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Ethanol Extract of Galangal (*Kaempferia Galanga*) Supplementation Inhibited the Elevation of Liver Steatosis Cells Number, Gamma Glutamyl Transferase, and Triglyceride Concentration in Male Wistar Rats (*Rattus norvegicus*) FED with Used Cooking Oil

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Abstract: ***Used cooking oil is unhealthy fat that caused damage to various organs in the body. Consumption of used cooking oil increases the synthesis and decreases triglyceride oxidation, resulting in its accumulation in the liver, which is a trigger of liver steatosis. Galangal (Kaempferia galanga) contains flavonoids, polyphenols, and tannins, and it has an anti- hyperlipidemic effect. The study aims to prove that the ethanol extract of galangal inhibits the elevation of liver steatosis cells number, Gamma Glutamyl Transferase (GGT), and triglycerides concentration in male Wistar rats (Rattus Norvegicus) fed with used cooking oil. A post-test-only control group design was conducted. The samples were 36 healthy male Wistar rats, 3-4 months old, weighed 200-210 gram, divided randomly into two groups. The control group was administrated with 1.5 ml of used cooking oil and 1 ml of aquadest placebo, while the intervention group was supplemented with 1.5 ml of used cooking oil and 100 mg of galangal ethanol extract that dissolved in 1 ml aquadest, once daily orally, for 28 days. The number of the liver steatosis cell, the plasma GGT, and TG concentration were measured after the intervention. The number of steatosis cells in the intervention group was found significantly lower than the control group (4.79 ± 2.40 fg/5 HPF vs. 25.55 ± 7.43 fg/5 HPF; p<0.001). The GGT concentration in the intervention group was also significantly lower than the GGT in the control group (5.00 (3.53 – 5.35) ng/ml vs. 5.83 (4.22-10.15)) ng/ml ; p<0.001). The TG concentration in the intervention group was significantly lower than the mean TG in the control group (78.12 mg/dL ± 21.86 vs. 142 mg/dL ± 27.18; p≤0.001). In conclusion, the ethanol extract of galangal inhibited the elevation of liver's steatosis number, plasma's GGT, and triglycerides concentration in male Wistar rats fed with used cooking oil.***

Keywords: used cooking oil, steatosis, gamma-glutamyl transferase, triglyceride, galangal rhizome

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of diseases from *simple steatosis* to *non-alcoholic steatohepatitis* (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma[1]. *Simple steatosis* can be detected and cured early; however, the treatment of late-stage is not effective and will lead to progressive disease, hepatocyte cells lose their regeneration ability, and accelerate the aging process[2]. The prevalence of NAFLD in Indonesia is estimated at 30.6%, higher than other countries in Asia, such as India (24.6%) and China (20%)[3]. The prevalence of NAFLD increases with age, 1 to 3% in children, 5% in adolescents, 18% between 20 and 40 years, 39% in 40 to 50 years, and more than 40% in the population aged 70 years and over[4].

One of the risk factors for NAFLD is an unhealthy diet. Indonesian people from lower-middle economies tend to use cooking oil repeatedly (used cooking oil)[5]. A study on rats reported that administration of oxidized soybean oil caused hepatocyte hypertrophy, fat deposition, inflammatory cell infiltration in the liver, and hepatocyte degeneration in the form of swelling and cell necrosis[6]. Free radicals from used cooking oil also trigger damage to various organs, including liver damage characterized by the swelling of the liver, damage to cell membranes, endoplasmic reticulum, and destruction of the oxidation process[7].

