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Thymoquinone reverses nonalcoholic fatty liver disease (NAFLD) associated with experimental hypothyroidism

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Abstract

Objectives: To assess the efficacy of thymoquinone (TQ), the most active constituent in *Nigella sativa*, which is a medicinal plant from the *Ranunculaceae* family, in restoring the normal liver structure after 6-propyl-2-thiouracil (PTU)-induced hypothyroidism and explore the mechanism behind this. *Materials and Methods*: Hypothyroidism was induced in rats by injection of PTU [6 mg/kg body weight (b.w.)] for six weeks. Twenty-four adult male Wistar rats were divided into four groups; the control, TQ-treated at the dose 400 mg/kg b.w., untreated hypothyroidism and TQ-treated hypothyroid groups. Serum levels of thyroid hormones and antioxidant profile were measured. Real-time polymerase chain reaction was used to assess gene expression of catalase (*CAT*). Liver was histopathologically examined using routine and immunohistochemical techniques. *Results*: Livers of rats with hypothyroidism displayed nonalcoholic fatty liver disease (NAFLD) in the form of steatosis as well as nonalcoholic steatohepatitis (NASH). Moreover, there was an intralobular inflammatory reaction associated with significant (p<0.05) increases in the density of resident hepatic macrophages [cluster of differentiation 68 (CD68)$^+$ cells], as well as in activated hepatic stellate cells, alpha-smooth muscle actin (α-SMA) index in livers with hypothyroidism. Resolution of hypothyroid NAFLD was observed in livers after treatment with TQ. The significantly increased (p<0.05) steatosis, lobular inflammation, NAFLD activity scores, α-SMA index as well as CD68$^+$ cells induced by hypothyroidism were corrected after TQ administration. Up-regulation of the CAT gene in livers with hypothyroidism after treatment with TQ supported our hypothesis of its antioxidant mechanistic hepatoprotective action. *Conclusions*: TQ efficiently restores the normal liver histology in hypothyroid rats with up-regulation of the antioxidant *CAT* gene.

Keywords: thymoquinone, hypothyroidism, 6-propyl-2-thiouracil, antioxidants, catalase, rat.

Introduction

Hypothyroidism is a common health problem worldwide. In Arabic countries, the incidence of hypothyroidism was reported to increase in the last decade. In a recent study conducted in Saudi Arabia, one of the Arabic Gulf countries, congenital hypothyroidism was the most frequently noticed disorders among the newborn with an incidence of 1:7175 [1].

Among the "thyroid-disrupting chemicals" that was frequently investigated for their effect on the health is 6-propyl-2-thiouracil (PTU), an anti-thyroid drug that was utilized in treating hyperthyroidism and Graves' disease [2]. In addition, it is frequently used in induction of an animal model of hypothyroidism in order to explore the effect induced by hypothyroidism on the different tissues, as well as to test the efficacy of different treatments on this model [3]. Hepatotoxicity was among the injurious effects induced by PTU as was previous described [4]. Until now, there is no specific therapeutic agent protect against such PTU-induced hepatic injury.

Liver is the key organ involved in the metabolism, enormous amount of hydrogen peroxide, one of the reactive oxygen species (ROS), was continuously produced during the different metabolic processes [4]. When produced in large among, hydrogen peroxide induces oxidation of the cellular components, with subsequent oxidative stress [5]. Liver has a potent defense mechanism for protection against the harmful effect of hydrogen peroxide exerted by catalase (CAT) enzyme that breakdown hydrogen peroxide into water and oxygen [4].

Nigella sativa (NG), a medicinal plant from the *Ranunculaceae* family, was reported to possess significant beneficial effects on the health, including antioxidant, anti-inflammatory and immunomodulatory properties, with no reported side effects [6]. Although the studies conducted to investigate the effect of NG on treating or improving thyroid disorders were scarce, it was found that one experimental study revealed a potent protective effect of NG on the thyroid cell, with subsequent relieve of the hypothyroidism-induced oxidative stress following PTU administration [7].

