result and Discussion

The results of the research conducted are as follows:

Table 1. High Density Lipoprotein (HDL) Cholesterol Levels Before and After Injection of DMPA and Lime Juice Without and With Dregs for 14 Days and 35 Days

	mean	Std.dev
PreTA - PostTA 14 days	9.25	6.90238
PreTA - PostTA 35 days	10.20	7.08439
PreDA - PostDA 14 days	16.13	4.38952
PreDA - PostDA 35 dys	18.61	3.39682

There was a very significant difference in HDL levels between in giving lime juice with pulp before and after on day 14 and day 35 before and after treatments.

Table 2. Low Density Lipoprotein (LDL) Levels Before and After Injection of DMPA and Lime Juice Without and With Dregs for 14 Days and 35 Days

	mean	**Std.dev**
PreTA – PostTA 14 days	5.38	3.41
PreTA – PostTA 35 days	6.46	2.55
PreDA – PostDA 14 days	5.38	3.61
PreDA – PostDA 35 days	12.48	3.54

Table 2 showed that there was significant difference between before and after treatment on the 14th and 35th days. This means that the administration of lime juice without and with pulp shows that there is no difference in LDL levels in the blood of mice.

Table 3. Trigliserida Levels Before and After Injection of DMPA and Lime Juice Without and With Dregs for 14 Days and 35 Days

	mean	Std.dev
PreTA – PostTA 14 days	5.13	1.39
PreTA – PostTA 35 days	10.38	1.34
PreDA – PostDA 14 days	3.83	0.65
PreDA – PostDA 35 days	10.10	1.81

The table 3 showed that there is no difference in Triglyceride levels between before and after treatment. However, there was a significant difference between triglyceride levels before and after lime juice was given without pulp.

Table 4. Total Cholesterol Levels Before and After Injection of DMPA and Lime Juice Without and With Dregs for 14 Days and 35 Days

	mean	Std.dev
PreTA – PostTA 14 days	153.0[a] 148.0[a]	38.73
PreTA – PostTA 35 days	31.00	10.53
PreDA – PostDA 14 days	5.25	0.16
PreDA – PostDA 35 days	51.38	8.32

Table 4 showed that there is no difference in cholesterol levels before and after treatment. There was no difference in cholesterol levels between before and after being given lime juice treatment without pulp on day 35.

Menwhile, there is a significant difference in total cholesterol levels between before and after being given DMPA injection treatment and lime juice with dregs on day 14 and day 35.

Discussion

High Density Lipoprotein (HDL) is cholesterol that serves to clean excess cholesterol that makes harm in the blood and bring it back to the liver to be removed from the body. Therefore, HDL is also known as the 'good cholesterol'. In addition to helping to remove excess bad cholesterol, HDL also functions to prevent damage to blood vessel walls due to fat accumulation and keep them healthy. So that HDL cholesterol levels actually need to be increased considering its function is needed by the body.

The treatment group after being given DMPA injections in mice, their HDL cholesterol decreased, the longer the administration decreased. The content of DMPA injections is progesterone

with a failure rate of <1%. Hormonal contraceptives are injected intramuscularly once every 3 months as much as 150 mg (Suratiah, et al. 2021). The hormone progesterone works by stimulating the appetite control center in the hypothalamus which causes appetite to increase. If the increase in appetite is not matched by body movement, it will cause an increase in body fat which is the result of the metabolism of carbohydrates and fats consumed (Hirschberg 2012). So it is very important for DMPA users to pay attention to the food they eat and exercise regularly so that glycogen stored as body fat from food consumption can be metabolized again.

The function of HDL is to reduce the oxidation of LDL and VLDL. If HDL decreases, there will be an increase in LDL and VLDL because the role of HDL as a thrombotic is not able to prevent fibrosis and calcification, which causes disruption of the elasticity of blood vessels (Cuchel and Rader, 2002). If this continues for a long time, atherosclerosis will occur. From this study, it was also found that the duration of administration of DMPA injections caused HDL levels to decrease.

This study appointed that HDL cholesterol levels before were different from HDL cholesterol levels after DMPA injection on day 14, as well as levels after injection on day 15. The results of the significance test also showed that there was a significant difference between the pre test and post test on day 15. the 14th and 15th days. This means that there is an effect of giving DMPA injections to HDL levels in the blood of mice and also the effect of the duration of DMPA injections. The longer the DMPA injection, the lower the HDL level in the blood.