Although cases of NAFLD continue increasing, there has not been any approved specific therapy for NAFLD. It is essential to treat NAFLD in the reversible stage to prevent disease progression[8]. Galangal (*Kaempferia galanga*) is a famous medicinal plant easily found in Indonesia9, usually used as cooking spices. It contains phytochemicals as an antioxidant with an antioxidant capacity of 108.41 mg/L with an IC50 of 928.13 ppm. Galangal extract also contains phenols (279.82 mg/100 grams), flavonoids (1559.69 mg/100 grams), and tannins (295.34 mg/100 grams). Ethyl-p- methoxycinnamate (EPMC) is the main compound from

the galangal rhizome that protects oxidative damage from chemotherapy side effects[10]. Previous studies on rats have shown that galangal has anti-hyperglycemic and anti-hyperlipidemic effects[11, 12]. The role of galangal in inhibiting NAFLD has not been investigated; therefore, it is essential to provide scientific evidence regarding its effect in inhibiting the increase in steatosis, GGT levels, and triglycerides due to exposure to used cooking oil.

2. Materials and Methods

The post-test-only control group study design was conducted in 36 male Wistar rats aged 3-4 months with a weight of 200-210 grams. The ethanol extract of galangal was made at the Postharvest Engineering Laboratory, Faculty of Agricultural Technology, Udayana University. Used cooking oil was prepared using palm oil that has been heated 180^0C for 8 minutes repeatedly six times with 15 minutes for frying tofu.

The rats were randomly divided into two groups: the control and intervention groups. The control group was administrated 1.5 ml of used cooking oil and 1 ml of aquadest placebo. The intervention group was exposed with 1.5 ml and supplemented with used cooking oil and 100 mg of galangal ethanol extract dissolved in 1 ml aquadest, once per day orally. Both groups were administrated for 28 days.

On the last day of the intervention, all group subjects were anesthetized with 16 mg of ketamine/200 gram BW and 2 mg of xylazine/200 gram BW before drawing the blood. One cc of blood was taken per rat and waited for 1 hour before centrifuging at 3000 rpm for 10 minutes. The blood serum was then taken using a micropipette and inserted into a 1.5 ml microtube for measuring GGT and TG levels. GGT was measured using GGT Elisa Assay Kit from BT Lab in ng/ml. TG was measured using TG Colorimetric kit from Elabscience.

Liver tissues were fixed in 10% buffered formaldehyde for 24 hours. Section of 3-5 mm thickness were cut and proceed using a tissue processor. The tissue was then embedded in paraffin. After that, the paraffin block was cut using microtome at 4-5 µm. Paraffin ribbon was placed in the water bath before putting in the object glass and then placed on a hot plate for few hours. Liver preparations were with Haematoxylin-eosin. The observation was carried out using microscope *Olympus CX21* with a 400x lens magnification and documented. The calculation of steatosis number using Image Raster software. The data was calculated from the average steatosis number in 5 HPF (high power field) of the hot spot area.

Statistical analysis was conducted using SPSS, with a significance level of α=0.05. The data with the normal distribution was analyzed using compare means (independent t-test), while data with non-normal distribution was analyzed using a non-parametric test (Man-Whitney test).

The study protocol was approved by the animal ethics committee of the Veterinary Faculty of Udayana University, with ethical clearance number: B/49/UN14.2.9/ PT.01.04/ 2021

3. Results

One rat from the control group died during the intervention, and one from the intervention group has extreme data (out layer) and was excluded for analysis, so the total number of each group to 17 subjects. The mean of steatosis number for the intervention group was significantly lower than the control group (4.79 ± 2.40 fg/5 HPF vs. 25.55 ± 7.43 fg/5 HPF; p<0.001). The median GGT value in the intervention group was significantly lower than the median GGT in the control group (5.00 (3.53 – 5.35) ng/ml vs. 5.83 (4.22-10.15) ng/ml ; p<0.001). The mean TG in the intervention group was significantly lower than the mean TG in the control group (78.12 mg/dL ± 21.86 vs. 142 mg/dL ± 27.18; p<0.001).

