

CHAPTER I INTRODUCTION

I.1. Background

Curcuma longa, a perennial herb and member of the Zingiberaceae (ginger) family, grows to a height of three to five feet and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. It has rectangle, pointed leaves and funnel-shaped yellow flowers. The rhizome, the portion of the plant used medicinally, is usually boiled, cleaned, and dried, yielding a yellow powder. Dried *Curcuma longa* is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow color.^{1,2}

Curcuma longa rhizome is used extensively in foods for both its flavor and color, as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. Turmeric can also be applied topically in poultices to relieve pain and inflammation. Recent researchs has focused on *curcuma longa* rhizome as antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders.³

Curcuma longa rhizome has hepatoprotective effect is mainly a result of its antioxidant properties as curcumin suppresses lipid peroxidation. Curcumin increases the expression of intracellular glutathione.^{4,5} Curcumin could also play an antioxidant role through its ability to bind iron, as well as its ability to decrease the formation of

proinflammatory cytokines, especially *curcumin* downregulates the expression of various inflammatory cytokines including TNF, IL-1, IL-6, IL-8, and chemokines.^{6,7} *Curcumin* has been shown to inhibit the action of TNF- α secretion.^{8,9} Turmeric and *curcumin* also reversed biliary hyperplasia, fatty changes, and necrosis induced by toxin. As most of the cancer-inducing chemicals in cigarette smoke are only carcinogenic during the period between activation by phase I and final detoxification by phase II. *Curcumin* in the *curcuma longa* rhizome can help prevent the cancer-causing effects of tobacco, Curcumin, the active ingredient found in turmeric, has a positive effect on the liver tissue. Even liver tissue that has been damaged by excessive exposure to alcohol or other damaging drugs can be positively affected by turmeric.

Turmeric can be used in food and it is readily available in powdered form. Curcumin extracts in liquid form are also available. Turmeric powder can be consumed with herb based teas, honey or hot water to treat gastric ailments. Dosage depends on whether turmeric is being consumed or its active ingredient, curcumin. Usually about a half to a quarter teaspoon of powdered turmeric should be consumed two to three times a day. Curcumin capsules with a dosage 250-500 mg can also be taken three times a day^{8,9}

Cigarette smoke has enormous negative health consequences worldwide, and the use of tobacco is still rising globally. Although approximately 4000 components in the cigarette. Most people are well aware of effects of smoking on the heart and

lungs. However, that smoking cigarettes can also severely affect liver. The numerous toxins found in cigarette tobacco lead to chronic inflammation and scarring in the liver, which in turn, increases risk for liver damage including diseases such as Hepatitis B and C, liver cancer and liver fibrosis. Additionally, smoking affects the liver processes alcohol and medications, which can increase risk for alcoholism as well as overall drug and alcohol tolerance levels.⁹

Science Daily (Sep. 10, 2009) — A team of scientists at the University of California, found that second-hand tobacco smoke exposure can result in nonalcoholic fatty liver disease (NAFLD), a common disease and rising cause of chronic liver injury in which fat accumulates in the liver. The researchers found fat accumulated in liver cells of mice exposed to second-hand cigarette smoke for a year in the lab. Such fat buildup is a sign of NAFLD, leading eventually to liver dysfunction. In their study, the researchers focused on two key regulators of lipid (fat) metabolism that are found in many human cells as well: SREBP (sterol regulatory element-binding protein) that stimulates synthesis of fatty acids in the liver, and AMPK (adenosine monophosphate kinase) that turns SREBP on and off. That Found the second-hand smoke exposure inhibits AMPK activity, which, in turn, causes an increase in activity of SREBP. When SREBP is more active, more fatty acids get synthesized. The result is NAFLD induced by second-hand smoke.

The liver as body's natural filter. It's the liver's responsibility to prevent harmful toxins from entering the bloodstream. In addition, The liver helps body metabolize sugars and carbohydrates, and regulates the flow of bile, the substance that aids in the digestion process. Humans cannot survive without a liver and when the organ is agitated, the body's immune system weakens and becomes more susceptible to disease and infection. Additionally, N-Nitrosodiethylamine, one of the many chemicals found in cigarettes, can cause liver tumors. Another chemical, N-Nitrosopyrrolidine, is proven to contribute to liver, cancer. Arsenic, another ingredient found in the smoke inhale, is also known to increase risk of developing liver cancer.

Oddly enough, The liver is also partly responsible for nicotine addiction. When inhale smoke, The liver produces enzymes that help body clear out the toxins through urine. One particular enzyme is specifically responsible for filtering out nicotine. Therefore, as liver produces more and more of this enzyme, the nicotine leaves body much faster, This means that, as smoke more cigarettes, more enzymes are created and nicotine leaves body more quickly. While this fact seems beneficial, it actually contributes to addiction--because the nicotine is leaving so quickly,the body demands more, which leads to that overwhelming sensation to smoke.

