Research Article

Effect of refined coconut oil intake on blood glucose, cholesterol, and leukocyte count of rats (*Rattus norvegicus*)

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ABSTRACT

Background: Medium chain fatty acids (MCFAs) of virgin coconut oil can be used as antimicrobial properties. The combination of MCFAs can act as a highly powerful defense against diseases. **Aim:** This study aims to investigate the effect of refined mandar oil (RMO) intake on plasma glucose level after high glucose induction as well as the immune response in rats. **Materials and Methods:** Hyperglycemic progression was induced in male albino Wistar rats by feeding them 21% w/v of glucose. The body weight, fasting and direct blood glucose level, 2h-pp blood glucose, and blood total cholesterol level were recorded for 30 days. The total white blood cell (WBC) count was calculated by an improved Neubauer counting chamber, and the differential count was performed by peripheral blood smear examination. **Results:** The blood glucose levels were increased significantly 30 and 120 min after high glucose feeding; however, the RMO diet restricted the elevation of blood glucose in comparison with the high glucose-fed control group without interfering with the total cholesterol level of rats. **Conclusion:** The present study showed that RMO might be beneficial in boosting the immune system by increasing WBC count, notably neutrophil percentage.

KEY WORDS: Coconut oil, Differential count, Neutrophil, Total white blood cell count

BACKGROUND

Coconut oil holds an important role as a functional food. Studies have revealed that populations who traditionally consume large quantities of coconut as a part of their diet have a low incidence of health problems associated with blood clotting, including heart disease and stroke.[1] Coconut oil is very stable and does not need to be refrigerated since it contains a saturated fatty acid (SFA) because all of the carbon atom linkages are filled or saturated with hydrogen. Coconut oil is produced by crushing copra, the dried kernel, which contains about 60–65% of the oil. There are different types of coconut oil based on the methods of production, including virgin coconut oil from wet coconuts (unrefined grade), coconut oil from dry coconuts (unrefined grade), and coconut oil by the solvent extraction method (refined from coconut expeller cake).[2]

Coconut oil contains mainly SFAs (93%), with lauric acid (C12:0) (50–55%) being the most prevalent fatty acid present. It also contains medium chain fatty acids (MCFAs) consisting of caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0), which can be easily burned for energy rather than being stored in the body.[3]

In 1966, Jon Kabara discovered that MCFAs of virgin coconut oil are incredible for their antimicrobial properties that kill harmful viruses, bacteria, fungi, and parasites. When MCFAs are digested, they break down into free fatty acids and monoglycerides. Lauric acid, capric acid, and caprylic acid are the most important MCFA present in coconut oil that possesses antimicrobial activity. Their monoglyceride forms, monolaurin, monocaprylin, and monocaprin, prevent microbes from terrorizing the immune system. Individually, these fatty acids act on microbes in

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different ways. Some may kill a particular organism that causes fungal infections but may not be as useful against other microbes. In combination, however, they act as a highly powerful defense against diseases. Monolaurin (the monoglyceride form of lauric acid) is considered to have the best antiviral, antifungal, and antibacterial effects.[4]

A study on the insulinotropic potency of lauric acid: A metabolic rationale for MCFA in total parenteral nutrition formulation by Garfinkel *et al*. proved that the effect of MCFA on insulin secretion depends on its chain length. Among all of the MCFAs, capric acid (C10) and lauric acid were observed to display the most potent effects on insulin secretion. Another study proved that, compared to other oils, coconut oil in the diet enhanced insulin action and improved binding affinity.[4]

Laga developed refined Mandar oil (RMO) which is produced from crude coconut oil, derived from Mandar district. This refining process aims to prevent the hydrolysis of free fatty acids and other soluble compounds to obtain a better quality of coconut oil with a more appealing taste and odor. The obtained coconut oil contains 92% SFAs, 48% lauric acid, 59% medium chain triglyceride, 0.05% water content, and 0.05% free fatty acids.[5] The use of coconut oil is, nowadays, widely considered an alternative food supplement. Therefore, the present study is required to evaluate the effect of RMO against plasma glucose level following the induction of 40% glucose and immune system by performing total and differential white blood cell (WBC) counts to increase scientific data regarding the health benefits of RMO.

