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The Effect of Turmeric Rhizome (*Curcuma Longa Linn*)On Decreasing Non-Alcoholic Steatohepatitis in Deep Frying Oil Induced Male Wistar Rats

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Abstract : Deep Frying oil/ DFO is cooking oil from high and repetitive heating temperatures. The use of DFO can increase steatosis hepatocyte with inflammation and hepatocellular injury (balloning hepatocytes) which is a typical sign ofNon-alcoholic steatohepatitis/ NASH. This disease must be treated as quickly as it can cause complications of liver cancer and liver cirrhosis. Turmeric rhizomes containing curcumin was active anti-oxidants which can inhibit fat accumulation intra-hepatic. The present study was conducted to evaluate the effect of turmeric rhizome to reduce fat degeneration in male Wistar rats in the NASH model. This study was experimental with a randomized post-test only control group design. The number of 24 samples consisted of four treatment groups. Group I (control): normal diet + DFO (10 µl/g /day), Group II, III and IV: normal diet + DFO + turmeric extract doses (100, 200, and 400 mg /kg BW/day, orally for 30 day at 6 times frying. The results of the Kruskal-Wallis test showed significant differences between the four treatment groups in steatosis ($P < 0.05$) and there were no significant differences in hepatocyte ballooning and lobular inflammation. The results of Mann-Whitney test showed significant differences in hepatocyte stestosis between the control group (P1) and the treatment group (P3) with a dose of 200 mg / kg BW.It showed that the dose was effective inhibit of NASH.
Keywords: Turmeric, NASH, DFO, fat degeneration.

Introduction and Experimental

Non-alcoholic steatohepatitis is a chronic liver disease characterized by steatosis and inflammation accompanied by hepatocellular injury (balloning)[1]. The incidence of this disease increases every year, in America 10-25% of patients who have steatosis histologically have NASH, with complications of liver cancer and hepatic chirrosis[2].

This disease can be caused by a high fat diet that affects lipid intra-hepatic accumulation. Lipid Intra-hepatic elevation caused hepatic steatosis. Quality of diet can play an important role in the development of NASH such as a diet rich in saturated fat, cholesterol, low in polyunsaturated fats and fiber lace and low in antioxidants such as vitamins C and E[3].

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Trans fat diet can also caused liver damage due to an increase in lipid peroxidation in the liver and a decrease in the antioxidant enzyme (superoxide dismutase, catalase, and glutathione peroxidase) which caused oxidative stress that leads to the development of NASH[4].

Food intake which contains high trans fat did not produce leptin secretion but increases de novo lipogenesis, causedlipotoxic, insulin resistance, increases TNF expression and ATP depletion which then increases the development of steatosis[5].

The results of the study conducted on mice showed that giving a high diet in trans fats increased body weight, abdominal fat accumulation, fat accumulation in the liver, adipose cells, and muscles that can induce insulin resistance. Fat accumulation in the liver given by trans oil is associated with an increase in cholesterol and trigleserides due to decreased lipid oxidation and increased synthesis of fatty acids in the liver. This can cause obesity, metabolic syndrome, steatohepatis, and lipotoxicity[6].

Turmeric rhizome (Curcuma Longa Linn) contains components such as 2-5% curcumin which act as antioxidants that can inhibit the development of NASH due to injury caused by ROS[7]. The antioxidant is as strongas vitamins, C, E and beta-carotene, so turmeric is the main choice of consumers[8].

Vitamin C can act as an antioxidant, can reduce ROS generation, stimulate anti-oxidant hormones such as glutathione peroxidase, can act as cholesterol biosynthetics, and as an anti-inflammatory[9].

While secondary metabolites such as tannins can play a role in overcoming chronic diseases such as antioxidants, anti-inflammatory, anti-tumor, overcome diarrhea and as deuretics[10].

