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**RESEARCH ARTICLE**

# THE EFFECTS OF THYMOQUINONE ON DIETHYLNITROSAMINE INDUCED HEPATIC INJURY IN RATS: A STEREOLOGICAL STUDY*

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*Hawar Ahmed MHAMED AMIN's Master Thesis

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**ABSTRACT**

This investigation has been conducted to study the protective effect of Thymoquinone against Diethylnitrosamine induced hepatic injury in rats. Twenty-eight of male Wistar albino rats (180-220g) were divided into four groups. The control group received intraperitoneal injection of saline (0.5 ml/kg) for seven days, Thymoquinone group was given thymoquinone (4mg/kg/day) for seven days by drinking water, diethylnitrosamine group was injected a single dose of diethylnitrosamine at the 5th day (intra-peritoneal, 100 mg/kg) thymoquinone+diethylnitrosamine group was given thymoquinone (4mg/kg/day) for seven days by drinking water and was injected a single dose of diethylnitrosamine at the 5th day (intra-peritoneal 100 mg/kg). The rats were anesthetized then livers were taken for process was followed for light microscopic research. The photo was taken by using light microscopy. The hepatocyte number were calculated using physical fractionator method. It was observed a significant decrease of the hepatocyte number in diethylnitrosamine group and thymoquinone+diethylnitrosamine group. In conclusion, diethylnitrosamine can cause liver damage and thymoquinone do not changed that diethylnitrosamine damage in liver.

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## INTRODUCTION

Diethylnitrosamine (DENA) is noted to induce liver cancer in experimental animal models throughout inhibition of many enzymes occupied in DNA repair mechanism. In rats, DENA is a strong hepatocarcinogen affecting the beginning stage of carcinogenesis throughout a period of developed cell production accompanied by hepatocellular necrosis and induces DNA carcinogen adducts, DNA-strand splits and in turn hepatocellular carcinomas without cirrhosis during the improvement of putative preneoplastic focal lesions. While there are many mechanisms for the treatment of liver cancer, its therapeutic outcome stays very poor. Therefore, prevention looks to be the best strategy for lowering the occurrence of this disease. In this piont, many compounds have been tested and demonstrated value against experimentally-induced hepatocarcinogenesis including morin, silymarin, garlic, star anise, ganfujian granule, apigenin and unprocessed extracts of agarcusblazei (Sayed-Ahmed *et al.,* 2010; Shirakami *et al.,* 2012). Thymoquinone (TQ), the most important constituent of the volatile oil from *Negella sativa* seeds is accounted to possess a strong antioxidant property. Thymoquinone defends organs against oxidative damage stimulated by a range of free radical generating agents including doxorubicin induced cardiotoxicity, carbon tetrachloride evoked hepatotoxicity, nephropathy generated by cisplatin autoimmune as well as allergic encephalomyelitis and gastric mucosal injury stimulated by ischemia reperfusion (Al-Majed *et al.,* 2006).

## MATERIALS AND METHODS

**Animals:** We obtained ethical approval for our study from Van Yüzüncü Yıl University Animal Research Local Ethic Committee under number TYL-2016-5369. 28 healthy wistar albino rats, weighing 180–220 g and averaging 20 weeks old were utilized in this study. Rats were housed in cages under controlled environmental conditions (25°Cand a 12 h light/darkcycleandad libitum). The animals were divided into four groups (n=7). The control group received in traperitoneal injection of saline (0.5 ml/kg) for seven days. TQ group was given thymoquinone (4mg/kg/day) for seven days by drinking water, DENA group was injected a single dose of diethyl nitrosamine at the 5thday (intra-peritoneal, 100 mg/kg). TQ+DENA group was given thymoquinone (4mg/kg/day) for seven days by drinking

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water and was injected a single dose of diethyl nitrosamine at the 5th day (intra-peritoneal 100 mg/kg). After the rats were perfuse after being anesthetized with ketamine 50mg/kg and xylazine 4mg/kg.

**Histology:** Following perfusion, liver was removed and fixed in 10% neutral buffered formalin liver was dehydrated through a graded alcohol series, shined in xylene and infiltrated with paraffin for embedding. Serial sections of each liver were cut at 5µm using a rotary microtome. Selected sections were mounted on slides, hydrated through descending concentrations of alcohol series and stained with hematoxylin and eosin (H&E). Photos were taken by using a light microscope (Zeiss axioskop 40 Carl Zeiss Göttingen, Germany) with a digital color camera attachment.

