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Hypoglycemic Effect of Hydrolyzed Palm Kernel Oil in Rats

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ABSTRACT

Palm Kernel Oil (PKO) is a medium chain triglyceride (MCT) oil with a high value of health and medical benefits. MCT contains medium chain fatty acids (MCFAs) especially lauric acid (C12:0) as mayor fatty acid (~50%) so that this oil is also called lauric oil. Of the fatty acids, lauric acid reported to be the most effective as antibacterial agent. MCT oil is quickly absorbed and metabolized to produce energy and hence may have physiological effect. The purposes of this study was to investigate the hypoglycemic effect of hydrolyzed PKO. Lipase enzyme from Rhizomucor miehei specifically active on sn-1 and sn-3 position was used to partially hydrolyze PKO, and hydrolyzed product (HPKO) was tested for hypoglycemic effect in rats. The optimum condition of hydrolysis was at pH of 8 and 50^{o}C for 10 hours. The results also showed that after 10 hours of incubation acid value (98.20 ± 0.62 NaOH/ g oil) and percentage of free fatty acid (49.12%), remained constant. Blood glucose level was decreased after treatment with PKO and HPKO for 15 days. This study indicated that hydrolyzed PKO was more effective as hypoglycemic agent compared with unhydrolyzed PKO and antidiabetic metformin.

Keywords: Palm Kernel Oil, hydrolysis, hypoglycemic, rats

INTRODUCTION

Oil palm trees are grown for their cluster of fruits which can weigh 40-50 kg. They are commonly planted in four tropical regions: Africa, South East Asia, Latin America and South Pacific. The world's largest producer and exporter of palm oil today is Malaysia producing about 47% of the world's total supply. Indonesia is the second largest world's producers of palm oil producing approximately 36% of the world palm oil volume. Upon harvest, the drupe, pericarp and seeds (kernels) are used for production of soap and edible vegetable oil. Different grades of oil quality are obtained from the pericarp and kernel with the pericarp oil used mainly for cooking and the kernel oil used in processed foods [1-5].

The fruit of palm yields two kinds of oil, palm oil and kernel oil (PKO) with different in fatty acid composition and hence different properties and applications. PKO is similar to coconut oil in fatty acid composition and both are the only source of lauric oil available in the world market [6]. The cake residue obtained after extracting oil is used in livestock feeds, while the palm and kernel oils are used for soap production, cooking oil and margarine. These oils are used as body creams, cooking oil and medication [7].

PKO contains about 50% of lauric acid containing 12 carbons atom belongs to medium chain fatty acids (MCFA) present in the form of triglycerides, so that this oil called mediun chain triglycerides (MCT) oil. In the human body

trygleceride is hydrolyzed by lipase enzyme specifically active on sn-1,3 positions and converted into monoglycerides and free fatty acids mainly as 2-monolaurin and lauric acid that have many pharmacological effects [8-10]. The PKO is not antibacterial but combination or mixture of 2-monolaurin and lauric acid reported to be potential antibacterial [9,11]. MCT is also a potential agent used to prevent obesity [10,12] and hence decrease incidence or prevent diabetes and induce insulin sensitivity [13].

Diabetes mellitus (DM) is a metabolic syndrome that all the symptoms are characterized by high level of blood sugar due to the dysfunction of blood glucose regulation related to insulin insufficiency secreted from pancreas and insulin resistance/activity due to the obesity. DM is divided by the need for insulin, i.e: insulin diabetes dependent diabetes mellitus (IDDM), called Type 1, and non insulin dependent diabetes mellitus (NIDDM), called Type 2. DM is a dangerous degenerative disease that may cause complication such cardiovascular disease, retinopathy, neuropathy and nephropathy, so that considered as high risk disease because it can cause death [6]. The purpose of this study was to investigate the hypoglycemic effect of hydrolyzed PKO in rats. PKO was hydrolyzed by lipase from *Rhizomucor miehei*.

MATERIALS AND METHODS

Materials

The chemicals used in this study were pro-analyst quality by E.merck (Germany) which are ethanol, n-hexane, sodium hydroxide, tris-(hydroxymethyl)-aminometan (TRIS-buffer), hydrochloric acid, calcium chloride, sodium sulfate anhydrous, potassium biphthalate, phenolphtalein indicator (1% in ethanol), Lipase from *Rhizomucor miehei* (Sigma), glucose kit (Easy touch) and Palm Kernel Oil (PT. Wilmar).