Aim

The aim of this study was to investigate the effect of refined mandar oil (RMO) intake on plasma glucose level after high glucose induction as well as the immune response in rats.

MATERIALS AND METHODS

Materials

The materials used in this study were dextrose monohydrate, distilled water, eosin, 70% ethanol, glucose strip test (Nesco® multicheck), methanol, methylene blue, Turk solution (gentian violet, glacial acetic acid), and RMO.

Research Design

The male albino Wistar rats were fed by 21% w/v of glucose. The body weight, fasting and direct blood glucose level, 2h-pp blood glucose, and blood total cholesterol level were recorded for 30 days. The total WBC count was calculated by an improved Neubauer counting chamber and the differential count was performed by peripheral blood smear examination.

Conformity Criteria

Male Sprague Dawley rats weighing 150–200 g were used. They were fed a standard pellet diet and tap water *ad libitum* and maintained under standard laboratory conditions.

Research Facilities

This research was done in the Laboratory of Clinical Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar.

Research Duration

This research was conducted for 2 months.

Total and Differential WBC Count

The animals were divided into two treatment groups, namely Groups 1 and 2. Group 1 (healthy control) consisted of three healthy rats that were fed pellets and drinks without the administration of RMO, while Group 2 consisted of three rats that were fed pellets and drinks with the administration of 0.9 ml/200 g BW of RMO for 8 days. On the 10th day, blood collection was performed. Total WBC count was performed using the improved Neubauer counting chamber while the differential WBC count was done using peripheral blood smear count.

Plasma Glucose Level

To induce a hyperglycemic effect in rats, 40% w/v dextrose monohydrate solution was used. 15 male Wistar rats were divided into five treatment groups, each consisting of three rats that were determined randomly. Before treatment, drinks were given to rats for 10–14 h and fasting glucose level (Fasting blood glucose [FBG]) was measured. Group 1, as a healthy control, was given 2 ml/200 g BW distilled water, Group 2, as the hyperglycemic control, was given 2 ml/200 g BW of 40% dextrose monohydrate solution, Group 3, as the control without glucose induction, was given 1.8 ml/200 g BW of RMO, Group 4 was given 0.9 ml/200 g BW of RMO + 2 ml/200 g BW of 40% dextrose monohydrate solution, and Group 5 was given 1.8 ml/200 g BW of RMO + 2 ml/200 g BW of 40% dextrose monohydrate solution. Plasma glucose levels were examined at 30 min (GD30) and 120 min (GD120) after treatment in each group using the Nesco® multicheck glucose meter.

Ethical Review

All protocols were approved by the ethics committee of the university instituted for animal handling.

Statistical Data Analysis

We calculated the means with standard deviations for data with normal distribution.

Table 1: Rat plasma glucose levels

Treatment groups	Parameters			Percentage decrease in plasma glucose levels from min 30 to 120 (%)
	FBG	GD30	GD120	
G1	69 g/dl	75 g/dl	74 mg/dl	−6.58
	80 mg/dl	58 mg/dl	72 mg/dl	
	63 mg/dl	63 mg/dl	63 mg/dl	
Mean	70.6 mg/dl	65.3 mg/dl	69.6 mg/dl	
G2	65 mg/dl	112 mg/dl	85 mg/dl	12.83
	67 mg/dl	106 mg/dl	90 mg/dl	
	69 mg/dl	93 mg/dl	96 mg/dl	
Mean	67 mg/dl	103.6 mg/dl	90.3 mg/dl	
G3	60 mg/dl	77 mg/dl	48 mg/dl	39.71
	60 mg/dl	71 mg/dl	53 mg/dl	
	58 mg/dl	56 mg/dl	22 mg/dl	
Mean	59.3 mg/dl	68 mg/dl	41 mg/dl	
G4	87 mg/dl	104 mg/dl	83 mg/dl	3.89
	62 mg/dl	90 mg/dl	131 mg/dl	
	53 mg/dl	115 mg/dl	83 mg/dl	
Mean	67.3 mg/dl	103 mg/dl	99 mg/dl	
G5	76 mg/dl	83 mg/dl	57 mg/dl	21
	65 mg/dl	131 mg/dl	95 mg/dl	
	75 mg/dl	83 mg/dl	83 mg/dl	
Mean	72 mg/dl	99 mg/dl	78.3 mg/dl	