Though cigarette smoke does not directly come into contact with the liver, it does indirectly affect the liver. The chemicals in cigarette smoke eventually make

their way to the liver. These chemicals cause oxidative stress on the liver, which leads to damage to the liver cells and fibrosis .

Oxidative stress is involved in the aging of all the organs of the body. Oxidation produces free radicals that damage the cells of the body, During exposure to cigarette smoke, large amounts of oxygen free radicals are generated; these radicals could damage the lipid components of the cell membranes as well as the matrix components of the lung , by induces vitamin A depletion in the lung and liver. Fibrosis is the development of excess tissue during the body's attempt at repairing an organ or tissue. This is similar to scar tissue and it can adversely affect the liver. The chemicals that are present in cigarette smoke prevent the liver from performing its main function. Over time, the liver becomes less efficient at removing the toxins from body. This can also prevent the proper uptake of medications that may be taking for a particular illness. If body suffering from liver disease, smoking can hasten the further development of this disease.

The evidence that cigarette smoking may negatively impact the incidence, severity, and clinical course of many types of chronic liver diseases. Chronic liver diseases are commonly characterized by continuous inflammation and oxidative stress in the hepatic parenchyma, which are well-characterized systemic consequences of continuous exposure to cigarette smoking. It is then plausible that

prolonged exposure to cigarette smoke negatively impacts key pathogenic events implicated in chronic liver injury.

The liver's third role in detoxification involves a two-step enzymatic process for the neutralization of unwanted chemical compounds. This pathway converts a toxic chemical into a less harmful chemical. This is achieved by various chemical reactions (such as oxidation, reduction and hydrolysis), and during this process free radicals are produced which, if excessive, can damage the liver cells. Phase 1 is carried out by the cytochrome P 450 enzyme system and consists of oxidation and reduction reaction, Excessive amounts of toxic chemicals can disrupt the P-450 enzyme system by causing hyper activity or what is called 'induction' of this pathway. This will result in high levels of damaging free radicals being produced. Substances that may cause hyperactivity of the P- 450 enzymes like nicotine and arsenic compound in cigarette smoking.

This study have been conducted to advice the people they cannot quit smoking even after advice them also be educate them to use curcuma longa rhizome as a treatment and protector of the liver symptoms of smoking and until the that time which people can quit smoking will sensitization them to using curcuma longa rhizome, especially some studies that pointed to the proper use of curcuma longa rhizome in the treatment of symptoms smoking on the liver.

I.2. Research Questions

I.2.1. Research Question.

Based on previous several studies that reported the negative effect of smoking to liver cell and the potency *curcuma longa* rhizome extract as antioxidant and anti inflammation, the major research question of this study:

Does *curcuma longa* rhizome extract has regenerative effect to SpragueDawely rat's liver cell damage induced by passive cigarette smoking?

The major research question is apart to minor research questions:

- a. Does Sprague Dawely Rats that received *curcuma longa* rhizome extract have liver change cell lower than those SD Rats did not received *curcuma longa rhizome extract* after exposure to passive cigarette smoke ?
- b. Does Sprague Dawely Rats that received *curcuma longa* rhizome extract have liver cell TNF- α expression score lower than those SD Rats did not received *curcuma longa rhizome extract* after exposure to passive cigarette smoke ?

I.3. Objectives of the study

I.3.1. General Objectives

To determine the effect *curcuma longa rhizome extract* to liver cell change induced by passive cigarette smoking. To explain the effect *curcuma longa rhizoma* extract against cigarette smoking induced hepatic tissue damage through examining the effect of *curcuma longa rhizoma* extract on reducing the severity of

liver damage under microscopic examination by using routine histopathological staining H&E ,and examining the regenerative effect of *curcuma longa rhizoma* extract on reducing inflammation marker TNF α by using immunohistochemistry method.

I.3.2. Specific Objectives.

- c. To analyze the difference of liver cell change between Sprague Dawely Rats that received *curcuma longa rhizome extract* and those SD Rats did not received *curcuma longa rhizome extract* after exposure to passive cigaratte smoke
- d. To analyze the difference of liver cell TNF- α expression between Sprague Dawely Rats that received *curcuma longa rhizome extract* and those SD Rats did not received *curcuma longa rhizome extract* after exposure to passive cigaratte smoke

I.4. Study benefit

I.4.1. Benefit for science

The results of this study may give additional evidence of adverse effect of smoking especially to liver tissue. This study is provide scientific evidence the benefit of *curcuma L* for protection and treatment of liver injury especially due to cigarette smoke exposure.