Methods

Pre-liminary study of enzymatic hydrolysis was done to determine the optimum condition at temperature of 50°C with pH of 7 and 8 for 8 hours. PKO was hydrolyzed by enzyme lipase from *Rhizomucor miehei* (Sigma) which is active at the position of sn-1,3. Optimal pH condition achieved was then applied for hydrolysis of PKO. Thirty (30) grams of PKO transferred into erlenmeyer and added 30 mL of distilled water, 12.5 mL of 0.063M $CaCl_2$, 25 mL of buffer-Tris-HCl 1M pH 7 or 8, 10% of lipase, shaken for 10 minutes at speed of 200 rpm and incubated at 50°C. Observation of the fatty acid value was carried out in every 2 hours within 14 hours [9,11,14,15]. To obtain the optimal condition of incubation period for PKO hydrolysis was carried out for 14 hours with acid value determination in every 2 hours. The optimum incubation period was indicated by the constant acid value obtained during the time of hydrolysis (0-14 hours).

Acid value determination

Acid value determination was carried out for un-hydrolyzed PKO and hydrolyzed PKO. Five (5) g oil transferred to an erlenmeyer, added 25 ml neutral ethanol of 90% and then heated for 10 minutes in waterbath while being stirred. Then 3-5 drops of phenolphtalein were added to this solution. Then titration with 0.1 N NaOH was done until the solution turned pink (color didn't change for 15 minutes). Acid value and percentage of free fatty acid of PKO hydrolysates were calculated using the following equation [9,15,16]:

$$\text{Acid value} = \frac{\text{A x N x BM NaOH}}{\text{G}}$$

where,
A = NaOH solution volume (ml)
N = normality of NaOH solution
G = sample mass (g)
Molecular weight of NaOH = 40

Percentage of free fatty acid

$$\text{CFFA (wt\%)} = \frac{\text{VNaOH x MNaOH x MMFFA}}{\text{10 x Ms}}$$

where,
C FFA = content of free fatty acid (wt%)
V NaOH= NaOH solution volume (ml)
M NaOH = molarity of NaOH solution (mol/L)
MM FFA = average molar mass of fatty acids (200.32 g/mol)
Ms = sample mass (g)

Hypoglycemic Effect Assay

Animal Preparation

The animals used in this study were male rats weighing 150-200 grams. Before the experiment, rats were maintained for 2 weeks in a good cage for environmental adaptation, i.e., the reception of light, 12 hours dark and 12 hours light [17,18].

Preparation of STZ Induced Diabetic Mice

Solution of STZ was prepared by dissolving STZ in distilled water. The rats were induced with STZ solution of 55 mg/kg administrated intra-peritoneally. The blood glucose level (BGL) of mice was measured on the third day. On the third day, rats with blood glucose level (BGL) higher than 200 mg/dl were separated and used as test animals. Animals with BGL lower than 200 mg/dl, were induced back with STZ. If on the third day the BGL of the mice higher than 200 mg/dl, the animal were included in test.

Assay of the antidiabetic effect of PKO and HPKO was conducted using STZ induced diabetic rats with single dose of oil. Rats were divided into 4 groups and each group consisting of 5 rats.

Group I : Diabetic rats were given suspension of 0.5 % CMC, dose 1 % of body weight (BW)
Group II : Diabetic rats were given suspension of Metformin® with dose of 65 mg/kg BW
Group III and IV : Diabetic rats were given PKO and HPKO with of dose 2 mL/Kg BW
Suspension of tested material (oils) was administered orally for 15 consecutive days and the BGL of rats were measured on the day 1, day 3, day, day7, day 9, day 11, day13 and day 15 after administration of the oils.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis of PKO

Data and graph of fatty acid value of enzymatic hydrolysis of PKO measured at pH 7 and 8 for 8 hours can be seen in Table 1.

Table 1. Acid value of enzymatic hydrolyzed PKO at pH 7 and 8 for 8 hours

pH	Acid Value (mg NaOH/g oil)	Free Fatty Acid (%)
7	69.52 ± 0.57	35.07
8	91.80 ± 0.24	45.98

Table 1 indicated that enzymatic hydrolysis of PKO was found to better at the pH of 8 than at pH of 7, so that this condition (pH 8) was applied for hydrolysis to find out the optimal incubation time. Enzymatic hydrolysis of PKO performed during 14 hours of incubation with interval of observation of fatty acid value every 2 hours, to determine the optimum incubation time for enzymatic hydrolysis of PKO using lipase from *Rhizomucor miehei* indicated by constant acid value at which no longer increased in free fatty acids. The results from hydrolysis with various incubation time presented in **Table 2** and **Figure 1**.