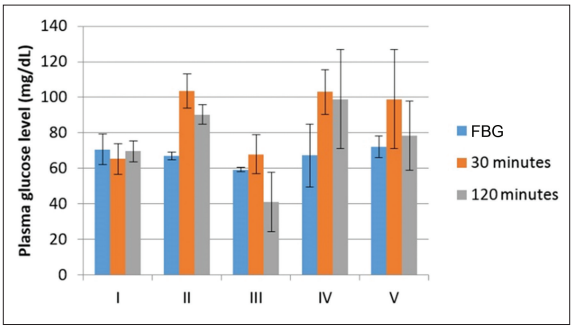

Figure 1: Plasma glucose level (I = Group 1, II = Group 2, III = Group 3, IV = Group 4, V = Group 5)

RESULTS

Plasma Glucose Level

Table 1 and Figure 1 show the results of this study. Based on the results, all experimental animals used in this study showed fasting glucose levels that did not differ significantly, with an average of 67.26 ± 9.04 mg/dL. Furthermore, 30 min after, 40% dextrose monohydrate induction in Groups 2, 4, and 5, plasma glucose levels (GD30) increased to 35.85% compared to fasting blood glucose (FBG) of each group with the average of 101.9 ± 16.11 mg/dL.

In G3, it was shown that fasting glucose levels decreased from 59.3 ± 1.15 mg/dL to 41 ± 16.64 mg/dL at min 120 (GD120) or decreased as much as 44.6%, while the fasting glucose levels of G1 (control group) were relatively stable. This indicated that coconut oil can lower glucose-induced hyperglycemia. Furthermore, the results of this study also showed a difference in GD30 and GD120 plasma glucose levels as following: G1 showed an increase of 6.58%, G2 showed a decrease of 12.83%, G3 showed a decrease of 39.71%, G4 showed a decrease of 3.89%, and G5 showed a decrease of 21%.

Based on these results, there appeared to be a decrease in plasma glucose levels at min 120 in groups that were given 40% glucose induction, namely G2, G4, and G5. However, based on the statistical analysis, there was not a significant difference between the three groups. Thus, it can be concluded that the administration of RMO either at the dose of 0.9 ml/200 g BW or 1.8 ml/200 g BW has not been able to lower plasma glucose levels significantly.

Total and Differential WBC Count

Table 2, and Figures 2 and 3 show the results of this study. The results of this study have shown that coconut oil X® has an immunostimulatory effect. Based on the results, it can be observed that the administration of RMO at a dose of 0.9 ml/200 g BW showed a significant increase in mean total WBC count (15.9 ± 0.8 × 10^3 cells/µL) compared to healthy controls (9.63 ± 1.5 × 10^3 cells/µL) [Table 2 and Figure 2].

In this study, the mean of lymphocyte and monocyte percentage in Group 2 (82 ± 2.0%) had no significant difference compared to healthy control (82.6 ± 3.78%) [Table 2 and Figure 3].

