

CHAPTER VI

DISCUSSION

According to previous studies, which were aimed to investigate whether the comparable liver injury can be achieved by oral low-carbohydrate ethanol diets intake in male Sprague rats (weighing 300g). Both oral and intragastric administration of low carbohydrate ethanol diets at dose 12 g/kg/day of ethanol for 36–42 days 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

In hepatocytes the most important pathways is oxidation of ethanol to acetaldehyde via ADH, which is a predominant biological pathway for alcohol metabolism, CYP2E1, which is a second pathway of alcohol metabolism. In mitochondria, acetaldehyde is converted to acetate and in turn acetate is converted to acetyl CoA which leads the two-carbon molecule into the TCA. This oxidation generates lead to reducing NAD, which contribute to ROS production. The changes in the NADH–NAD⁺ potential in the liver inhibit both of the oxidation of triglycerides, fatty acids and may thereby increase lipogenesis.^{27,31} In addition, long term of ethanol consumption activates of oxidative stress, through the activity of cytochrome P-450 2E1, that alters the intracellular balance between levels of S_{AMe} and S_{AH}, resulting in a decrease in hepatic methionine levels, decrease in the activity of methionine adenosyl transferase, the enzyme which converts methionine to S_{AMe}, and decrease in the ratio of S_{AMe} to S_{AH}, that

lead to increase in SAH, homocysteine. Increases in concentration of SAH, stressing the endoplasmic reticulum (ER stress), that lead to release of SREBP-1c from ER stress. Hepatic SREBP-1c initiates the transcription of genes involved in triglyceride and fatty-acid synthesis.^{28, 29}

Thymoquinone (TQ) in *Nigella sativa* seeds has found react with GSH, NADH and NADPH chemically, that indicate a possible intracellular non-enzymatic activation of TQ dependent upon GSH, NADH and NADPH representing perhaps the 'cellular switch' for modulating cellular antioxidant defenses.⁵⁸ In additionally, Thymoquinone was observed to work as general free radical scavengers.⁵⁵ These indicators suggests that TQ may able to reduce NADH, thereby reducing the NADH–NAD changes, which caused to increase in the process of lipogenesis. Also TQ was observed to decrease cellular oxidative stress by inducing glutathione.⁴⁹ According to previous study which indicated that active antioxidant components of black seeds of *Nigella sativa* plants capable of rendering protection against the development of methionine-induced hyperhomocysteinemia (Hhcy) and its associated state of oxidative stress.⁵⁹ These results suggest that TQ capable to provide balance between SAME and SAH, and may thereby it provided protection against ER stress and SREBP-1c activation respectively, that include transcription of genes involved in triglyceride and fatty-acid synthesis.

There is another pathway to induce hepatic inflammation caused by oxidative stress through ethanol metabolism in the liver. In hepatocytes, ethanol is converted acetaldehyde through three mechanisms: ADH, CYP2E1 and catalase.³¹

Some of the acetaldehyde interacts with proteins resulted from Lipid-peroxidation in the cells such as malondialdehyde and formation MAA which located on the membranes of hepatocytes to produce neoantigens, that can stimulate certain immune cells to produce various cytokines, that in turn attacks healthy liver cells, resulting in tissue damage.^{38,39}

According the results of previous study that indicate TQ was found to increase the red blood cell glutathione, and inhibit the activation of NF- κ B. In addition to this, there is another previous study was indicated that TQ have been shown inhibit iron-dependent microsomal lipid peroxidation efficiently. Also TQ was shown to work as a scavenger of various ROS including superoxide radical anion and hydroxyl radicals.⁵⁵ According to the results of another study which were indicated that there is a possible intracellular non-enzymatic activation of TQ dependent upon GSH, NADH and NADPH representing perhaps the 'cellular switch' for modulating cellular antioxidant defenses.⁵⁸ Addition other studies indicate that TQ has a role in reducing oxidative stress by increasing glutathione.⁴⁹ These effects of TQ indicate that TQ has a role in reducing inflammation caused by ethanol through its ability to decrease malondialdehyde, lipid peroxidation products, reduction in amount of cytokines via inhibiting activity of NF- κ B and to reducing cytochrome c production from the mitochondria via inhibition of generates ROS.

In hepatocyte, ethanol is converted to acetaldehyde by ADH and CYP2E1. An increase of CYP2E1 lead to increased electron leakage and release of ROS by CYP2E1 and mitochondria, that cause lipid peroxidation and produce

protein carbonyls, that lead to inhibition of proteasomes, leading to reduces the catabolism of damaged proteins and may contribute to accumulation of an abnormal form of ubiquitin called Ub 1. When these abnormal ubiquitin–protein conjugates called cytokeratin filaments, and formation of Mallory bodies on liver histology.^{27,42}

Active component of *Nigella sativa* seeds (TQ). TQ have inhibit iron-dependent microsomal lipid peroxidation efficiently, as well as TQ has been shown to work as a scavenger of various ROS, superoxide radical anion and hydroxyl radicals.⁵⁵ In addition to that, The compound was observed to decrease cellular oxidative stress by inducing glutathione (GSH).⁴⁹ These effects of *Nigella sativa* seeds extract are capable of rendering protection against formation of Mallory bodies in hepatocytes.

In the present study, the dose of ethanol was high (total of 12 gm/kg). This may explain not great decreased hepatic steatosis and which is probably due to decreased synthesis resulting from extensive damage of the hepatic cells as illustrated in (Fig.9 D). This damage appeared to have been inhibited or modulated by administration of *Nigella sativa* prior and during treatment with ethanol (Fig. 8A). Indeed, degree of liver steatosis was significantly decreased in these animals when compared to that of animals treated with ethanol, that indicating the restoration of hepatic cell function.

There were not previous studies have conducted to investigate hepatoprotective effects of *Nigella sativa* seeds extract against hepatic steatosis, hepatic inflammation and Mallory bodies induced by other hepatotoxic agent.

Although In one of slides in group 3 was indicated to none steatosis, but the most of the slides have mild. In addition there is a difference in response to the antioxidant effect of *Nigella sativa* between the immune systems of rats, which lead to difference the histological finding, However, there were significant protection towards ethanol induced hepatic tissue changes.

Furthermore, the findings of this study are in agreement with a previous study, which it was aimed to protective effect of *Nigella sativa* seeds against liver damage induced by carbon tetrachloride, which reported that daily administration of *Nigella sativa* oil (250 mg/kg or 500 mg/kg orally for 5 days) may be successful to decrease liver damage induced by Carbon Tetrachloride in animals pretreated with *Nigella sativa* seeds.¹⁵

In conclusion, *Nigella sativa* seeds appeared to be having a significant effect against ethanol induced hepatic tissue changes. However, further studies may still be needed to clarify its effects on the hepatic fibrosis, hepatic cirrhosis, prior to supporting its use in the fields of contemporary medicine and folk medicine for hepatic illnesses.