**Stereology:** We used the physical fractionator and disector counting method for the total hepatocyte number. The liver was sampled at 1/4 ratioby fractionator instrument that consist of equally (5mm) spaced knifes. The physical disector consists of two parallel cross-sectional planes separated by a certain distance "t". One counts the particles that are visible on the reference section, but not the look-up section by unbiased counting frame (Figure 1). To achieve systematic random sampling for stereology (Sterio, 1984)

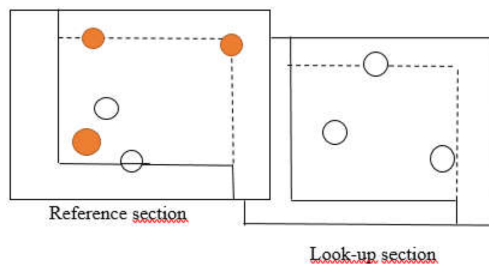


**Figure 1. Unbiased counting frame. The orange particle seen in the reference section but not seen in the look-upsection was counted. Thus, a disectorparticle ($Q^{-}$)=3**

The total hepatocyte number in the liver estimate during the formula:

$$N = N_v \, x \, V_{ref}$$

$N_v$ is the numerical density of the particle of interest (hepatocytes/unit volume) and $V_{ref}$is the total (reference) volume of the object.

We used physical fractionator and disectorfor total hepatocite calculation. With this method, the liver was divided into parallel slices at equal intervals (t) with systematic random sampling from beginning to end. To achieve systematicrandom sampling for stereology, the first section was chosen randomly while the next section was taken after cutting every 180. section. About 11 sections were taken.

**Statistical analysis:** Statistical Analyses were performed using Microsoft SPSS Version 13.0 for statistical analyses. Kruskal-Wallis test was performed to compare groups.

## RESULTS

The liver histological structure of the groups was examined microscopically and structural changes were observed in the Diethylnitrosamine (DENA) group (Figure 3).

**Total hepatocyte number:** The total hepatocyte number that showed in table 1 and in Figure 2. In Diethylnitrosamine (DENA) and Thymoquinone + Diethylnitrosamine group the hepatocyte number were decreased compared to control group. (P=0.01).

The coefficient variation (CV) of total hepatocytecounts for all groups; control: 0.07, TQ: 0.1, DENA: 0.2, TQ+DENA: 0.03 and Coefficienterror (CE) for all groups; control: 0.05, TQ: 0.05, DENA: 0.07, TQ+DENA: 0.06.

**Table 1. Total hepatocyte number**

| | Groups | Median | Mean | St. Dev. | Min. | Max. | p.* |
|---|---|---|---|---|---|---|---|
| Total hepatocyte number | Control | a9081107200 | 9109220723 | 79108595 | 9006694023,000 | 9236747520,000 | ,001 |
| | TQ | b7271409600 | 7311617352 | 1161590456 | 5262156900,000 | 8810605440,000 | |
| | DENA | d 456215760 | 492564379 | 179436549 | 214053840,000 | 779456680,000 | |
| | TQ+ DENA | c4153927050 | 4294131660 | 1893575294 | 2305943640,000 | 7117044480,000 | |