DISCUSSION

The prime objective of the study was to investigate the effect of RMO on plasma glucose level and total and differential WBC count. RMO that was used in this study is Mandar Coconut oil that has been purified using zeolite and activated charcoal, meaning that it has a clear transparent color with a distinctive smell of coconut oil. This pure coconut oil contains 92% SFA,

Table 2: Rat plasma leukocyte levels

Rats groups	Leukocyte (sel/µL)	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)
Group 1 (healthy control)				
T1	11.2×10^3	84	13	2
T2	9.5×10^3	80	12	4
T3	8.2×10^3	82	14	1
Average	$9.63\pm1.5\times10^3$	82±2.0	13±1.0	2.3±1.5
Group 2 (Coconut oil×0.9 ml/200 g)				
T1	15.1×10^3	80	12	18
T2	15.8×10^3	87	15	11
T3	16.8×10^3	81	13	8

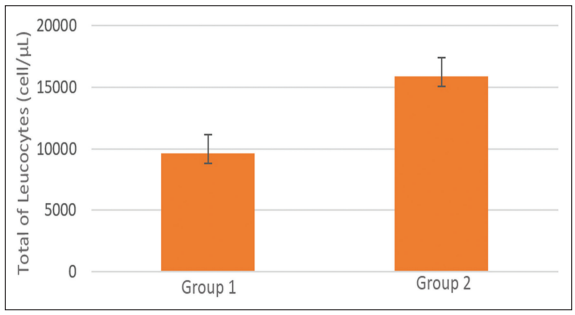

Figure 2: The mean profile of total white blood cell count after treatment (Group 1 = healthy control and Group 2 = RMO 0.9 ml/200 g BW)

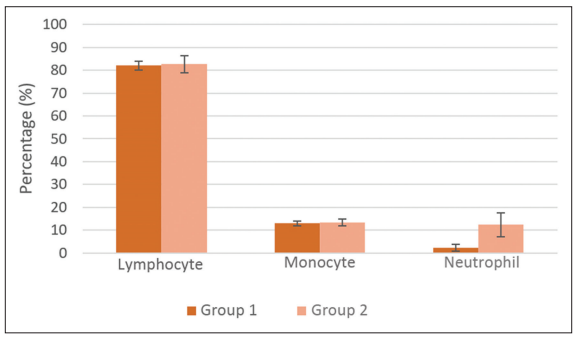

Figure 3: The mean profile of differential white blood cell count (Group 1 = healthy control and Group 2 = RMO 0.9 ml/200 g BW)

up to 48% lauric acid, a peroxide value of 0% O_2/kg, 59% medium chain triglyceride, 0.05% water content, and 0.05% free fatty acids.

The result of this study showed that fasting glucose levels did not differ significantly. The level plasma glucose in Groups 2, 4, and 5 increased compared to fasting blood glucose (FBG). There was difference in GD30 and GD120 plasma glucose levels, which in G1 has increased, while G2, G3, G4, and G5 have decreased.

These results were in *accordance* with the previous study reported by Ira *et al*. which stated that the administration of 40% dextrose monohydrate may increase plasma glucose levels by utilizing glucose as a source of energy and synthesizing fat, resulting in glucose accumulation in the blood and hyperglycemia.[6]

Leukocytes or WBCs consist of five different cell types. Each cell type has its own function and is responsible for the body's defense mechanisms. In humans, neutrophils are the greatest in number followed by lymphocytes, monocytes, eosinophils, and basophils, in that order.[7] However, this study obtained different results in rats, in which the dominant WBC was lymphocytes followed by neutrophils. This is in accordance with some previous studies conducted by Thewlis and Meyer and Shugaba *et al*., who stated that the percentage of lymphocytes is more dominant than neutrophils in rats.[8,9]

Modulation of the immune response to stimulation or suppression can help to maintain a disease-free state. The ingredients that activate the host defense system when the immune response is impaired can provide supportive therapy to conventional chemotherapy.[10] Some studies of total WBC count showed that an increase in WBC count can help to improve the immune system. An increase in the WBC count suggests that the immune system produces sufficient leukocytes and is able to help the body to fight against infections as well as foreign bodies. Leukocytes play an important role in protecting the body from attacks by microorganisms and other foreign bodies.[11] It can be observed that an increase in the WBC count may indicate an increase in rat's immune status.