Table 1: The Comparison of Steatosis Number, GGT, and TG concentration between Group Post-Intervention

Parameter	Group	n	Mean ± SE	p
Steatosis	Control	17	25.55 mg/dL ± 7.43	<0.001
(fg/5 HPF)	Intervention	17	4.79 mg/dL ± 2.40	
GGT*)	Control	17	5.83 (4.22 - 10.15)	<0.001
(ng/mL)	Intervention	17	5.00 (3.53 – 5.35)	
Triglyceride	Control	17	142 mg/dL ± 27.18	<0.001
(mg/dL)	Intervention	17	78.12 mg/dL ± 21.86	

Steatosis and triglyceride were tested using an independent t-test, presented in mean±SD*) GGT was tested using Man-Whitney, presented in median (min-max).

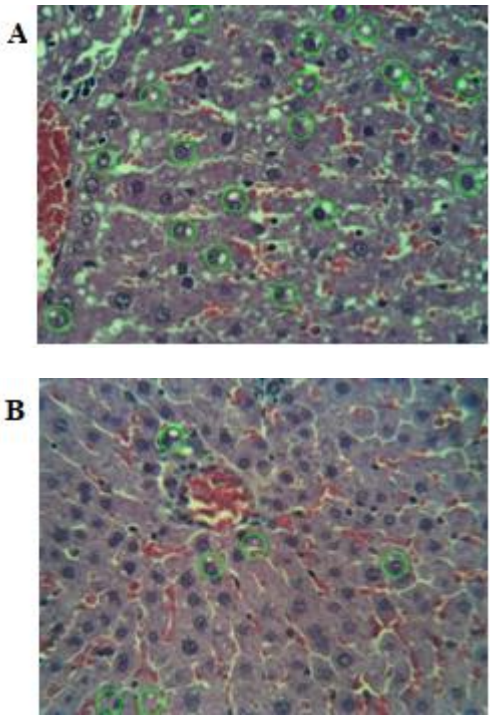

Figure 1: Histopathologic Examination of Steatosis (Haematoxylin Eosin, 400x)
(A) The liver steatosis in the control group with the administration of aquadest and used cooking oil. (B) The liver steatosis in the intervention group with ethanol extract of galangal and used cooking oil.

4. Discussion

Effect of the Ethanol Extract of Galangal on Steatosis Number

Repeated heating of the oil will increase the level of transfat[14]. Diets high in trans fats increase the synthesis of free fatty acids and reduce lipid oxidation, thereby causing triglyceride accumulation in the liver, triggering hepatic steatosis and lipotoxicity[15]. Supplementation with 2: 1 ratio of N-6: N-3 polyunsaturated fatty acid improves liver steatosis with OR = 0.064 (p=0.001) [16]. It shows that PUFA supplementation or good fat can inhibit steatosis, while the administration of used cooking oil in this study resulted in steatosis.

The effect of galangal extract in inhibiting steatosis is influenced by its anti- hyperlipidemic and anti-inflammatory effects[17] (Subbaian and Ragavan, 2020). *Galangal extract* can inhibit various inflammatory mediators, such as TNF-α, IL- 1, TGF beta, and Nitric Oxide (NO) [17]. In addition, the role of galangal in inhibiting steatosis is also influenced by its compound content, such as flavonoids, polyphenols, and tannins.

Flavonoids stimulate Peroxisome Proliferator-Activated Receptor (PPAR)-a thereby reducing steatosis by promoting beta-oxidation and reducing inflammation[19]. Peroxisome proliferator-activated receptor gamma (PPARg) is activated partially by flavonoids, triggering adipocyte differentiation and insulin sensitization. This causes the redistribution of visceral fat to the subcutaneous fat depot and reduces free fatty acids to the liver[20]. Flavonoids can inhibit (Sterol regulatory-element binding proteins) SREBP-1c, therefore decreasing the rate of de novo lipogenesis[21, 22]. Flavonoids have the role as the antioxidant by reducing the free radical productions, inhibit pro-oxidant enzymes, and enhance endogenous antioxidant defenses[20]. The anti-inflammatory effect of flavonoids is mainly influenced by their ability to inhibit the NF-B pathway. In addition, flavonoids also inhibit the activity of regulatory enzymes involved in inducing inflammatory responses, such as protein tyrosine kinase, PKC, phosphodiesterase, phospholipase A2, lipoxygenases, and COX[21].