In a more recent randomized clinical trial, NG was reported to have a potent beneficial effect in improving thyroid function and anthropometric variables in patients suffering from Hashimoto's thyroiditis, the most common cause of hypothyroidism [8].

The majority of the pharmacological properties and therapeutic effects of NG is due to its main constituent, thymoquinone (TQ) [9]. These include antioxidant, anti-inflammatory, immunomodulatory, antihistaminic, anti-microbial and anti-tumor effects that impose a protective function for almost all organs [10].

In this study, we hypothesized that PTU-induced hypothyroidism is associated with hepatic injury that might be caused by defect in hepatic oxidative stress and so the use of TQ can alleviate this effect on the liver tissue. Additionally, the mechanism behind TQ-induced effects was explored regarding oxidative stress and CAT pathway.

Materials and Methods

The study design was reviewed and permitted by the Biomedical Research Ethics Committee at the Faculty of Medicine, King Abdul Aziz University, Jeddah, Saudi Arabia. The Guidelines of the Care and Use of Laboratory Animals set at the King Fahd Medical Research Centre (KFMRC) were followed during the experiment conduction.

Experimental design

Twenty-four adult male Wistar rats (180–200 g) purchased from the animal house at KFMRC were kept to acclimatize for two weeks in the standard laboratory conditions. The rats were randomly divided into four groups (n=6). Rats of the control group received 1 mL of dimethyl sulfoxide (DMSO) saline through the intragastric intubation (IGI) for six weeks. The positive control group (TQ) was given thymoquinone at the dose 400 mg/kg body weight (b.w.) through IGI for six weeks. TQ extracted from NG was purchased from Frinton Laboratories, Inc. and dissolved in DMSO (at the 1:100 dilution). Hypothyroidism was induced in the other two groups by administration of PTU (6 mg/kg b.w.) for six weeks through IGI as was described by Villar *et al.* [11]. PTU was purchased from Sigma Aldrich, Inc. (St. Louis, Missouri, USA).

After two weeks of PTU administration, the hypothyroid state was confirmed in the rats by assessing the triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH) levels in the serum. Rats were then further divided into two groups; hypothyroid continued on PTU alone for another four weeks and hypothyroid treated with TQ (while they were continued on PTU) for four weeks [12]. Rats were fed the standard diet and water during the whole experiment.

Histological and immunohistochemical techniques

At the end of the 6th week, rats were lightly anesthetized using diethyl ether and decapitated. The chest wall was opened and blood was obtained rapidly from the heart. The liver was immediately and gently dissected out and fixed in 10% neutral buffered formalin to be further processed for obtaining of paraffin blocks. Paraffin sections at 4-μm thickness were prepared and stained with Hematoxylin and Eosin (HE). In addition, another set of paraffin sections at same thickness was immunohistochemically stained using the Streptavidin–Biotin–Peroxidase technique.

The histopathological features observed in the liver were semi-quantitatively assessed in accordance with the NAFLD activity score (NAS) [13]. A total of five fields (at the ×40 magnification) per slide, from each rat were examined using Image-Pro Plus analysis software (version 6.0) (Media Cybernetics, Inc., Rockville, MD, USA).

Immunohistochemical (IHC) staining for alpha-smooth muscle actin (α-SMA) was performed to detect presence of collagen IV. Presence of collagen IV beneath the endothelium of the hepatic sinusoids provides an IHC marker of basement membrane formation indicating a significant pathology found in advanced fibrosis [14].