Based on previous study that turmeric rhizome extract is an alternative herb in reducing fat levels in the blood or antihypertriglyceridemia[11].In this study, to find out the improvement of liver tissue by means of histopathological examination by assessing the degree of steatosis, hepatocyte balloning and lobular inflammation, so that it is hoped that the ethanol extract of turmeric rhizome can improve liver tissue in NASH disease.

Research design and sampling

This study was an experiment with randomized post-test only control group design with a total sample of 24 male Wistar rats.The rats were divided by simple randomization into 4 groups of 6 animals per group, by using the formula of Federer: $(t-1)(n-1) \geq 15$[12].The study was conducted in the animal breeding house in the Pharmacy Laboratory, while the histopathology examination was conducted in the Laboratory of Histology University of North Sumatra.

Plant material and extraction

Turmeric rhizomes were collected from Tanah Seribu Binjai.Turmeric rhizomes are cleaned and then dried with a drying cabinet with a temperature of 60° C. Then mashed with an electric blender. The results of the dried powder of turmeric rhizome were then macerated with 96% ethanol. The filtrate obtained was then evaporated using an evaporator at a temperature range of 40-60^{o}C. To get a thick extract, evaporation with a evaporating cup

Standardization extract to calculate water content, water soluble extract content, soluble ethanol extract, total ash content and acid insoluble ash content based on pharcopeia Indonesia[13]. Phytochemical identification to identify flavonoids, alkaloids, tannins, saponins, steroids, terpenoids and glycosides[14].

Deep frying oil preparation

DFO is made by heating 2 liters of palm oil to fry 4 kg of kentucky fried chickenat 200ºC (measured with a cooking thermometer) 6 times frying, each for 45 minutes. Each time the frying is cooled ± 5 hours.

Animal preparation and experimental procedures

Healthy male Wistar rats aged 2-3 months weighing between 150-200 (n = 24) divided into four treatment groups. Animals were obtained from the Laboratory of the Faculty of Pharmacy, University of North

Sumatra. Then acclimatized at room temperature (25-30°C) for one week with 12 hours of light and 12 hours of darkness. Food was given ad-libitum orally with the normal CP 551 diet standard from PT. Charoen Pokphan Tanjung Morawa Medan.

The interventions were based on the treatment group. Group P1 (control) was given a standard diet and DFO + aquadest + 1 ml Na-CMC 1%, and the treatment group P2, P3, P4 were given a standard diet and DFO + aquadest + 1ml Na-CMC 1% and turmeric rhizome extract with a doses of 100, 200 and 400 mg / kg BW are taken orally for 30 days.

The rats were anesthetized by using ketamine xylazine at doses of 75-100 mg / kg + 5-10 mg /kg intraperitoneally with a duration of 10-30 minutes at the end of the treatment on the 37th day (acclimatization and treatment), then intraperitoneal surgery was performed to remove the liver, which was prepared for histological analysis.

Histopathology analysis

Preparation of histopathological analysis with H & E staining method for microscopic assessment of steatosis, hepatocyte balloning and lobular inflammation. Preparation of preparations starts from fixation, washing, dehydration, clearing, embedding, sectioning, affixing, staining, mounting and labeling[15].

Statistic analysis

Processing data by using SPSS version 20 software. Data were statistically tested with Kruskal-Wallis. Continued Mann-Whitney test with a value of $\alpha < 0.05$[16].

Ethical clearance

The study was conducted according to the Ethical Clearance of the Animal Ethics Committee FMIPA USU Medan with the letter number:0020/KEPH-FMIPA/ 2018.

Results and Discussion

The standardization of the Turmeric rhizomes extract yielded the following results: water content of simplicia (7.17%), extract (5.00%), water soluble content (21.70%), ethanol soluble concentration (10.39%), total ash content (3.42%) and acid soluble ash (1.27%).The phytochemical screening of simplicia and extract traced the presence of alkaloids, saponins, tannins, flavonoids, steroids, triterpenes and glycosides.

Water content of extract has met the requirements of less than 10%, because if the water content of more than 10% will result in the growth of fungi, microbes, enzymatic reactions or hydrolysis processes[17].

