

CHAPTER III

THEORETICAL FRAMEWORK LITERATURE

III.1. Hepatoprotective effects of *Nigella sativa* seeds against ethanol-induced Hepatic tissue changes

III.1.1. Hepatoprotective effect of *Nigella sativa* seeds against ethanol-induced hepatic steatosis

In hepatocytes the most important pathway is oxidation of ethanol via alcohol dehydrogenase (ADH) to acetaldehyde. In mitochondria, acetaldehyde is converted to acetate and in turn acetate is converted to acetyl CoA, Which leads ultimately to reduced nicotinamide adenine dinucleotide (NAD), thereby contribute to reactive oxygen species production (ROS). The changes in the NADH–NAD⁺ potential in the liver inhibit both the oxidation of triglycerides, fatty acids and may thereby increase lipogenesis.²⁷

Protective effects of *Nigella sativa* seeds extract against the effects' above of ethanol. According to previous studies indicated that TQ has reacted with GSH, NADH and NADPH chemicals, which lead to forming two products, viz. glutathione dihydrothymoquinone and dihydrothymoquinone (DHTQ), respectively. These results 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 addition, TQ was observed to work as general free radical scavengers.⁵⁵ These indicators

suggests that TQ may be able to reduce NADH, thereby reducing the NADH–NAD⁺ changes, which leads ultimately to inhibiting the process of lipogenesis.

Oxidative stress activation via long term ethanol consumption, through the activity of cytochrome P-450 2E1, that alters the intracellular balance between levels of S-adenosylmethionine (SAMe) and S-adenosylhomocysteine (SAH), resulting in a decrease in hepatic methionine levels, decrease in the activity of methionine adenosyl transferase, the enzyme which converts methionine to SAMe, that lead to increase in SAH, homocysteine. Increases in concentration of SAH, stressing the endoplasmic reticulum (ER stress), that lead to the release of SREBP-1c from ER stress, which in turn initiates the transcription of genes involved in triglyceride and fatty-acid synthesis.^{28,29}

According to previous studies that, the protective effects of *Nigella sativa* seeds extract, it may be produced by inhibiting iron-dependent microsomal lipid peroxidation efficiently via TQ, additionally TQ was observed to decrease cellular oxidative stress by inducing glutathione.^{55,56} TQ capable of rendering protection against the development of methionine-induced hyperhomocysteinemia (Hhcy) and its associated state of oxidative stress.⁵⁹ These indicators suggest that TQ may be able to provide balancing between SAMe and SAH, that lead to providing protection against ER stress and SREBP-1c activation respectively, which leads ultimately to inhibit the process of lipogenesis via inhibit transcription of genes involved in triglyceride and fatty-acid synthesis.

III.1.2. Hepatoprotective effect of *Nigella sativa* seeds against ethanol-induced hepatic inflammation.

Heavy ethanol ingestion contributes to activate Kupffer cells induced by LPS-endotoxin which lead to release large amounts of TNF- α and NF- κ B. NF- κ B is a transcription factor that is translocate to the nucleus in response to the promoter region of pro-inflammatory, thereby activating multiple cytokine genes, which may further contribute to hepatic inflammations.^{33,35}

Protective effects of *Nigella sativa* seeds extract against these effects' above of ethanol. According to previous studies indicated that TQ has ability to increasing the red blood cells' glutathione. In addition, that NF- κ B is a molecular target of TQ.^{48,49} These indicators suggest that TQ has a role in reducing inflammation through its ability to increase intracellular antioxidant via induction of glutathione, and reduction of amount of cytokines via inhibit activity of NF- κ B.

Hepatocytes convert ethanol to acetaldehyde through three mechanisms: ADH, CYP2E1, and catalase. Some of the acetaldehyde interacts with proteins resulted from Lipid-peroxidation in the cells such as malondialdehydete, formation MAA located on the membranes of hepatocytes to produce neoantigens, which can stimulate certain immune cells to produce various cytokines, that in turn attacks healthy liver cells, resulting in tissue damage.^{38,39}

According to previous studies indicated that TQ has the ability to inhibit iron-dependent microsomal lipid peroxidation efficiently.⁵⁵ This effect of TQ lead to decrease in malondialdehyde, lipid peroxidation products, thereby lead to

inhibition neo antigens production, cytokines activation caused by MAA, which in turn lead to decrease in hepatic inflammation caused by alcohol.