Anti-α-SMA antibody (Biocare Medical, Pacheco, USA, at 1/100 dilution), and anti-cluster of differentiation 68 (CD68) antibody (Biocare Medical, Pacheco, USA, at 1/100 dilution) were utilized in this study. The primary antibody was omitted while the secondary antibody immunoglobulin G (IgG) was added during staining of some slides to act as negative control ones. The nuclei were counterstained with Hematoxylin. Brown cytoplasmic staining was considered positive reaction in anti-α-SMA and anti-CD68 antibodies. Olympus BX-51 microscope (Olympus) connected to a digital camera and a computer was used for photographing. Image-Pro Plus software was used for semiquantitative analysis of antibody immunoreactivity. The area percentage of α-SMA expression was assessed in 30 fields using a ×40 objective lens and ×10 ocular lens and used as an indicator of the extension of the reaction [15]. The α-SMA index was calculated according to Namisaki *et al.* [16].

The number of $CD68^+$ cells was counted in 0.125 mm^2 of the hepatic tissue, at a magnification of ×400, in serial sections, according to the method described by McGuinness *et al.* [17]. At least five fields from each slide were examined and the mean was calculated. In order to verify the counting, this process was done by two independent histologists.

Biochemical techniques

Blood samples were obtained for biochemical assessment from the intra-orbital sinus during the experiment and from the heart at the end of the experiment [18]. Centrifugation was performed at 3000 rpm for 15 minutes, at 4°C, to obtain the serum from the blood samples and was kept at -18°C. Biochemical assessment was performed at El-Safwa Laboratory, Tanta, Egypt.

Serum levels of T3, T4, and TSH were measured using ADVIA Centaur automated competitive chemiluminescence immunoassay Bayer HealthCare (Kanawha River, Western West Virginia, USA). Levels of reduced glutathione (GSH), nitric oxide (NO) and malondialdehyde (MDA) were assessed in the plasma using kits purchased from Biodiagnostic (Giza, Egypt). Plasma levels of superoxide dismutase (SOD), CAT, glutathione peroxidase (GPX) activities were also assessed using kits provided by Biodiagnostic.

Gene expression of CAT

Ribonucleic acid (RNA) was extracted from 100 mg of formalin-fixed paraffin-embedded (FFPE) section; deparaffinization was performed with 1 mL of xylene, incubated at 56°C for 15 minutes, centrifuged for 10 minutes at 13 000 g. The supernatant was discarded and the pellet washed twice with 1 mL 100% ethanol,

centrifuged, the supernatant was discarded, and 1 mL Trizol was added to the pellet [19]. Extraction of total RNA using Trizol was done according to the supplier instruction (Invitrogen Life Technologies, Carlsbad, CA, USA). Genomic deoxyribonucleic acid (DNA) contamination was removed from RNA samples in the presence of ribonuclease inhibitor. NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA) was used to ensure purity of extracted RNA.

Complementary DNA (cDNA) was synthesized as described by Bunker *et al.* [20], using the RevertAid First Strand cDNA Synthesis Kit. Total RNA was reverse transcribed to single stranded cDNA (ss-cDNA) in 20 µL reaction volume containing 2.5 µg total RNA, 1 mM deoxynucleoside triphosphates (dNTPs), 200 ng random hexamer, 20 U ribonuclease inhibitor, and 40 U of M-MuLV reverse transcriptase, at 37°C, for one hour, followed by 70°C for 10 minutes. The synthesized cDNA was stored at -20°C until use for reverse transcription–polymerase chain reaction (RT-PCR).

The RT-PCR assay was performed on real-time PCR system (Applied Biosystems, 7500, USA), 2X SYBR Green PCR Master Mix (Applied Biosystem, USA). The primer sequences for *CAT* gene were forward GAAT GGCTATGGCTCACACA, backward CAAGTTTTTGA TGCCCTGGT. The housekeeping gene – glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) – was used as a control for normalization with primer sequence forward ATGGAGAAGGCTGGGGCTCACCT and backward AGCCCTTCCACGATGCCAAAGTTGT. Optimization of the primer concentration was performed to determine the primer concentration that give the lowest cycle threshold (CT) and to minimize the non-specific amplification in real time RT-PCR reactions.