Fatty degeneration was found in 24 male Wistar rat liver tissue samples with H & E staining, The histologic sections in control groups showed steatosis grade 2 after 30 days of DFO administration(Figure 1a). Figure 1b showed lobular inflammation grade 1 after turmeric rhizomes extract at the dose of 200 mg/kg BWadministration. Figure 1cshowed hepatocyte balloninggrade 1 after 30 days of DFO administration (control). Figure 1d showsincreased in hepatocyte balloning and sinusoid dilationafter Turmeric rhizomes extract at the dose of 400 mg/kg BWadministration.

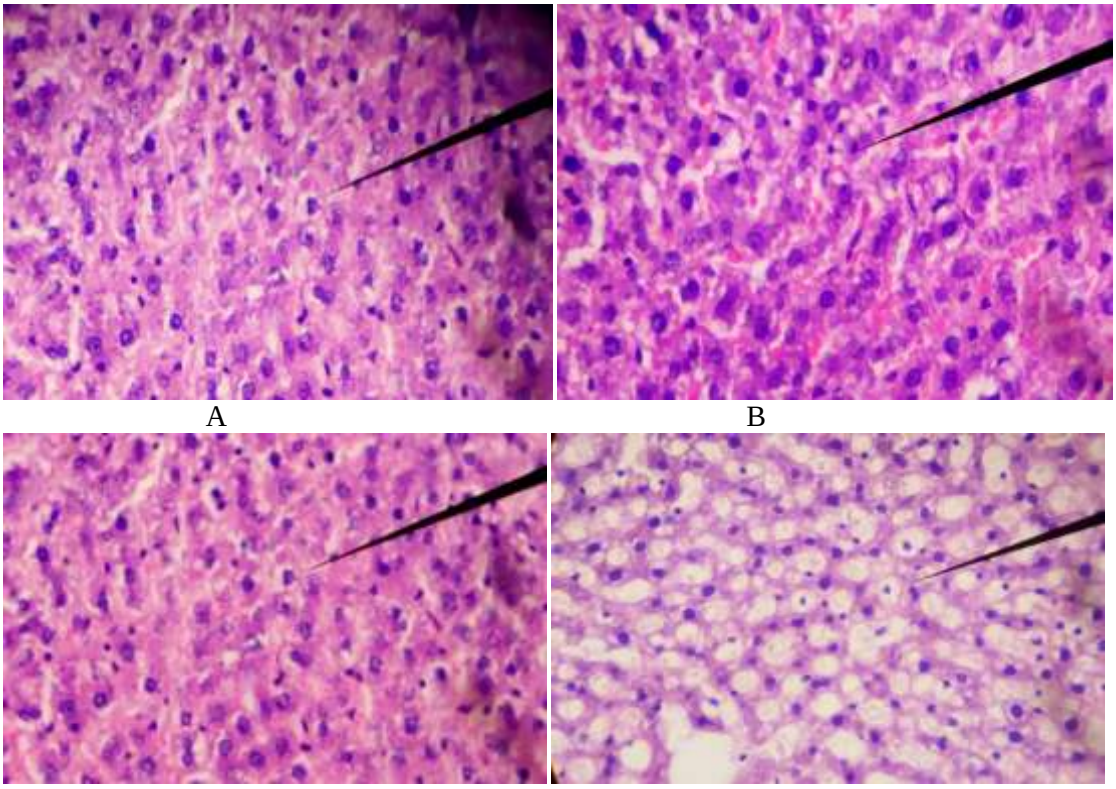

Figure1.Histopathology of lipid degeneration with H & E staining, arrows show:A.Steatosis grade 2 after 30 days of DFO administration (control)B.lobular inflammation grade 1 after Turmeric rhizomes extract dose of 200 mg/kg BW administrationC.hepatocyte balloninggrade 1 after 30 days of DFO administration (control) D. hepatocyte balloninggrade 2 and sinusoid dilationafter Turmeric rhizomes extract at the dose of 400 mg/kg BW administration

The result of Kruskal-Wallis test showed that the number of hepatocytes with steatosis (p=0,019), hepatocyte ballooning (p=0,392) and lobular inflammation (p=0,260), were significantly different between the four groups.