Neutrophils, lymphocytes, and monocytes were observed on the differential WBC count, whereas eosinophils and basophils were not found. The number of eosinophils and basophils in the circulation is very small. Eosinophils have an important role in parasitic infections and allergic inflammatory reactions while basophils release heparin that prevents blood coagulation and plays an important role in some types of allergic reactions.[11]

Lymphocytes play an important role in the formation of antibodies, and an increased number of lymphocytes are generally caused by viral infections such as infectious mononucleosis, mumps, and rubella. In contrast, monocytes play a role in eliminating foreign antigens and dead cells and other debris, and an increased monocyte number may be observed in mononucleosis, subacute bacterial endocarditis,

malaria, and tuberculosis, as well as during the recovery phase of some infections.[7]

One of the other cell components in the immune system is neutrophils, which are capable of performing various immune responses, especially chemotaxis, phagocytosis, exocytosis, and the destruction of intracellular or extracellular foreign objects.[10] An increase in the number of neutrophils can be found in bacterial or fungal infections because these cells play an important role in killing invading microorganisms. In addition, neutrophils also play a role in the pathogenesis of tissue damage in non-infectious diseases such as rheumatoid arthritis, inflammatory bowel disease, asthma, and gout.[7] The present study has also shown an increase in the mean percentage of neutrophils in Group 1 compared to Group 2. Neutrophils that were found in the peripheral blood smears were segmented neutrophils (mature neutrophil cells) and no immature neutrophils (bands) were found. When the bone marrow increases the production of new leukocytes, there is also an increase in the number of bands which is called the "left shift" phenomenon and indicates a bacterial infection.[7] This suggested that the increased percentage of neutrophils was not caused by infection or inflammation. Based on these results, it can be observed that coconut oil can enhance the body's immune system and can help to fight bacterial infections.[10] According to de Pablo and Alvarez de Cienfuegos, a diet containing SFAs helps with the elimination of microorganisms such as *Listeria monocytogenes* more efficiently than diets containing unsaturated fatty acids.[12] Kono *et al.* also observed that the administration of medium chain SFAs has a positive effect on antigens, in which the administration of medium chain SFAs increases IgA secretion in the ileum after lipopolysaccharide stimulation.[13]

In addition, lauric acid is one of the medium chains SFAs that are known to have antimicrobial activity. Based on research conducted by Batovska *et al.,* the monoglyceride form of lauric acid, monolaurin, is known to be able to inhibit the growth of *Staphylococcus aureus*.[14] The antimicrobial activity of lauric acid is caused by acid penetration into the cell membrane of the bacteria, which causes the cellular pH to become more acidic and leads to cell death by suppressing cytoplasmic enzymes and nutrient transport systems.[15] Based on the research that has been done, it can be observed that RMO has an immunostimulatory effect by increasing the total WBC count and the percentage of neutrophils in rats.

CONCLUSION

Based on the results, it can be concluded that the administration of RMO, either 1.8 ml/200 g BW or 0.9 ml/200 g BW, did not show a decrease in plasma glucose level at minute 120 after 40% glucose induction. Meanwhile, the administration of RMO 0.9 ml/200 g BW showed a significant increase in total WBC count and neutrophil percentage.

AUTHORS' CONTRIBUTIONS

Marianti A. Manggau, Syaharuddin, and Elly Wahyudin contributed to the development of the research concept and study design, editing, and final approval of the manuscript. Andi Dirpan, Rezky Aphrodyta, and Fajrin Alamsyah performed laboratory testing. Sumarheni contributed to the development of the research concept and study design, data collection, data analysis, data interpretation, and drafting of the manuscript.

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