The assay was performed as defined by Bunker *et al.* [20], in a 25 µL reaction combination consisting of 12.5 µL 2X SYBR green PCR master mix, 2 µL cDNA, 10 pmol of both forward and reverse primer. After optimization, the thermal cycling was performed as adheres to: initial hold at 50°C for 2 minutes, initial denaturation at 95°C for 10 minutes, followed by 40 amplification cycles (95°C 15 seconds and also 60°C for 1 minute) and ultimately an added action (60°C for 15 seconds, 95°C for 15 seconds, as well as 37°C for 2 minutes). A negative control response without any template was run with each assay. The PCR response was duplicated in three reproduces in six animals.

The PCR reactions were kept track of by determining the strength of the fluorescence brought on by SYBR Green Dye intercalation to the double-stranded DNA (dsDNA), melting curve evaluation was done (Figure 1, a and b) to verify the specificity of the products.

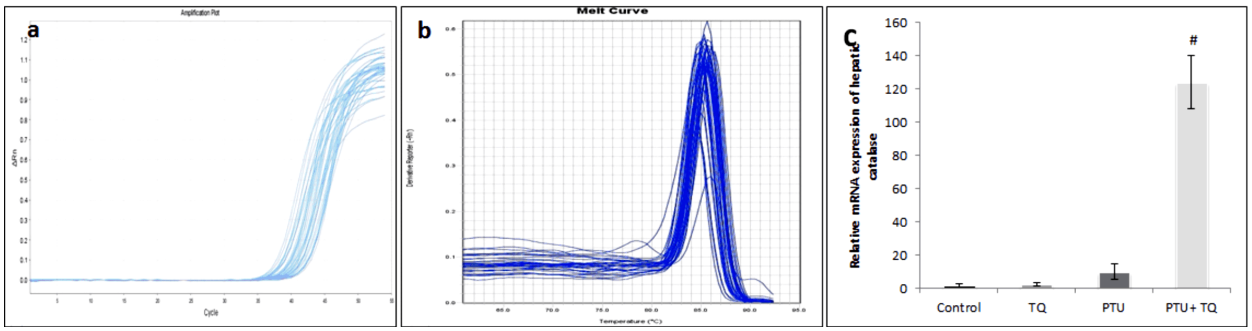

Figure 1 – ***CAT gene real-time PCR amplification plot (a), melting curve (b), (c) hepatic expression. Data was presented as mean and standard deviation (n=6 rats).*** $^{\#}$***Significance*** **versus** ***the hypothyroid group. Significance is considered at p<0.05. CAT: Catalase; PCR: Polymerase chain reaction; mRNA: Messenger ribonucleic acid; TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil.***

The CT values were figured out after that relative quantification using –ΔCT method with reference gene; The calculations differences between CT value for *GAPDH* (endogenous reference gene) and *CAT* gene for every sample was computed then the ratio of relative expression was determined.

Statistical analysis

The data of this study were analyzed using the Statistical Package for the Social Sciences (SPSS, version 16; SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean ± standard deviation (SD). For parametric data, the different groups were compared using Analysis of Variance (ANOVA) (*f* test), followed by a Bonferroni *post-hoc* test to avoid a multiple comparison effect. For nonparametric data, Kruskal–Wallis ANOVA followed by a *post-hoc* test (based on the Dunn procedure) was used to compare between each pair of groups. Statistical significance was accepted at *p*-value <0.05.

Results

Effect of TQ on serum level of thyroid hormones and TSH

A significant reduction in the serum levels of T3 and T4 was recorded in rats given PTU for six weeks, compared to those of the control rats. On the other hand, TSH level was significantly increased in rats given PTU, compared to the control rats. It was found that administration of TQ to rats could induce a significant increase in the levels of T3 and T4, as well as a significant reduction in the level of TSH, compared to those of the untreated group (Figure 2, a–c).