The results of Mann Whitney test showed a significant difference between the control group with treatment groups dose of 200 mg / kg BW (p = 0.030) (Table 1). This means that there is a significant decrease in the number of hepatocyte steatosis with an effective dose of turmeric extract 200 mg / kg BW, so there was no significant difference in the amount of steatosis, hepatocyte ballooning, lobular inflammation between the control group with treatment group doses 100 and 400 mg/kg BW.

Table 1.The effect of ethanol extract of turmeric rhizome on histopathology in DFO-induced male Wistar rats

Treatment Group	N	Steatosis (P value)	Hepatosit *Balloning* (P value)	Lobular *Inflamation* (P value)
P1-P2	6	0, 056	1,000	0, 138
P1-P3	6	*0, 030	1, 000	0, 138
P1-P4	6	0, 575	0, 317	0, 523

Data presented as *Mann-Whitney* test; * *Significant at p< 0.05 by Mann-Whitney test*; PI: (control) Normal Diet + DFO (10µl/g/day), P2: Normal Diet + DFO + Tumeric rhizomes ekstrak 100 mg/kg BW/ day, P3: Normal Diet + DFO + Tumeric rhizomes ekstrak 200 mg/kg BW/day, P4: Normal Diet + DFO + Tumeric rhizomes ekstrak 400 mg/kg BW/ day

Discussion

Liver steatosis accompanied by inflammation and injury (ballooning) is a typical sign in NASH. Ballooning hepatocytes are characterized by clear hepatocyte cytoplasm and hepatocellular injury. Hepatocytes appear to be bubbly by the presence of lipid droplets and Mallory's body[18].

Frying at high temperatures and repeated use of cooking oil will damage the double bonds of fatty acids to form trans fatty acids, form toxic acrolein compounds and various free radicals known as ROS (reactive oxygen species) [19].

ROS oxidizes unsaturated fatty acids which cause lipid peroxidation to form products such as 4-hydroxynoneal (HNE) and malondialdehyde (MDA). ROS and aldehyde reactive lipid peroxidation products directly damage mitochondrial DNA and increase the expression of proinflammatory cytokines such as (TNF-α, TGF-β, IL-8) will activate caspase and increase mitochondrial permebility, malori body formation and collagen synthesis in stellate cells . ROS will also cause apoptosis directly by means of activation of Nf-kB[20,21].

Turmeric has the main active substances namely curcuminoids and essential oils, as well as other ingredients such as carbohydrates, proteins, fats, vitamins and selenium[22]. Curcumin which is a natural product of turmeric rhizomes has proven to be antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, anticancer activity and has the potential for a variety of malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, etc[23].

Curcumin has a strong anti-oxidant 2-6% as free radical scavenger so it can inhibit free radical disease[24]. Curcumin in turmeric rhizomes can suppress proinflammatory pathways that can inhibit TNF production which leads to inflammatory processes associated with chronic diseases such as NASH[25].

The antioxidant power by curcumin is the same as vitamins C and E is able to capture superoxide ions and break the chain between superoxide ions (O2-) so inhibit liver cell damage. Curcumin is also able to increase gluthation S-transferase (GST)so it can inhibit some proinflammatory factors, gene expression and hepatitis B virus replication by means of down regulation from PGC-1a, so curcumin can be made an alternative as a hepatoprotector[26].

Flavonoids found in the tissues of turmericas a component of secondary metabolites are phenolic groups, phenolic has one aromatic ring A, one aromatic ring B, and the middle ring contains oxygen and contains one or more hydroxyl groups[27]. The hydroxyl group in phenolic is a group that has antioxidant activity and plays an important role in capturing free radicals, because these hydroxyl groups donate hydrogen atoms to stabilize free radical compounds[28,29].