Polyphenols show their hepatoprotective effects by increasing fatty acid oxidation, modulating insulin resistance, and inhibiting oxidative stress and inflammation. Polyphenols have also been reported to decrease NF-kB activity on peripheral blood mononuclear cells. Polyphenols can donate hydrogen to free radicals and produce unreactive radicals that can suppress lipid peroxidation[22]. Tannins can decrease the expression of messenger RNA (mRNA) genes related to lipogenesis and lipid accumulation[23].

Effect of the Ethanol Extract of Galangal on GGT

GGT is the main enzyme of extracellular glutathione catabolism[24]. GGT release indicates oxidative stress and is closely associated with body fat, especially visceral fat, contributing to accelerated NAFLD progression[16].

Decreased intracellular glutathione triggers GGT secretion[25]. Flavonoids increase intracellular glutathione concentrations by about 50% through transactivation of the gamma subunit promoter glutamylcysteine synthetase[26].

Galangal inhibited the increase of GGT because of its flavonoid compounds that can increase the concentration of intracellular glutathione, its hypolipidemic and anti-hyperglycemic effects. Administration of extracts with hypolipidemic and anti-hyperglycemic effects, such as the hydroalcoholic extract of *Peganum harmala* and extract of *Curculigo latifolia*, can reduce the level of GGT. Anti-hyperglycemic and hypolipidemic effects are thought to ameliorate hepatotoxicity complications of diabetes mellitus. The reduction of hepatocyte damage in the treatment group decreases the GGT enzyme that entered into circulation[27, 28].

Effect of the Ethanol Extract of Galangal on Triglyceride

The ability of galangal extract in inhibiting the increase of triglycerides in this study is in line with the previous studies. The effect of the ethanolic extract of Kaempferia galanga with doses of 500 mg/kg BW and 1000 mg/kg BW for 30 days in female rats that underwent ovariectomy showed a significant decrease in total cholesterol and LDL levels, but there was no significant difference in triglyceride levels[12]. The study that administers ethanol extract of Kaempferia galangal with 250 mg/kg BW and 500 mg/kg BW for 28 days in diabetic rats showed a significant decrease in triglyceride, LDL, VLDL, and an increase in HDL levels[18].

The mechanism of galangal extract in reducing triglyceride levels is due to the content of flavonoids, tannins, and polyphenols. Flavonoids can increase lipoprotein lipase activity; therefore, they could reduce triglyceride[29].

Flavonoids and tannins can inhibit cholesterol absorption in the intestine and increase the formation of bile acids from cholesterol[29]. In addition, flavonoids and tannins can inhibit the HMG-COA reductase enzyme, resulting in the reduction of Apo-B synthesis and the increase of LDL receptors on the liver surface[30].

Polyphenols can reduce LDL by inhibiting cholesterol absorption in the small intestine, preventing LDL biosynthesis and microsomal transfer of protein in the liver, decreasing apolipoprotein B-100 secretion, and increasing LDL receptors in the liver liver[31]. An increase in LDL receptors will redistribute the blood cholesterol to the liver, thereby reducing LDL and VLDL levels, reducing blood triglycerides. A decrease in Apo-B levels will interfere with the formation of chylomicrons, VLDL, IDL, and LDL, resulting in reduced triglyceride levels[30].

5. Conclusion

Supplementation of Ethanol extract of galangal (*Kaempferia galanga*) inhibited the elevation of liver steatosis cell number, GGT, and TG concentration in male Wistar rats (*Rattus norvegicus*) fed with used cooking oil.