There are other sources of oxidative stress caused by alcohol to induce inflammation, that include granulocytes from the inflammatory process catalyzed by the enzymes NADPH oxidase and myeloperoxidase, which cause the production of ROS, and a decrease in antioxidants. This process leads to the release of cytochrome c from the mitochondria which then activates enzymes called caspases and promotes production of IL-8 in the cell.^{40,41}

According results that indicate a possible intracellular non-enzymatic activation of Thymoquinone (TQ) dependent upon GSH, NADH and NADPH representing perhaps the 'cellular switch' for modulating cellular antioxidant defenses.⁵⁸ In addition that TQ has been shown to act as a scavenger of various reactive oxygen species including (ROS) superoxide radical anion and hydroxyl radicals.⁵⁵ In addition, there were other studies indicated that Thymoquinone have a role in reducing oxidative stress by increasing glutathione.⁵⁶ These effects lead to inhibition of generates reactive oxygen species (ROS), that lead to reducing cytochrome c production from the mitochondria wich finally lead to reducing inflammation and oxidative stress.

III.1.3. Hepatoprotective effect of *Nigella sativa* seeds against ethanol-induced Mallory bodies.

CYP2E1 may increase in patients with Alcoholic hepatitis, leading to increases in the release of ROS by CYP2E1 and mitochondria cause lipid peroxidation and produce protein carbonyls, that lead to inhibition of

proteasomes. Inhibition of the proteasomes leads ultimately to the formation of Mallory bodies on liver histology.^{27, 42}

According to previous study was indicated that TQ has ability to inhibiting iron-dependent microsomal lipid peroxidation efficiently via active component of *Nigella sativa* seeds extract, as well as TQ showed to work as a scavenger of various reactive oxygen species, that include (ROS) superoxide radical anion and hydroxyl radicals.⁵⁵ In addition TQ showed increase GSH levels.⁵⁶ These effects of *Nigella sativa* seeds extract are capable of rendering protection against formation of Mallory bodies in hepatocytes.

III.2. Theoretical framework :-



Figure 4. Theoretical framework

III.3. Conceptual framework

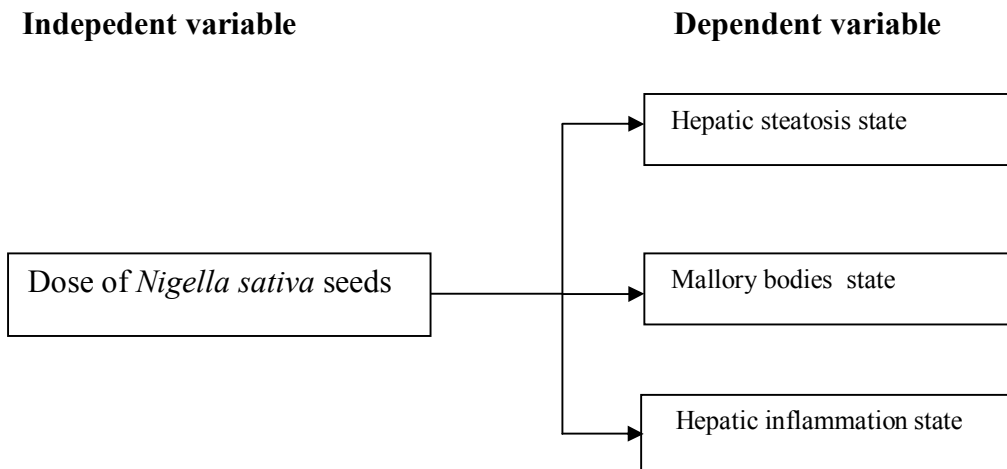


Figure 5. Conceptual framework of the study

III.4. Hypothesis

III.4.1. Major hypotheses

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

III.4.2. Minor hypothesis

1. There's 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. There's 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. There's an association between 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).