The results of the current study by giving lime juice without pulp on the 14th day showed there was no significant, but in HDL levels in the blood of mice. From the results of giving lime juice with dregs, it turned out that on day 14 and day 35 showed a significance value of 0.000, which means there was a very significant difference. This shows that there was an effect of giving lime juice to increase HDL in mice using DMPA injection. However, giving lime juice with dregs gives a better and faster effect than lime juice without pulp.

Low Density Lipoprotein (LDL) is the "bad cholesterol" that can cause the buildup of cholesterol in the blood throughout the body. LDL serves to carry cholesterol from the liver into the blood vessels, while HDL functions to carry cholesterol from the blood vessels to the liver to be removed from the body. So if the state of LDL cholesterol increases it will result in a lot of cholesterol in the blood because a lot of it transports cholesterol from the liver to the blood vessels. On the other hand, if HDL decreases, it will not be able to return the excess cholesterol carried by LDL to the blood vessels because the amount is less. So that cholesterol in the blood increases. An increase in LDL levels is a barometer of an increase in total cholesterol levels in the blood.

The research of Suratiah, et al (2021) showed that an increase in LDL cholesterol levels in the treatment group after DMPA was injected and increased if injected for a longer time. The same study was also found by Yadav (2011) that various synthetic progesterone used as contraception can affect lipid and lipoprotein fractions. Total cholesterol (TC) and LDL-C levels were significantly higher in DMPA users compared to those not using DMPA, so the use of DMPA can induce changes in lipid metabolism that will increase the risk of cardiovascular disease. (Yadav, 2011).

The research of Suratiah, et al (2021) indicate a significant number for the duration of DMPA injection. The longer the administration, the higher the LDL level in the blood.

Tthere is no difference in the value of LDL levels after giving lime juice without and with pulp. However, significance value of day 35 is smaller than day 14. This indicates a possibility if it continues to be accompanied by giving lime juice without dregs the possibility of getting a significant difference value. In other words, the longer administration of lime juice will be able to affect the reduction of LDL levels in mice with DMPA injections.

Triglycerides are a type of fat that are often found in the blood. Triglycerides are produced in the liver, but most of them come from food. Fat obtained from food will be broken down and then converted into triglycerides. Excess and unused fat will become triglycerides which are stored in fat cells. If needed the body will convert it in the form of energy. If there are too many triglycerides in the blood, it can lead to thickening of the walls of blood vessels.

There was a significant difference between the pretest and posttest group on day 35. It was probably due to standard food provided and not foraging freely. In the current study, on day 14

there was no difference in triglyceride levels in the blood of mice with DMPA injections, but on day 35 there was a value very significant difference. This means that there is a different value of triglyceride levels in the blood of mice with DMPA injections (Berenson et al, 2009). This shows that lime juice with pulp can be recommended for use in lowering triglyceride levels in the blood.

Total cholesterol is a fat compound in the body that is produced by various body cells, and the most cholesterol produced by the body is produced by the liver. In other words, the body still needs cholesterol to stay healthy. Total cholesterol is a combination of HDL, LDL and triglycerides in the blood.

DMPA contains artificial progesterone or synthetic steroids that can stimulate the acceptor to eat so that it stimulates hunger and eats more. In addition, progesterone also causes carbohydrates and sugars to easily accumulate into fat. According to Zainatul (2019), there is a significant relationship between the duration of contraceptive use at Depo Medroxy Progesterone Acetan and the incidence of hypertension.

In the current study, giving lime juice without pulp obtained an average mean value of no difference on the 14th day, while on the 35th day a value of 0.022 was obtained, meaning there was no difference in total cholesterol levels. Meanwhile, by administering lime juice with pulp, a value of 0,000 was obtained both on day 14 and on day 35. This means that there was a significant difference in total cholesterol levels in the blood of DMPA-injected mice. This study complied with study conducted by Rahayu and Munjidah (2020). So giving lime juice with pulp can be recommended to lower total cholesterol in the blood.

Conclusion

There is an effect of giving Lime Juice Without Dregs on HDL and Triglycerides. Meanwhile, administration of Lime Juice with Dregs had an effect on HDL, Triglycerides and Total Cholesterol in the blood of DMPA-injected mice. Giving lime juice with pulp can be recommended as a product that can lower blood cholesterol levels.

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