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7. Conflict of interest

The author declares no conflict of interest

References

[1] Adiwinata R, Kristanto A, Christianty F, Richard T, Edbert D. Tatalaksana Terkini Perlemakan Hati Non Alkoholik. *Jurnal Penyakit Dalam Indonesia*. 2017;2(1):53. doi:10.7454/jpdi.v2i1.65

[2] Younossi ZM. Non-alcoholic fatty liver disease – A global public health perspective. *Journal of Hepatology*. 2019;70(3):531-544. doi:10.1016/j.jhep.2018.10.033

[3] Sufyan DL. Pengaruh Pemberian Jus Terong Ungu terhadap Perlemakan Hati Tikus Wistar. *Jurnal Ilmiah Kesehatan*. 2019;18(2):59-63. doi:10.33221/jikes.v18i2.301

[4] Gan L, Chitturi S, Farrell GC. Mechanisms and implications of age- related changes in the liver: Non-alcoholic fatty liver disease in the elderly. *Current Gerontology and Geriatrics Research*. 2011;2011. doi:10.1155/2011/831536

[5] Suroso AS. Kualitas Minyak Goreng Habis Pakai Ditinjau dari Bilangan Peroksida , Bilangan Asam dan Kadar Air. *Jurnal Kefarmasian Indonesia*. 2013;Vol 3(2):77-88.

[6] Dhibi M, Brahmi F, Mnari A, et al. The intake of a high-fat diet with different trans fatty acid levels differentially induces oxidative stress and non-alcoholic fatty liver disease (NAFLD) rats. *Nutrition and Metabolism*. 2011;8(1):65. doi:10.1186/1743-7075-8-65

[7] Megawati M. Konsumsi Minyak Jelantah dan Pengaruhnya terhadap Kesehatan. 2019;8:259-264.

[8] Ferramosca A, Di Giacomo M, Zara V. Antioxidant dietary approach in treatment of fatty liver: New insights and updates. *World Journal of Gastroenterology*. 2017;23(23):4146- 4157. doi:10.3748/wjg.v23.i23.4146

[9] Silalahi M. Kencur (Kaempferia galanga) dan Bioaktivitasnya. *Jurnal Pendidikan Informatika dan Sains*. 2019;8(1):127. doi:10.31571/saintek.v8i1.1178

[10] Srivastava N, Ranjana, Singh S, et al. Aromatic ginger (Kaempferia galanga L.) extracts with ameliorative and protective potential as a functional food, beyond its flavor and nutritional benefits. *Toxicology Reports*. 2019;6(May):521-528. doi:10.1016/j.toxrep.2019.05.014

[11] Sudatri NIW, Wirasiti N, Suartini NIM, Gusti I. Anti-diabetic and anti- cholesterol activity of Kaempferia galanga L . herbal medicine rhizome in albino rats. 2019;6(5):13-17.

[12] Handayani S, Fita FE, Istatoah S, Indah E. Potensi Rimpang Kencur (Kaempferia Galanga L.) Sebagai Pencegah Osteoporosis Dan Penurun Kolesterol Melaui Studi In-Vivo Dan In-Silico. Published online 2015:125- 133.

[13] Kochuthressia K, Britto S. In vitro antimicrobial evaluation of Kaempferia galanga L. rhizome extract. *American Journal of Biotechnology and Molecular Sciences*. 2012;2(1):1-5. doi:10.5251/ajbms.2012.2.1.1.5

[14] Sartika RAD. Pengaruh Asam Lemak Jenuh, Tidak Jenuh dan Asam Lemak Trans terhadap Kesehatan. *Kesmas: National Public Health Journal*. 2008;2(4):154. doi:10.21109/kesmas.v2i4.258

[15] Dorfman SE, Laurent D, Gounarides JS, et al. Metabolic implications of dietary trans-fatty acids. *Obesity*. 2009;17(6):1200-1207. doi:10.1038/oby.2008.662

[16] Weta W, Mahadewa T, Sutirtayasa G., et al. Supplementation with 2: 1 ratio of N-6: N-3 polyunsaturated fatty acid improves liver steatosis and serum cytokine levels in young obese balinese women: A randomized clinical trial. *Asian Journal of Pharmaceutical and Clinical Research*. 2017;10(12):74-79. doi:10.22159/ajpcr.2017.v10i12.20851

[17] Umar MI, Asmawi MZ, Sadikun A, et al. Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from Kaempferia galanga L. extracts. *Molecules*. 2012;17(7):8720-8734. doi:10.3390/molecules17078720

[18] Subbaian K, Ragavan B. Antihyperlipidemic effect of Kaempferia galanga in streptozotocin- induced diabetic rats Collection of Plant Material. *Drug Intervention Today*. 2020;14(3):7-10.