This is consistent with the results of the study, that the administration of turmeric extract can reduce hepatocyte steatosis, especially at doses of 100 and 200 mg/ kg BW, but at a dose of 400 mg / kg BB there is an increase in the number of steatosis, maybe the dose is a toxic dose of liver tissue.

This study directly assessed the Gold Standard, namely liver histology, especially hepatocyte degeneration (number of steatosis, belloning hepatocytes, and lobular inflammation). From the results of the above studies that turmeric rhizome can be used as an additional supplement to inhibit the occurrence of NASH.

Conclusions

Based on the results of the study it can be concluded as follows: Increased fat degeneration; steatosis, lobular inflammation and balloon hepatocytes in the group given DFO (control), and a significant decrease in the number of hepatocyte steatosis in the administration of turmeric rhizome extract at a dose of 200 mg / kg BW.

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References

1. Chalasani, N., Younossi, Z., Lavine, E.J., Diehl, M.A., Brunt, M.E., Cusi, K., Chartlton, M., Sanyal, A, J. The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease, Practice Guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology, and the American Gastroenterological Association. 2012, 55(6): 2005-2023
2. Geoffrey, F., Vincent, W., Chitturi, S., (2013). NAFLD in Asia—as Common and Important as in the West Riviews. *Gastroenterology dan Hepatology*. 10: 307-318
3. Schwenger, K. J. P. (2014). Clinical Approaches to Non-Alcoholic Fatty Liver Disease. *World Journal of Gastroenterology*, 20(7), 1712–1723. doi:10.3748/wjg.v20.i7.1712.
4. Dhibi, M., Brahmi, F., Mnari, A., Houas, Z., Chargui, I., Linda, B., . . ., & Hammami, M. (2011). The Intake of High Fat Diet With Different Trans Fatty Acid Levels Differentially Induces Oxidative Stress and Non-Alcoholic Fatty Liver Disease (NAFLD) in Rats. *BioMed Central*, 8(65), 1–12.
5. Attar, B. M., & Van Thiel, D. H. (2013). Current Concepts and Management Approaches in Non-Alcoholic Fatty Liver Disease. *The Scientific World Journal*, 2013, 1–10. doi:10.1155/2013/481893.
6. Dorfman, S. E., Laurent, D., Gounarides, J. S., Li, X., & Tara, L. (2009). Mullarkey, Rocheford. E.C, Farid, S., Erica, A., Thomas, E., Commerford, S.R. Metabolic Implications of Dietary Trans-Fatty Acids. *The Observatory Journal*, 17(6), 1200–1207.
7. Wong-Yee, Yusadli, Nurdiyana, Amarina. 2014. Extraction of Essential Oil FromCurcuma Longa. Journal of Food Chemistry andNutrition. 2014, 01-10.
8. Akram, M., Uddin, S., Ahmed, A., Usmanghani, K., Hannan, A., Mohiuddin, E., & Asif, M. (2010). Curcuma Longa And Curcumin: A Review Article. *J. Biol. Plant Biol.*, 55(2), 65–70.
9. Zeinab, A. (2016). Potentialhepatoprotective effect of combining vitamin C and L-Carnitine against acetaminophen induced hepatic injury and oxidative stress in rats. *International Journal of Pharm Tech Research*, 9(7), 33–47.
10. Jisha, M., Hukuman, Z., & Leena, P. (2017). Quantitative Analysis of SelectedSecondary Metabolites in the Leaves and Flowers of PogostemonQuadrifolius(Benth.) F. Muell. (Lamiaceae). *International Journal of Chemtech Research*, 10(2), 1079–1084.
11. Patonah, Y. (2016). Activity of Curcuma Longa And Curcumin: A Review Article. *Journal Farmasi Galenika*, 01(02), 54–60.
12. Wahyuningrum, M. R., & Probosari, E. (2012). The effect of papaya fruit (Carica papaya L.) on triglyceride levels in hypercholesterolemic Sprague Dawley rats. *Journal of Nutrition College*, 1, 192–198.
13. Departemen Kesehatan RI, Farmakope Indonesia. edition V. Jakarta: Departemen Kesehatan Republik Indonesia; 2014.
14. Yang, Z., Yang, W., Peng, Q., He, Q., Feng, Y., Luo, S., & Yu, Z. (2009). Volatile phytochemical composition of rhizome of ginger after extraction by headspace solid-phase microextraction, petrol ether extraction and steam distillation extraction. *Bangladesh Journal of Pharmacology*, 4(2), 136–143. doi:10.3329/bjp.v4i2.3232.
15. Brunt, E. M. (2016). Nonalcoholic Fatty Liver Disease: Pros and Cons of Histologic Systems of Evaluation Review. *International Journal of Molecular Sciences*, 17(1), 97. doi:10.3390/ijms17010097.
16. Dahlan, M. Statistics for Medicine and Health Edition 5. SalembaMedika. Jakarta, 2011.
17. Depkes.General Standard Parameters of Medicinal Plant Extracts. Directorate of Drug and Food Supervision and Traditional Drug Medicine. 2000.
18. Takahashi, Y., Fukusato, T. (2014). Histopathology of Non-Alcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. *World Journal of Gasrtoenterology*, 20 (42), 15539-15548.
19. Edwar, Z., Suyuthie, H., Yerizel, E., & Sulastri, D. (2011). Effect of Heating on Fatty Acid Saturation of Palm Cooking Oil and Corn Cooking Oil. *J. Indon. Med. Assoc.*, 61(6), 248–252.
20. Yusri, D. J., & Sayoeti, Y. (2014). Elfitrimelly. The Role of Anti-Oxidants in Non-Alcoholic Fatty Liver Disease (NAFLD). *Journal Health Andalas*, 3(1), 1–6.

