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 alcohol intoxication, some often-fatal medical consequences of long term alcohol abuse, such as liver disease or risk for cardiovascular disease. An association between liver disease and heavy alcohol consumption was recognized more than 200 years ago, Long-term heavy alcohol use is the most prevalent single cause of illness and death from liver disease in the United States.

The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. As alcohol 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 alcohol consumption clearly plays a major role in the development of alcohol-related liver damage [1].

Alcohol-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 nonalcoholics 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. The appearance of steatohepatitis is an important rate-limiting step in the development of progressive alcoholic liver disease[2].

Continued alcohol abuse in the presence of steatosis markedly increases the risk for 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 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.[2]

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.[3]

Large number of medicinal plants and their constituents has been shown beneficial therapeutic potentials. *Nigella sativa* (*N. sativa*) seed, called as 'Black Seed' in English language.[4] Seeds of *Nigella sativa* have been employed for thousands of years as a spice and food preservative. *Nigella sativa* L. (Black Seed) is grown throughout much of Asia and Mediterranean region for its seeds. [5] 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. Also the researchers concluded that black seed has a role in preventing the liver from the effects of carcinogens.[6]

Inflammation has been known to produce proinflammatory cytokines, one of the primary and most effective cytokines of inflammation is TNFα which

produce by phagocyte and injured liver cell and this cytokine further propagate the inflammatory effect of alcohol in liver tissue .

This study will be about hepatoprotective effects of *Nigella sativa* extract against ethanol induced hepatic tissue damage. liver tissue damage include inflammatory changes, steatosis, necrosis and ballooning Accumulation of either small or large fat droplets in hepatocytes called faty liver or steatosis[7]. Hepatic inflammation is an inflammation of the liver, accompanied by the destruction of individual liver cells and scarring.[8]The large irregular masses which occur in the cytoplasm of damaged liver cells called Mallory bodies also known (eosinophilic inclusion bodies) , often a sign of an alcohol-related disease, also they are most common in alcoholic hepatitis (prevalence of 65%).[9]

According to previous studies which were conducted about The intragastric administration of ethanol as part of a low carbohydrate diet results in alcohol hepatotoxicity ,wich it was aimed to investigate whether 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 Other group of rats were 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 also

Inflammation and necrosis were significantly greater in the livers of rats fed intragastrically than orally. [10]

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

I.2.RESEARCH QUESTIONS

Is there protective effect for *Nigella sativa* extract in various doses in reducing expression of TNFα and reducing liver tissue damage induce by ethanol?

I.3.RESEARCH OBJECTIVES

I.3.1.General Objectives

To explain the protective effect of Nigella sativa extract in various doses against ethanol induced hepatic tissue damage through examining the effect of

Nigella sativa extract on reducing the severity of liver alcoholic damage under microscopic examination by using routine histopathological staining H&E, and examining the effect of Nigella sativa extract on reducing inflammation marker TNFα by using immunohisochemistry method.

I.3.2.Specific Objectives

1. To analyze the difference in terms of hepatic inflammation through measuring the expression of TNFα after administration of Nigella sativa extract among the three groups of treatment (0.5 g/kg of BW, 1 g/kg of BW, 1.5 g/kg of BW and control).
2. To analyze the difference in terms of hepatic tissue damage after administration of Nigella sativa extract among the three groups of treatment (0.5 g/kg of BW, 1 g/kg of BW, 1.5 g/kg of BW and control).

I.5. Benefits of Research

I.5.1. for Study

1. To determine the dangerous effect of alcohol on liver and it's ultimately damage on liver tissue through assessing the liver tissue inflammation, necrosis and damage.
2. To assess the Protective effect of *Nigella sativa* extract for liver injury.

1.5.2. For Researchers.

1. As an enrichment material for science, especially in the field of of hepatology , treatment and prevention by herbal extract

2. To investigate more and more the pharmacological effects of NNigella sativa and its Protective efficacy as an antihepatotoxic

3. To give initial step for further study conducting in this field.

1.5.3.For Communities.

1. To give scientific application about the use of *Nigell sativa* as a Pharmacologic agent and this will increase and drw the attention of the agriculturists to grow Nigella sativa,pharma-ceutical industry .
2. To take advantage of the herbs available, the primary goal will be to use simple, available, not expensive herbal in preventing alcoholic liver damage.
3. Increase of the awareness about the toxic effects of alcohol and that would draw the attention of the people to dangers of alcoholism.

I.4.ORIGINALITY OF RESEARCH

Table 1. Previous study about protective 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 0.7 g/kg/day. Rats were pair-fed for 42 days.	Rats fed low carbohydrate diets plus ethanol orally developed pan-lobular microand macrovesicular steatosis,inflammatory infiltrates of monocytes, polymorphonucleated granulocytes, and occasional foci of necrosis.[10]	This study done to examine the damaging effect of ethanol on liver tissue as part of low carbohydrate diet, compared to current study which focus to alcohol tissue damage that unrelated to diet .