Effect of TQ on plasma MDA, NO, GSH in the studied groups

Rats given PTU for six weeks showed a significant (p<0.001) elevation in the levels of MDA and NO, while GSH level showed a non-significant reduction, compared to those of the control rat. Levels of NO and GSH were

significantly increased, while that of MDA was significantly decreased after administration of TQ along with PTU, compared to those of the hypothyroid rats (Table 1).

Table 1 – ***Levels of malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) in the studied groups***

Variables	Groups			
	Control	*TQ*	*PTU*	*PTU+TQ*
MDA [µmol/L]	6.6±0.4	2.2±0.3 *P1*<0.001	19.6±0.3 *P2*<0.001	13.6±0.5 *P3*<0.001
GSH [mmol/L]	0.41±0.08	0.33±0.05 *P1*=0.09	0.32±0.15 *P2*=0.32	0.53±0.15 *P3*=0.04
NO [µmol/L]	26.3±2.2	35.4±10.9 *P1*=0.07	48.6±6.3 *P2*<0.001	59.4±9.2 *P3*=0.03

TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil. Data are presented as mean ± standard deviation of the mean (SDM) of six rats. *P1*: *p*-value *versus* the control group; *P2*: *p*-value *versus* the control group; *P3*: *p*-value *versus* the PTU group; Significance is considered at $p<0.05$.

Effect of TQ on enzymatic antioxidant activities

The hypothyroid state produced after administration of PTU was found to be associated with a significant ($p<0.001$) reduction in SOD activity, while this activity significantly ($p=0.04$) elevated after the rats received TQ. On the other hand, plasma CAT activity significantly ($p<0.001$) elevated in PTU-induced hypothyroidism, while it showed a non-significantly increase in hypothyroid rats treated with TQ, compared to that of the hypothyroid rats. The level of GPX did not show any significant changes in any of the studied groups (Figure 2, d–f).

Hepatic *CAT* expression

The level of hepatic *CAT* transcription was upregulated by five-fold in the rats treated with PTU, however, its transcription showed a higher upregulation by 64-fold in rats treated with TQ after two weeks of PTU administration, compared to control group (Table 2). Although the mean expression of hepatic *CAT* gene was non-significantly ($p=0.9$, $p=0.11$) increased in TQ- and PTU-treated groups compared to the control, respectively, it was significantly increased ($p<0.001$) in hypothyroid rats treated with TQ, compared to PTU-treated group (Figure 1c).