I.4.2. Benefit for health care provider

The results of this study can be used as evidence to use *Curcuma L* as supplementary therapy for liver disease caused by passive cigarette smoke .

I.4.3. Benefit for research

The results of this study can be used for developing further research on prevention and therapy for adverse effect of smoking.

I.5. Originality of the study

Based on searching on research publication on Pubmed National Library of Medicine national Institute of Health USA and “Litbang Depkes” website, the study of reparative effect of *Curcuma L* on liver cell change and TNF- α expression has not conducted yet. There were several study related to current study as listed below:

Table (1) Originality of the study

		Method	Results
1.	Al-Khawaja, et al. The effect of nicotine on the liver and kidney of prepubertal Sprague Dawley rats. FASEB J. 2008;22:1123.8.) ¹⁰	Design: experimental Subjects:Prepubertal male Sprague Dawley rats(n=40), Treatment:Intra peritoneal injection of nicotine 6.25 ng/g for 1 (G2) and 2 weeks (G4) Measured parameters: Liver histopathology - ALT and AST - lipid profile,urea - IL-2, IL-6	Histological studies of the liver showed deleterious effects of nicotine on both liver and kidney in G4. ALT level showed a significant increase in G4, where a significant increase in AST was found in both G2 and G4. Urea showed a significant increase in G4. Lipids however, showed a significant decrease in cholesterol and HDL-C (in both G2 and G4, while triglyceride level significantly increased in G4. A significant increase in IL-2 (P<0.05) in both G2 and G4 was detected. However, IL-6 showed a significant increase in G4. Nicotine exposure increase the risk of damage that occurs in the liver and kidney with increasing the period of exposure to nicotine.

		Method	Results
2.	Kalpana C, et al. Modulatory effects of curcumin and curcumin analog on circulatory lipid profiles during nicotine-induced toxicity in Wistar rats. <i>J Med Food.</i> 2005;8(2):246-50. ¹¹	Design: experimental Subjects: Wistar rats Treatment: subcutaneous injection of nicotine (2.5 mg/kg BB for 5 days a week, for 22 weeks), and curcumin (80 mg/kg) simultaneously along with nicotine by intragastric intubation for 22 weeks Study parameters: -AST,ALT,Lipidprofile - AP,LDH	In nicotine-treated rats, enhanced plasma marker enzymes and lipid profiles were observed. Administration of curcumin or curcumin analog to nicotine-treated rats significantly reduced the activity of marker enzymes and plasma lipid levels
3.	Azzalini L, et al. Cigarette Smoking Exacerbates Nonalcoholic Fatty Liver Disease in Obese Rats. <i>Hepatology.</i> 2010 ;51(5):1567-76. ¹²	Design: Experimental study, Research subjects: Normal and obese rats. Treatments: rats were exposed to 2cigarettes/day,5 days/week for 4 weeks. Variabels: - Insulin resistance (HOMA-IR) - Lipid profile - Hepatic histological examination for assessing the degree of liver injury	Obese rats showed hypercholesterolemia, insulin resistance, and histological features of NAFLD. Smoking increased alanine aminotransferase serum levels and the degree of liver injury in obese rats. Smoking increased the histological severity of NAFLD in obese rats. Smoking increased the degree of oxidative stress and hepatocellular apoptosis in obeserats. Smoking increased the hepatic expression of tissue inhibitor of metalloproteinase-1 and procollagen-alpha2(I) in obese rats. Smoking causes oxidative stress and worsens the severity of NAFLD in obese rats

		Method	Results
4.	Valenca SS, et al. Effects of oral nicotine on rat liver stereology. <i>Int. J. Morphol.</i> ,2008 26(3):1013-1022 ¹³	Design: experimental study Study subjects: Male Wistar rats Treatment: oral nicotine (ON) diluted in drinking water during 32 days. Variabel: Liver tissue histopathology feature	Control group reserved hepatocyte with no presence of inflammatory cells. ON group showed varied size of hepatocytes with cloudy cellular limits and histoarchitecture loss. Capillaries were fully of red blood cells. observed also an increase of at globules with small size. Oral nicotine has harmful effects in liver induced by toxic mechanism with reduction of hepatocytes number and disturbance in lipid metabolism.

The current study is different with several previous studies that listed above. The difference as follow:

- In current study, research subjects were SD rats, previous studies used Wistar rats and genetically obese
- In current study the SD rats were exposed to cigarette smoke through direct exposure of cigarette smoke from cigarette that commercially available in local vendor to mimic real condition of passive smoker. The length of exposure in current study were 10 weeks and 13 weeks.
- In currennt study *curcuma L extract* were administered orally in the dose 80 mg/day by orogastric tube.
- Outcome in this study were liver tissue change and TNF- α expression Based on these facts, current study was different with several studies that have published previously.

