

# CHAPTER I

## INTRODUCTION

### I.1. BACKGROUND

Alcohol is a commonly used substance among people all over the world with its intoxicating effects and potential for abuse, that affects virtually every organ in the human body. Most people have general knowledge about the consequences of ethanol intoxication, some often-fatal medical consequences of long term ethanol abuse, such as liver disease or risk for cardiovascular disease.<sup>1</sup> An association between liver disease and heavy ethanol consumption was recognized more than 200 years ago. Long-term heavy ethanol use is the most prevalent single cause of illness and death from liver disease in the United States. The liver is particularly susceptible to ethanol-related injury because it is the primary site of alcohol metabolism. As ethanol is broken down in the liver, a number of potentially dangerous by-products are generated, such as acetaldehyde and highly reactive molecules called free radicals. Perhaps more so than alcohol itself, these products contribute to alcohol-induced liver damage. The liver is one of the largest organs in the body; it has not only considerable reserves but also the ability to regenerate itself. Consequently, symptoms of liver damage may not appear until damage to the organ is quite extensive. Heavy long-term ethanol consumption clearly plays a major role in the development of ethanol-related liver damage.

Ethanol-related liver damage can be divided into three categories or morphological changes range include; (1) Fatty liver; Some degree of fat deposition in the liver occurs in almost all heavy drinkers. It also may occur transiently in nonalcoholic after a single drinking session. Fatty liver is often unnoticed and reverses within a few weeks of sobriety (2) Alcoholic hepatitis; This disorder is characterized by widespread inflammation and destruction (i.e., Necrosis) of liver tissue. Scar tissue may begin to replace healthy liver tissue, a process called fibrosis. (3) Alcoholic cirrhosis. A cirrhotic liver is characterized by extensive fibrosis that stiffens blood vessels and distorts the internal structure of the liver. This structural damage results in severe functional impairment, which may lead secondarily to malfunction of other organs.<sup>2</sup>

The appearance of steatohepatitis is an important rate-limiting step in the development of progressive alcoholic liver disease. When there is inflammation and cell death associated with the fatty change it is called steatohepatitis. Steatosis is often but not exclusively an early histological feature of alcoholic liver disease (alcohol-related fatty liver) leading to alcohol-related steatohepatitis. Continued ethanol abuse in the presence of steatosis markedly increases the risk for the development of hepatitis, fibrosis and cirrhosis. About 10-30% of alcoholics develop acute alcoholic hepatitis, the frequency of which has been suggested to be even lower than that of alcoholic cirrhosis. Alcoholic hepatitis, also called sclerosing hyaline necrosis, is a highly characteristic histological condition. Alcoholic hepatitis in most cases is a reversible condition, and is not always clinically symptomatic. Hepatitis is considered to be the most important precursor

to cirrhosis, the progression of the disease appearing to require one or more antecedent episodes of steatohepatitis. The presence of alcoholic hepatitis in the initial biopsy may be of prognostic significance in the progression to cirrhosis; it is estimated that about 50% of patients with hepatitis develop cirrhosis within 10 years.<sup>2</sup> Epidemiological studies suggest that a threshold dose of alcohol must be consumed for serious liver injury to become apparent. For men, this dose amounts to 600 kilograms (kg) taken chronically over many years, an intake that can be achieved by consuming approximately, 1 liter of wine, or 8 oz distilled spirits daily for 20 years. For women, the threshold dose is one-fourth to one-half that amount. Yet, no more than one-half of heavy drinkers develop alcoholic hepatitis or cirrhosis.<sup>3</sup>

Large number of medicinal plants and their constituents have been shown beneficial therapeutic potentials. *Nigella sativa* seed, called as 'Black Seed' in English language.<sup>4</sup> Seeds of *Nigella sativa* have been employed for thousands of years as a spice and food preservative. *Nigella sativa* L. (Black Seed) has grown throughout much of Asia and Mediterranean region for its seeds.<sup>5</sup> The oil and the seed constituents have shown potential medicinal properties in traditional medicine. It is known that black seed oil has protective effects to the liver is protected from some types of liver poisoning. It is also known that the black seed itself is used in folk medicine in the treatment of liver diseases.<sup>6</sup>