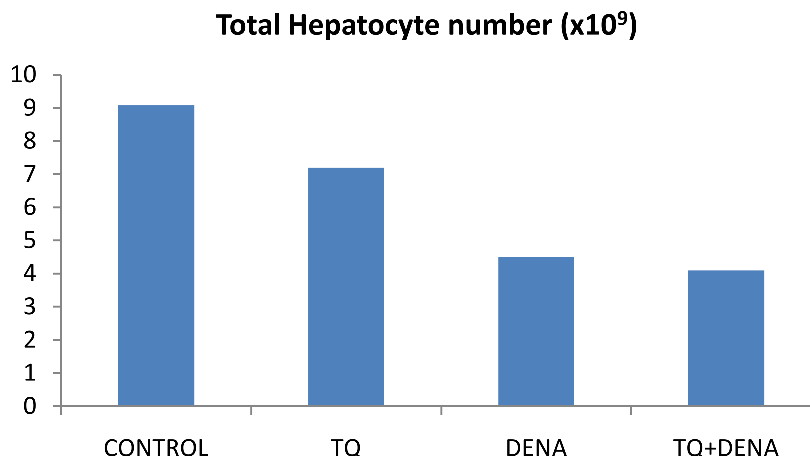


**Figure 2. Total Hepatocyte number ($x10^9$)**

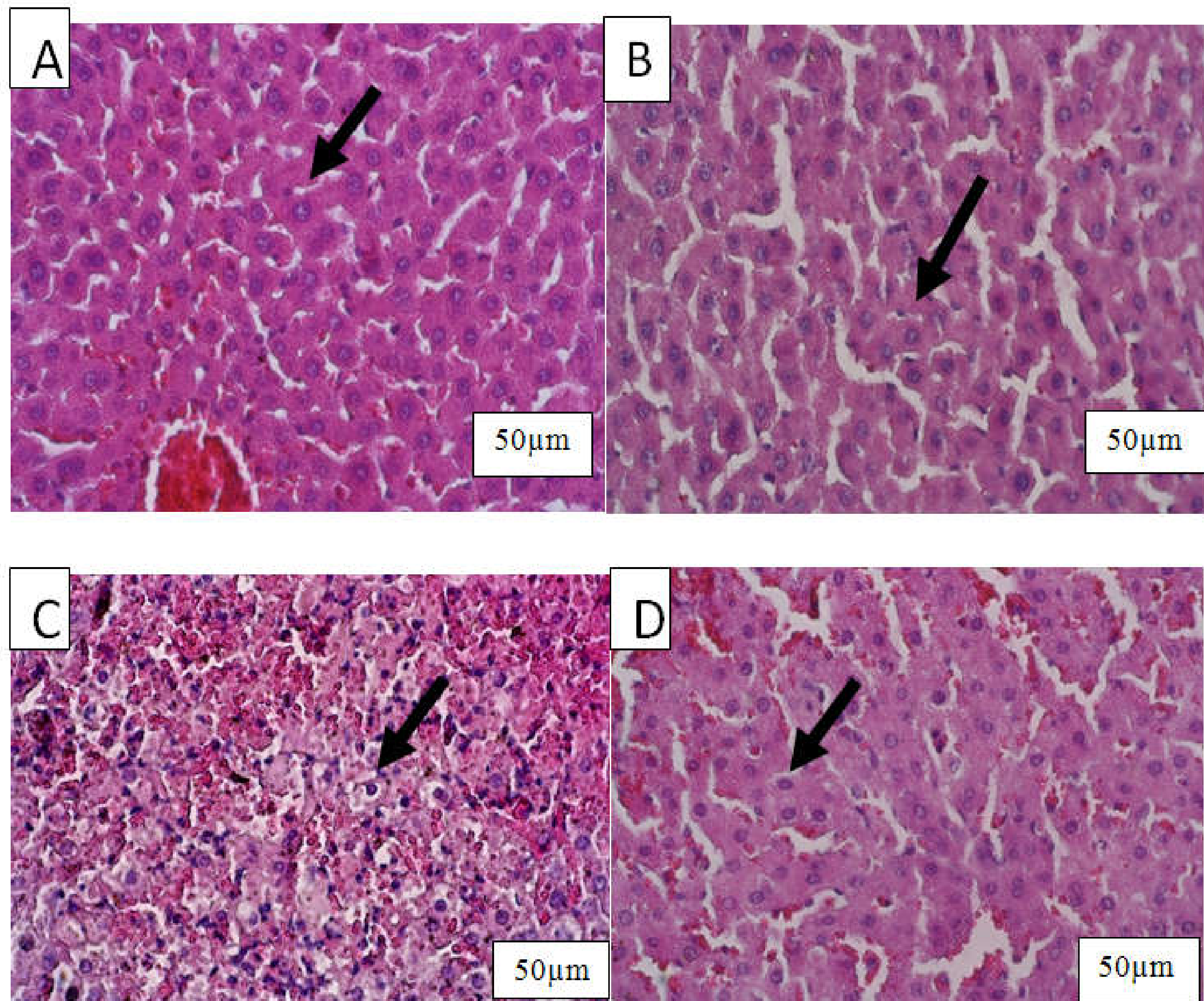


**Figure 3. Microscopicalsection of liver showing hepatocyte A; Control group, B; TQ group, C; DENA group, D; TQ+DENA group (Scale bars=50µm, H&E)**

## DISCUSSION AND CONCLUSION

According to Pradeep (2007a), DENA induced hepatotoxicity by increased serum AST, ALT, ALP and bilirubin levels and a simultaneous decrease in their levels in the liver tissue. Moreover, DENA induced oxidative stress by increased lipid peroxidation (LPO) and by decline in the activities of CAT, superoxide dismutase (SOD), glutathione peroxidase, catalase, glutathione reductase (GR) and Glutathione-S-transferase (GST) (Pradeep *et al.,* 2007a; Pradeep *et al.,* 2007b). Diethylnitrosamine (DENA) is induceoxidative stress and hepatic injury (Pradeep *et al.,* 2007; Shaban *et al.,* 2013). Diethylnitrosamine (DENA) is a general hepatocarcinogenic agent available in tobacco smoke, water, cured and fried meals, cheddar cheese, smoked, salted and dried fish, cure meat, agriculture chemicals and cosmetics and pharmaceutical products (Rezaie *et al.,* 2014). Thymoquinone could protect the liver against hepatotoxicity (Mansour *et al.,* 2001). The *Nigella sativa* has a hepatoprotection with antioxidant and anti-inflammatory characteristics against paracetamol toxicity (Yesmin *et al.,* 2013). TQ can reduce liver damage against chronic cyclosporine, which resets the oxidant/antioxidant balance of affected organs through scavenging free radicals and antilipoperoxidative effects (Farag *et al.,* 2015). In the present study, Diethylnitrosamine caused liver damage, and decreased hepatocyte number comparing to control group. In the thymoquinone+ diethylnitrosamine group, hepatocyte number were significantly decreased comparing to the control group, while hepatocyte number were increased comparing to diethylnitrosamine injected rats. Finally, diethylnitrosamine caused liver damage and thymoquinoneprobably may have induced apoptosis in the damaged hepatocytes.

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