Table 2. Acid value of enzymatic hydrolysis of PKO at pH of 8 for 14 hour

Samples	Incubation Time (h)	Acid Value (mg NaOH/g oil)	Free Fatty Acid (%)
Non-hydrolyzed PKO	-	10.27 ± 0.67	5.02
Enzymatic Hydrolysis of PKO	0	15.13 ± 0.41	7.54
	2	30.52 ± 0,57	15.24
	4	55.51 ± 0.48	27.78
	6	78.66 ± 0.44	39.37
	8	91.74 ± 0.95	45.98
	10	98.20 ± 0.62	49.12
	12	98.48 ± 0.50	49.31
	14	100.28 ± 0.85	50.13

Table 2 and **Figure 1** show that the acid values during hydrolysis increased with time but found to relatively constant after 10 hours to 14 hours of incubation. This result is also similar to those reported by previous studies [9,11].

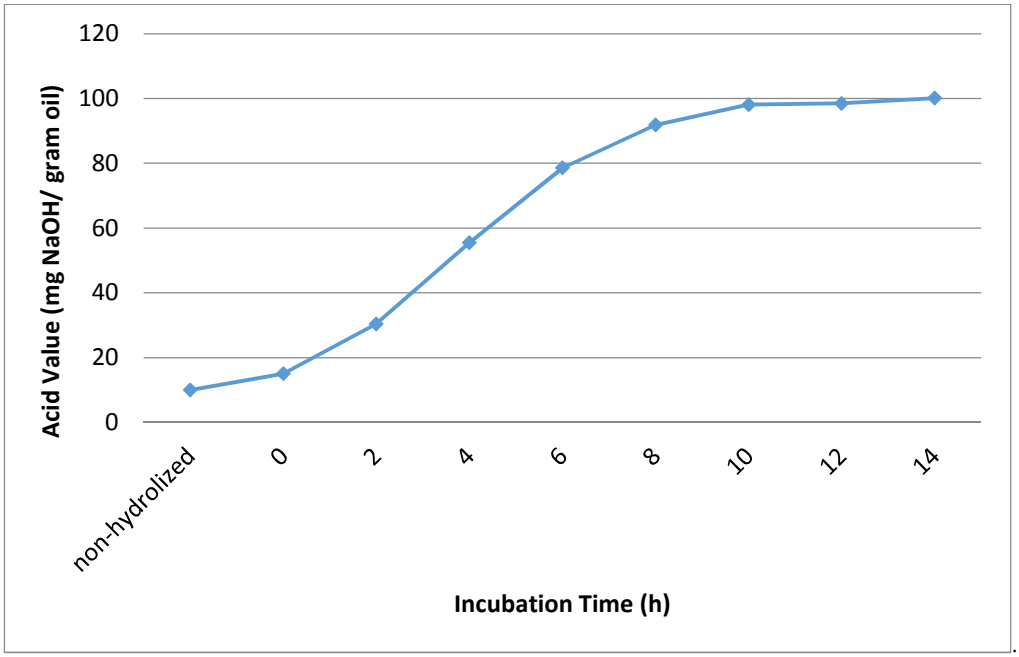

Figure 1. The effect of incubation time of hydrolysis of PKO on Acid Value

Acid value is the amount of mg of NaOH needed to neutralize free fatty acids contained in 1 gram oil or fat. Enzymatic hydrolysis PKO with lipase will produce 2 free fatty acids molecule and 1 monoglyceride from each tryglyceride molecule present in the PKO, because lipase from *Rhizomucor miehei* works specifically on position sn-1 and 3 of tryglyceride molecules [19]. Lipase from *Rhizomucor miehei* works like the lipase enzymes in the human gastrointestinal tract.

Hypoglycemic activity of PKO and HPKO observed in interval time of 3 days for 15 days and the results presented in **Table 3** and **Figure 2**. From **Table 3** and **Figure 2** can seen that blood glucose level (BGL) in control group (without treatment) increased with time, but the levels were decreased during treatments. PKO reduced BGL from initial level of 399 mg/dL to 302.6 mg/dL (~25%) after 15 hours, HPKO reduced from 405.2 mg/dL to 131.98 mg/dL (~60%), and treatment with antidiabetic drug metformin reduced BGL from 389.8 mg/dL to 196.4 mg/dL (~50%). This result indicates that HPKO is more effective to lower BGL than metformin.