This study was about hepatoprotective effects of *Nigella sativa* seeds aqueous mixture against ethanol induced hepatic tissue changes. Liver tissue changes include inflammatory changes, hepatic steatosis, Mallory bodies. Hepatic

steatosis is accumulation of either small or large fat droplets in hepatocytes.<sup>7</sup> Hepatic inflammation is an inflammation of the liver, accompanied by the destruction of individual liver cells and scarring.<sup>8</sup> Mallory bodies, which are intracellular perinuclear aggregations of intermediate filaments are eosinophilic inclusion bodies.<sup>9</sup>

According to previous studies which were conducted about the intragastric administration of ethanol as part of a low carbohydrate diet results in alcohol hepatotoxicity, which was aimed to investigate whether the comparable liver injury can be achieved by oral diet intake. Male Sprague rats (weighing 300 g) were fed ethanol as part of low-carbohydrate diets for 36–42 days either intragastrically or orally. Rats were fed at 10 g/kg/day of ethanol, as the ethanol infusion increased in 0.5 g/kg/day steps to; 12 g/kg/day by intragastric infusion. Another group of rats was fed with oral low-carbohydrate liquid diets that contained 40% carbohydrate (control) or 5.5% carbohydrate plus 34.5% ethanol (EtOH). Both oral and intragastric low-carbohydrate ethanol diets resulted in marked steatosis with additional inflammation and necrosis, that were significantly greater in the livers of rats fed intragastrically than orally.<sup>10</sup>

According to the side effects of *Nigella sativa* seeds. It should not be taken by pregnant, thereby was chosen male Wistar rats, additionally the most of previous studies that conducted on ethanol effects, which have used male rats instead of female rats.

There are few reports about a great potential in the *Nigella sativa* seeds and its active principles for the development of new anti-oxidant activities and

anti-inflammatory activities of *Nigella sativa*, this activity also needs to more attention. Although a lot of work has been done to demonstrate these effects, but hepatoprotective effects of *Nigella sativa* seeds against ethanol induced liver damage is not clear, therefore this study was aimed to find out if whole *Nigella sativa* seeds aqueous mixture possess hepatoprotective activities against ethanol induced hepatic tissue changes, and also to increase attention to the activities of *Nigella sativa* seeds antioxidants and anti-inflammatory activities, also this study does not support people to increase of alcohol consumption, but to make them aware of impacts the harmful drinking of alcohol, as well as giving an incentive for patients with alcoholic hepatitis for treatment this disease as long as they stopped drinking alcohol and this will be done by conducting studies that will help and support researchers to develop treatments for this disease.

## **I.2. RESEARCH QUESTION**

### **I.2.1. Major research question**

Is there a role of hepatoprotective effects of *Nigella sativa* seeds extract in various doses against ethanol induced hepatic tissue changes in Wistar rats?

### **I.2.2. Minor research question**

1. Is there any a difference in terms of hepatic steatosis after administrate with *Nigella sativa* seeds extract among the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control)?

2. Is there any a difference in terms of hepatic inflammation after administrate with *Nigella sativa* seeds extract among the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control)?
3. Is there any association between the presence of Mallory bodies and administration with *Nigella sativa* seeds extract among the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control )?

### **I.3. RESEARCH OBJECTIVES**

#### **I.3.1. General objectives**

To prove the role of hepatoprotective effects of *Nigella sativa* seeds extract in various doses against ethanol induced hepatic tissue changes in Wistar rats.

#### **I.3.2. Specific objectives**

1. To analyze the difference in terms of hepatic steatosis after administrate with of *Nigella sativa* seed extract among the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control).
2. To analyze the difference in terms of hepatic inflammation after administrate with *Nigella sativa* seeds extract among the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control).
3. To analyze the association between the presence of Mallory bodies and administrate with *Nigella sativa* seeds extract in the three groups of treatment (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w and control).