[19] Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: Potential therapeutic targets. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*. 2012;1821(5):809- 818. doi:10.1016/j.bbalip.2011.10.016

[20] De Wier B Van, Koek GH, Bast A, Haenen GRMM, Van De Wier B. Critical Reviews in Food Science and Nutrition The potential of flavonoids in the treatment of non-alcoholic fatty liver disease The potential of flavonoids in the treatment of non-alcoholic fatty liver disease. Published online 2017. doi:10.1080/10408398.2014.952399

[21] Ferré P, Foufelle F. Hepatic steatosis: A role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes, Obesity and Metabolism*. 2010; 12(SUPPL. 2):83-92. doi:10.1111/j.1463-1326.2010.01275.x

[22] Chojnacka K. Biologically Active Compounds in Seaweed Extracts - the Prospects for the Application. *The Open Conference Proceedings Journal*. 2012; 3(1):20-28. doi:10.2174/1876326x01203020020

[23] Chung MY, Song JH, Lee J, et al. Tannic acid, a novel histone acetyltransferase inhibitor, prevents non-alcoholic fatty liver disease both in vivo and in vitro model. *Molecular Metabolism*. 2019; 19 (November 2018):34-48. doi:10.1016/j.molmet.2018.11.001

[24] Gunawan S, Santoso A, Wijaya A. The Correlation of Gamma-Glutamyl Transferase (γ-GT), Glutathione Peroxidase (GPx), and Total Antioxidant Status (TAS) with Inflammatory Marker in Individuals

with Metabolic Syndrome. *The Indonesian Biomedical Journal*. 2011;3(1):57. doi:10.18585/inabj.v3i1.135

[25] Liss KHH, Finck BN. PPARs and non- alcoholic fatty liver disease. *Biochimie*. 2017; 136:65-74. doi:10.1016/j.biochi.2016.11.009

[26] Myhrstad MCW, Carlsen H, Nordström O, Blomhoff R, Moskaug JØ. Flavonoids increase the intracellular glutathione level by transactivation of the γ-glutamylcysteine synthetase catalytical subunit promoter. *Free Radical Biology and Medicine*. 2002; 32(5):386-393. doi:10.1016/S0891-5849(01)00812-7

[27] Komeili G, Hashemi M, Bameri- Niafar M. Evaluation of antidiabetic and anti-hyperlipidemic effects of peganum harmala seeds in diabetic rats. *Cholesterol*. 2016;2016. doi:10.1155/2016/7389864

[28] Ishak NA, Ismail M, Hamid M, Ahmad Z, Abd Ghafar SA. Antidiabetic and hypolipidemic activities of Curculigo latifolia fruit:Root extract in high fat fed diet and low dose STZ induced diabetic rats. *Evidence-based Complementary and Alternative Medicine*. 2013; 2013. doi:10.1155/2013/601838

[29] Yuliana AR, Ardiaria M. Journal of Nutrition Volume. *Journal of Nutrition College*. 2016; 5(4):428-437.

[30] Rahastuti, S., Tjahjani, S. dan Hartini E. Efek Infusa Daun Salam (Syzgium polyanthum) terhadap Penurunan Kadar Kolesterol Total Darah Tikus Model Dislipidemia Galur Wistar. *Jurnal Medika Planta*. 2011;4:28-32.

[31] Naufalina MD, Nuryanto N. Pengaruh Pemberian Susu Kacang Koro Pedang (Canavalia Ensiformis) Terhadap Kadar Kolesterol Ldl Dan Hdl Pada Tikus Dislipidemia. *Journal of Nutrition College*. 2014;3(4):456-464. doi:10.14710/jnc.v3i4.6827