21. Martín-Domínguez, V., González-Casas, R., Mendoza-Jiménez-Ridruejo, J., García-Buey, L., & Moreno-Otero, R. (2013). Pathogenesis, Diagnosis and Treatment of Non-Alcoholic FattyLiver Disease. *Revista Espanola de Enfermedades Digestivas*, 105(7), 409–420. doi:10.4321/S1130-01082013000700006.
22. Nasri, H., Sahinfard, N., Rafieian, M., Rafieian, S., Shirzad, M., & Rafieian-kopaei, M. (2014). Turmeric: A spice with multifunctional medicinal properties. *Journal of Herb Med. Pharmacology*, 3(1), 5–8.
23. Steffi, P. F., & Srinivasan, M. (2014). Preparation, Characterization and Stabilization of Curcumin Nanosuspension. *International Journal of Pharm Tech Research*, 6(2), 842–849.
24. Mohammed, R.S. (2016). Review article: Herbal sources as a remedy forrheumatoid arthritis. *International Journal of PharmTech Research*, 9(9), 25-50.
25. Aggarwal, B., Gupta, S., & Sung, B. (2013). Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *British Journal of Pharmacology*, 169(8), 1672–1692. doi:10.1111/bph.12131.
26. Marinda, F. (2014). Hepatoprotective Effect of CurcuminIn Chronic Hepatitis. *J. Majority*, 3(7), 52–56.
27. Redha, A. (2010). Flavonoids: Structure, Antioxidant Properties and Their Role in Biological System. *Journal Bellan*, 9(2), 196–202.
28. Adawiah, S. (2013). D., Muawanah, A. Antioxidant Activities and Content Bioactive Components oJuiceNamnam Fruits. *Journal Kimia Valensi*, 1(2), 127–122.
29. Vamanu, E. (2012). In Vitro Antimicrobial and Antioxidant Activities of Ethanolic Extract of Lyophilized Mycelium of Pleurotusostreatus PQMZ91109. Molecules (Basel, Switzerland), 17(4), 3653–3671. doi:10.3390/molecules17043653.

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