#### **I.4. BENEFITS OF RESEARCH**

##### **I.4.1. For study**

1. To find information about Protective effects of *Nigella sativa* seeds against liver injury by ethanol.

##### **I.4.2. For researchers.**

1. As an enrichment material for science, especially in the field of diseases of hepatology, treatment by herbal extract.
2. To investigate more and more the truth of *Nigella sativa* pharmacological effects and protective efficacy as an antihepatotoxic extract.

##### **I.4.3. For communities.**

1. To give the scientific application about the scientific effects of *Nigella sativa* as a pharmacological agent to that it will draw an increase or no increase in the attention of the agriculturists to grow *Nigella sativa*, pharmaceutical industry.
2. To take advantage of the herbs available.

## I.5. ORIGINALITY OF RESEARCH

**Table 1.** Previous studies about hepatoprotective effects of *Nigella sativa* against ethanol induced hepatic tissue changes.

No	Title, author, Journal	Materials	Result	Novelty of study
1.	Alcoholic Liver Disease In Rats Fed Ethanol As Part Of Oral Or Intragastric Low-Carbohydrate Liquid Diets. Ronis M J J , Hakkak R , Korourian S, et al. Expe Biol and Med.	Sprague-Dawley rats (weighing 300 g) The average ethanol intake for the infused group over the entire study was $11.5 \pm 0.7$ g/kg/day. Rats were pair fed for 42 days.	Rats fed low carbohydrate diets plus ethanol orally developed pan-lobular micro and macrovesicular steatosis, inflammatory infiltrates of monocytes, polymorphonucleated granulocytes, lymphocytes. <sup>10</sup>	This research to determine dosage , period and the effects of alcohol to induce steatosis , inflammation in rats. Also to determine of a kind, weight, age of rats.
2.	Mechanism of the alcohol cyclic pattern: role of catecholamines. Jun Li, Barbara A. French, Paul Fu, Fawzia Bardag-Gorce, and Samuel W. French . <i>Am J Physiol Gastrointest Liver Physiol</i>	Male Wistar rats weighing 300 g were fed diet and ethanol at a constant rate of 13 g/kg/ day by intragastrically continuously 24 h/day for 6 weeks together with pair-fed controls fed dextrose isocalorical to ethanol.	The result The histopathology of livers in ethanol fed without treatment showed steatohepatitis compared with the normal histology of the pair-fed control . <sup>11</sup>	This research to determine dosage , period and the effects of alcohol to induce steatosis , inflammation in rats. Also to determine of a kind, weight, age of rats.
3.	Effect Of <i>Cassia Auriculata</i> Leaf Extract On Lipids In Rats With Alcoholic Liver Injury Kumar R S , Ponmozhi M , Viswanathan P and Nalini N. <i>Asia Pacific J Clin Nutr</i>	Hepatotoxicity in male, adult Wistar rats (150–170 g). Rats received a standard pellet diet (15 g/150 g b.w/day), with 5 ml of 25% ethanol (2.5 ml in the morning and 2.5 ml in the afternoon), equivalent to 9.875 g/kg b.w as an aqueous solution, using an intragastric tube for 30 days. At the end of this period the animals were treated as follows for the next 30 days.	In the alcohol treated rat liver, the involvement of the liver was uniform. Fatty changes of both macro-and microvesicular type, and mononuclear cell infiltrates were observed (H&E) in all fields. <sup>12</sup>	This research to determine dosage , period and the effects of alcohol to induce steatosis , inflammation in rats. Also to determine of a kind, weight, age of rats.



