

CHAPTER V

DISCUSSION

Alcohol induced liver damage can be divided into three categories or morphological changes range (1) Fatty liver; some degree of fat deposition in the liver occurs in almost all heavy drinkers. It also may occur transiently in non alcoholics 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 and (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 organs².

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.⁴ 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

This research was aimed to identify and analyze the liver tissue damage induced by Ethanol in Wistar male rats, this study investigate the liver tissue changes using H&E staining and Immunohistochemistry evaluation of one of the inflammation markers which TNF α , in H&E staining this study evaluate the hepatic cell degeneration, necrosis of the hepatocyte, and the injury of endothelial cell around the central vein in addition to the fat vacuoles in hepatocyte , for the immunohistochemistry examination this study investigate the percentage and the intensity of the cells that express the TNF α in their cytoplasm .

According to previous studies which were conducted about The intragastric administration of ethanol with a low carbohydrate diet results in alcohol hepatotoxicity ,which it was aimed to investigate whether comparable liver injury can be achieved by oral diet intake . Male Sprague Dowly rats (weighing 300 g) were fed ethanol with 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/kgBW/day steps to ;12 g/kgBW/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 the potential in the *Nigella sativa* seeds and its active principles for the development of anti-oxidant activities and anti-inflammatory activities. 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 whole *Nigella sativa* seeds extract possess hepatoprotective activities against ethanol induced hepatic tissue changes, and also to highlight the activities of *Nigella sativa* seeds antioxidants and anti-inflammatory activities, 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 and protection for 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 and protection for this disease.

In this study after examining the liver tissue damage using H&E staining it's found that control group which just given alcohol has the more severe tissue damage (median=3), however group 3 has the least tissue damage (median=0.50) and the other groups ranging between these two groups, as conclusion the tissue damage get less and less when the *Nigella sativa* dose increase reaching the least tissue damage in group 3, this result is being confirmed by the TNF α expression as the control group having the most TNF α expression (median=3) and group 3 having the less TNF α expression (median=0), the TNF α expression has statistical

difference with the dose of *Nigella sativa* extract ,as increasing the dose the expression become less and less and the least expression was in group3 and the most expression was in control group.

Ethanol is one of direct hepatotoxins agents, ethanol ingested in high doses impairs tissues by a variety of mechanisms¹⁶ ,oxidative stress plays surely a crucial role . Most alcohol is broken down (i.e., metabolized) in the liver through a series of chemical reactions, known as oxidation reactions, which involve hydrogen and oxygen atoms . In the predominant biological pathway for alcohol metabolism, called the alcohol dehydrogenase pathway (ADH) ,a second pathway of alcohol metabolism, the microsomal ethanol-oxidizing system (MEOS), is activated by long-term heavy alcohol consumption .¹⁷

Hepatocytes convert ethanol to acetaldehyde through three mechanisms: Alcohol dehydrogenase(ADH), cytochrome P-450 isoenzyme-1 (CYP2E1), and catalase.³⁰ Lipid-peroxidation products can combine with acetaldehyde and with proteins to produce neoantigens, which can stimulate an autoimmune response. Against the background of steatosis and existing liver damage, heavy and continued drinking in some patients causes Alcohol hepatitis. Two main mechanisms are involved: inflammation and oxidative stress.

Patients with alcoholic hepatitis frequently have high levels of cytokines in their bloodstream, including tumor necrosis factor alpha (TNF- α). TNF- α , produced primarily by Kupffer cells, may cause liver injury directly or indirectly. First, evidence suggests that TNF- α might be directly toxic to liver cells. Second, TNF- α stimulates the liver to produce other cytokines, which attract white blood

cells to the liver and stimulate them to release free radicals and toxic enzymes. In experimental animals, TNF- α increase in the liver after 1 month of alcohol administration, timing that coincides with the onset of liver cell necrosis and inflammation. TNF- α production also leads to increased production of chemokines (e.g., IL-8), which attract inflammatory cells from the bloodstream to the liver, contributing to liver inflammation. Interleukin-8(IL-8) and monocyte chemoattractant protein 1 (MCP-1) have been shown to attract neutrophils and macrophages.³¹

The first stage of alcoholic liver damage is steatosis and its most severe in perivenular areas, foci of microvesicular steatosis may be present in addition, the cause of simple macrovesicular steatosis cannot be determined histologically, liver lesion in alcoholic include, steatosis with its two type Microvesicular (foamy degeneration) and Macrovesicular then its convert into steatohepatitis, which compose of many cell changes (steatosis, hepatocyte ballooning, hepatocyte apoptosis, Mallory body formation, inflammatory infiltration and finally fibrosis) followed by megamitochondria, siderosis and then fibrosis which include (pericellular, perivenular and portal) finally if the patient still drink alcohol he will develop irreversible cirrhosis and hepatocellular carcinoma adding to all of this the effect of non-hepatic alcohol-related diseases.⁵³

It should be mentioned that the H&E examination of the liver tissue shows changes that not already mentioned in the score of reading slides that is the cell express microvesicular fatty changes and fatty cytoplasmic accumulation in

hepatocyte decrease gradually from control group till group 3 ,also there was inflammatory cell like lymphocyte , some slides in control group and group 1 was found to contain Mallory body which is nonspecific indication of liver tissue inflammation, also there was ballooning of the cells and central vein and sinusoidal dilatation in these groups and its notice that these changes occur mostly in zone 2 and zone 3 in liver tissue, the other effects of alcoholic liver damage like fibrosis did not occur in this experiment because these change need more time to occur, its consider as long term complication of alcoholic liver damage.

This study has the limitation of the rats weight since not all the rats has the same weight the weight range from 250-300 mg and hence the dose calculated according the mean of the upper and lower weight and didn't calculate for each rat alone .

It should be mentioned that the correlation between the liver tissue damage and the TNF α expression was statistically examined and found to be statistically significant with Spearman's rho correlation coefficient (0.847)