2.	Effect Of *Cassia Auriculata* Leaf Extract On Lipids In Rats With Alcoholic Liver Injury Kumar R S , Ponmozhi M , Viswanathan P and Nalini N. *Asia Pacific J Clin Nutr*	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.[11]	This study done to examine the therapeutic effect of *Cassia Auriculata* Leaf Extract on already liver tissue that is damaged due to alcohol , compared to current study that use Nigella sativa instead of *Cassia Auriculata* Leaf

3.	Mechanism of the alcohol cyclic pattern: role of catecholamines. Jun Li, Barbara A. French, Paul Fu, Fawzia Bardag-Gorce, and Samuel W. French . *Am J Physiol Gastrointest Liver Physiol*	Male Wistar rats weighing 300 g were fed diet and ethanol at a constant rate of 13 g/kg/ day at which 40% of the total calories were derived from ethanol or isocaloric dextrose intragastrically continuously 24 h/day for 6 wk 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 . One liver in *group 3* had one small focus of centrilobular necrosis.[12]	This study done with alcohol as part of the diet given for rats and then examine the histopathological picture ,compared to current study that use alcohol as the initiator of liver tissue damage.
4.	Hepatoprotective activity of *Phyllanthus amarus* Schum.Thonn.extract in ethanol treated rats: *In vitro* and *in vivo*	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	Histopathological changes included hepatocyte swelling, liver cell degeneration, active Kupffer cells and fatty liver.[13]	This study use different herbal extract and compare their effect on hepatic liver damage induced by ethanol, the

	studies. Pramyothin P, Ngamtin C, Poungshompoo S, Chaichantipyuth C. Chulalongkorn University	with ethanol (4 g/kg /day), p.o.) enhanced liver cell recovery .		current study compare different dose of Nigella sativa on alcoholic liver damage
5.	Protection Against Ethanol Induced Hepatotoxicity By Silymarin in Albino Rats. *M.H-ur-Rehman,et al.*Original articalArticle	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 aqueous solution of ethanol containing 0.6ml (0.5gm) of ethanol .	The hepatocytes of the ethanol group were larger and their cytoplasm contained large number of micro and macro vacuoles involving 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.[14]	This research to examine the effect of Silymarin on hepatic damage induced by ethanol, compared to current study that uses Nigella Sativa instead of Silymarin.

6.	Protective Effect of *Nigella sativa* Seeds Against Carbon Tetrachloride-induced Liver Damage. Al-Ghamdi M S. The American Journal of Chinese Medicine.	Adult Albino Wistar male rats (age > 24 months), weighing 250–300 g. Aqueous suspension of the black seeds was given orally at two dose levels (250 mg/kg and 500 mg/kg) for five days.	Histopathological or biochemical changes were not evident following administration of *N. sativa* alone. In conclusion, *N. sativa* seeds appeared to be safe and possibly protective against CCL4-induced hepatotoxicity.[15]	This research to determine various doses of Nigella sativa ,its antioxidant activities against hepatotoxic agent (CCl4) induced liver damage,compared to current study that will depend on alcohol to induce the liver damage.
7.	Nigella sativa thymoquinone-rich fraction greatly improves plasma antioxidant Capacity and	The antioxidant activities of the thymoquinone-rich fraction (TQRF) extracted from Nigella sativa and its Bioactive compound,	liver antioxidant enzyme levels, including SOD1 and GPX, were also apparently increased in the TQRF- and TQ-treated rats compared to	This research to determine various doses of Nigella sativa ,its antioxidant activities in hypercholesterole

	expression of antioxidant genes in hypercholesterolemia rats. Ismail M, Al-Naqeep G, Chan K W.	thymoquinone (TQ), in rats with induced hypercholesterolemia was investigated. Rats were fed a semi purified diet supplemented with 1% (w/w) cholesterol and were treated with TQRF and TQ at Dosages ranging from 0.5 to 1.5 g/kg and 20 to 100 mg/kg body wt, respectively, for 8 weeks.	Untreated rats (Pb0.05). In conclusion, TQRF and TQ effectively improved the plasma and liver antioxidant capacity and enhanced the expression of liver antioxidant genes of hypercholesterolemia rats.[49]	mia rats, compare to this study that will use normal rats to induce the damage

As a conclusion from these previous study its notice that alcohol has destructive effect over liver tissue by inducing inflammation some other studies show other material having the destruction effect in liver cells such as CCL4, also others study show the anti-inflammatory effect of Nigella sativa on liver cells by various destructive material, although there is no previous study indicate the effect of Nigella Sativa extract on TNFα.