<p>4. Hepatoprotective activity of <i>Phyllanthus amarus</i> Schum.Thonn.extract in ethanol treated rats: <i>In vitro</i> and <i>in vivo</i> studies. Pramyothin P, Ngamtin C, Pougshompoo S, Chaichantipyuth C. Chulalongkorn University</p>	<p>Male Wistar rats (180–200 g), 6–8 weeks old. In sub acute treatment of rats with PA (75 mg/kg day), p.o.) or SL (5g/kg day), p.o.) for 7 days after 21 days with ethanol (4 g/kg /day), p.o.) enhanced liver cell recovery .</p>	<p>Histopathological changes included hepatocyte swelling, liver cell degeneration, active Kupffer cells and fatty liver.<sup>13</sup></p>	<p>This research to determine dosage , period and the effects of alcohol to induce steatosis , inflammation in rats. Also to determine of a kind, weight, age of rats.</p>
<p>5. Protection Against Ethanol Induced Hepatotoxicity By Silymarin in Albino Rats. <i>M. H-ur-Rehman, et al.</i> Original Article</p>	<p>Male albino rats of 6-8 weeks old, weighing 150-200 gm. Rats received 2ml/100gm body weight per day of 30%v/v of an aqueous solution of ethanol containing 0.6ml (0.5gm) of ethanol .</p>	<p>The hepatocytes of ethanol group were larger and their cytoplasm contained a large number of micro and macro vacuoles involving the whole of the hepatic lobule. Nuclei of hepatocytes of ethanol group appeared vesicular with a distinct nuclear envelope containing one or two prominent nucleoli and scattered chromatin.<sup>14</sup></p>	<p>This research to determine dosage, period and the effects of alcohol to induce steatosis, inflammation in rats. Also to determine of a kind, weight, age of rats.</p>
<p>6. Protective Effect of <i>Nigella sativa</i> Seeds Against Carbon Tetrachloride-induced Liver Damage. Al-Ghamdi M S. The American Journal of Chinese Medicine.</p>	<p>Adult Albino Wistar male rats (age &gt; 24 months), weighing 250–300 g. An aqueous suspension of the black seeds was given orally at two dose levels (250 mg/kg and 500 mg/kg) for five days.</p>	<p>Histopathological or biochemical changes were not evident following administration of <i>Nigella sativa</i> alone. In conclusion, <i>Nigella sativa</i> seeds appeared to be safe and possibly protective against CCL4-induced hepatotoxicity.<sup>15</sup></p>	<p>This research to determine various doses of <i>Nigella sativa</i>, its antioxidant activities against hepatotoxic agent (CCl4) induced liver damage.</p>

<p>7. <i>Nigella sativa</i> thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats.</p> <p>Ismail M, Al-Naqeep G, Chan K W.</p>	<p>The antioxidant activities of the thymoquinone-rich fraction (TQRF) extracted from <i>Nigella sativa</i> and its bioactive compound, TQ, in rats with induced hypercholesterolemia were investigated. Rats were fed a semipurified diet supplemented with 1% (w/w) cholesterol and were treated with TQRF and TQ at dosages ranging from 0.5, 1, 1.5 g/kg and 20 to 100 mg/kg b.w, respectively, for 8 weeks.</p>	<p>Liver antioxidant enzyme levels, including SOD1 and GPX, were also apparently increased in the TQRF- and TQ-treated rats compared to untreated rats . In conclusion, TQRF and TQ effectively improved the plasma and liver antioxidant capacity and enhanced the expression of liver antioxidant genes of hypercholesterolemic rats.<sup>16</sup></p>	<p>This research to determine various doses of <i>Nigella sativa</i>, its antioxidant activities in hypercholesterolemic rats .</p>
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This research is original and different from previous studies regarding the things below :-

1. This study measured the histopathological changes in terms both of hepatic steatosis and hepatic inflammation induced by ethanol which pretreated with various doses of *Nigella sativa* seeds extract, whereas that the previous study was used a different hepatotoxic agent such as CCl4 to induce liver tissue damage, additionally it has used different doses of *Nigella sativa* seeds extract.
2. There is no previous study indicated to prove the role of hepatoprotective of *Nigella sativa* seeds extract against hepatic tissue changes and particularly against hepatic steatosis, hepatic inflammation and the present of Mallory bodies induced by ethanol, additionally this study indicated that these of Histopathological changes were not evident in the liver sections after pretreated with various doses of *Nigella sativa* seeds extract.