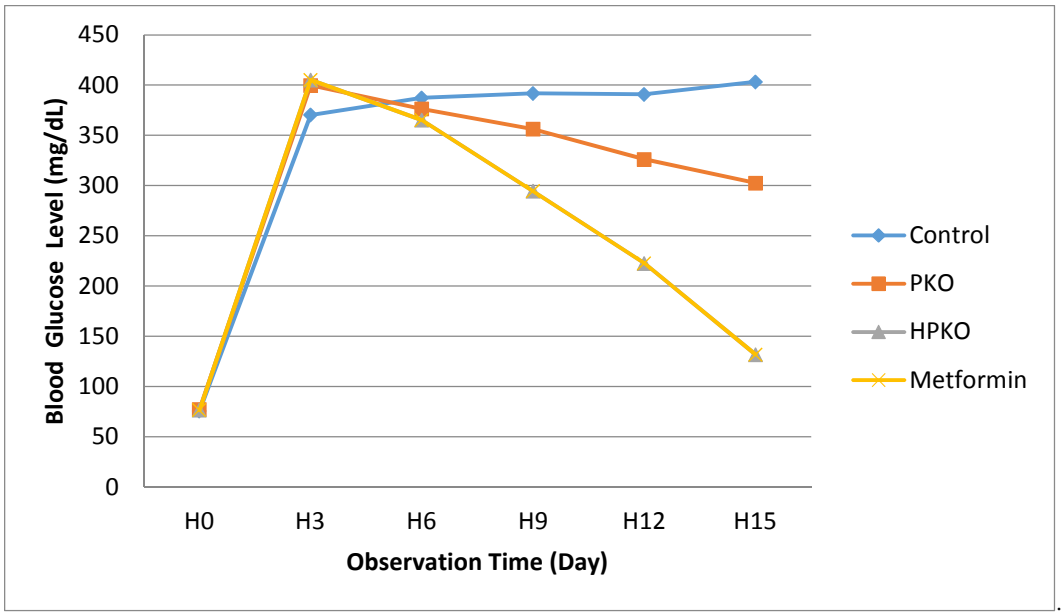

Figure 2. The hypoglycemic effect of PKO and HPKO in rats

Table 3. Hypoglycemic effect of PKO and HPKO in STZ induced rats

Treatments	Blood Glucose Level (mg/dL) with interval time (days)					
	H0	H3	H6	H9	H12	H15
Control	75.8 ± 1.48	370.2± 16.45	387.2 ± 3.56	391.8 ± 3.43	390.8 ± 3.96	403.2 ± 4.82
PKO	77.4± 1.67	399.8± 7.56	376.4 ± 12.66	356.2 ±9.28	326.2 ± 9.57	302.6 ± 5.03
HPKO	76.6± 1.52	405.2± 5.63	365.2 ± 7.29	294.4 ± 7.30	222.6 ± 6.19	131.8 ± 6.9477
Metformin	76.4 ± 0.55	386.8 ± 5.26	335.8 ± 5.93	282.6 ± 6.35	235.8 ± 4.87	195.8 ± 4.97

Note: Data is the means of three replicates

Streptozotocin has been shown to cause direct irreversible damage to β-cells of pancreatic islet of Langerhans, resulting in degranulation and loss of insulin secretion. Clarification of the regenerating potential in experimentally-induced diabetic animals would be of interest as an alternative therapy for diabetes [20]. STZ mechanism work by generating oxygen free radicals, which causes a decrease in plasma GSH concentration, and plasma GSH/GSSG ratio [21].

Pancreas has a relatively weak antioxidant defense system against oxidative stress, which can be externally strengthened [22]. Lauric acid is one of medium chain fatty acid (MCFA) which have antioxidant activity. It can be scavenging free radicals, inhibit lipid peroxidation and reduce nitrit oxide level [23]. The amount of MCFA in PKO are lower than HPKO resulted from hydrolysis process in PKO. This condition gave more effective in reducing blood glucose level in hyperglycemic rats.

CONCLUSION

This study indicated that palm kernel oil (PKO) was active to decrease blood glucose level and hydrolysis of PKO increased hypoglycemic activity which was shown to be similar with antidiabetic metformin.

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