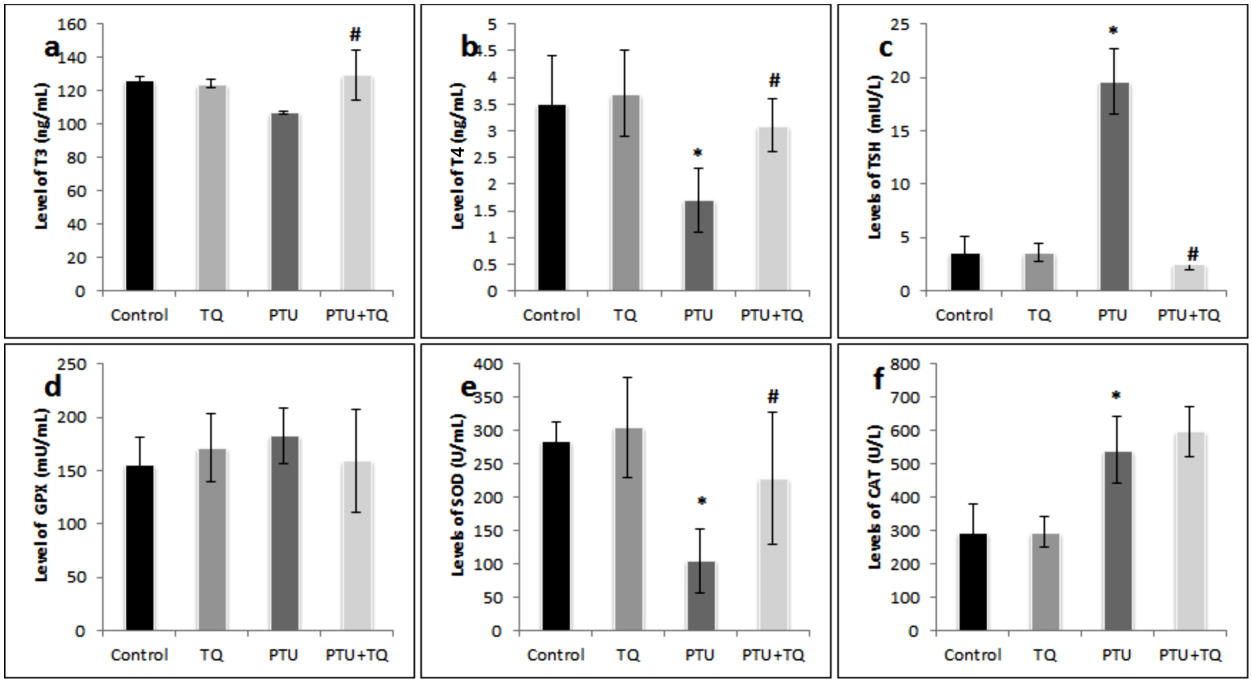

Figure 2 – ***Effect of TQ on serum level of thyroid hormones (a and b), TSH (c) and enzymatic antioxidant activities (d–f). TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil; T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid-stimulating hormone; GPX: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase.***

Table 2 – ***Hepatic CAT gene expression***

Groups	Mean 2^-ΔCT	ΔΔCq expression (Fold)
Control	1.94	Reference
TQ	2.41	1.24
PTU	10.19	5.26
PTU+TQ	123.88	63.97

CAT: Catalase; TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil; CT: Cycle threshold; Cq: Constant query expression.

Results of histopathology and immunohistochemistry

By HE staining, although their general architecture was not severely affected, livers of the PTU group showed fatty degeneration in the form of steatosis that included microvesicles and macrovesicles that were frequently and diffusely seen within the hepatocytes, when compared to those of the control (Figure 3, a–c). In addition, nonalcoholic steatohepatitis (NASH) was observed in the form of the inflammatory reaction that was located mainly intralobular alongside the classical steatotic lesion. Resolution of both steatosis and steatohepatitis was observed in livers treated with PTU+TQ (Figure 3d), which appeared similar to those of the control. Moreover, the significantly increased ($p<0.05$) steatosis, lobular inflammation and NAFLD activity scores in livers with hypothyroidism were all come down in the PTU+TQ treated groups (Figure 3, e–g). As expected, the histology of livers of TQ group was nearly identical to that of the control.

A minimal α-SMA reaction in livers of control group was observed (Figure 4a), whereas livers of PTU showed intense and significant increase ($p<0.05$) in this reaction mainly in portal region (Figure 4, c and e) together with areas of focal inflammatory response located intralobular in the vicinity of the obvious diffuse steatosis (Figure 4b). Interestingly, administrating TQ after PTU (Figure 4, d and e) restored α-SMA index to the control level.

In order to evaluate the density of resident hepatic macrophages in of various groups, we immunohistochemically stained liver sections of various group with anti-CD68 antibody. Obvious increase in the number of $CD68^+$ cells was demarcated in the PTU group both in lobular (Figure 5b) as well as in portal (Figure 5c) regions. On the other hand, in the PTU+TQ group, the $CD68^+$ cells (Figure 5d) were scarcely seen, similar to those in the control group (Figure 5a). These morphological data were statistically validated demonstrating a significant increase ($p<0.05$) in the intralobular as well as portal $CD68^+$ cells, compared to those of the control and PTU+TQ groups (Figure 5, e and f).

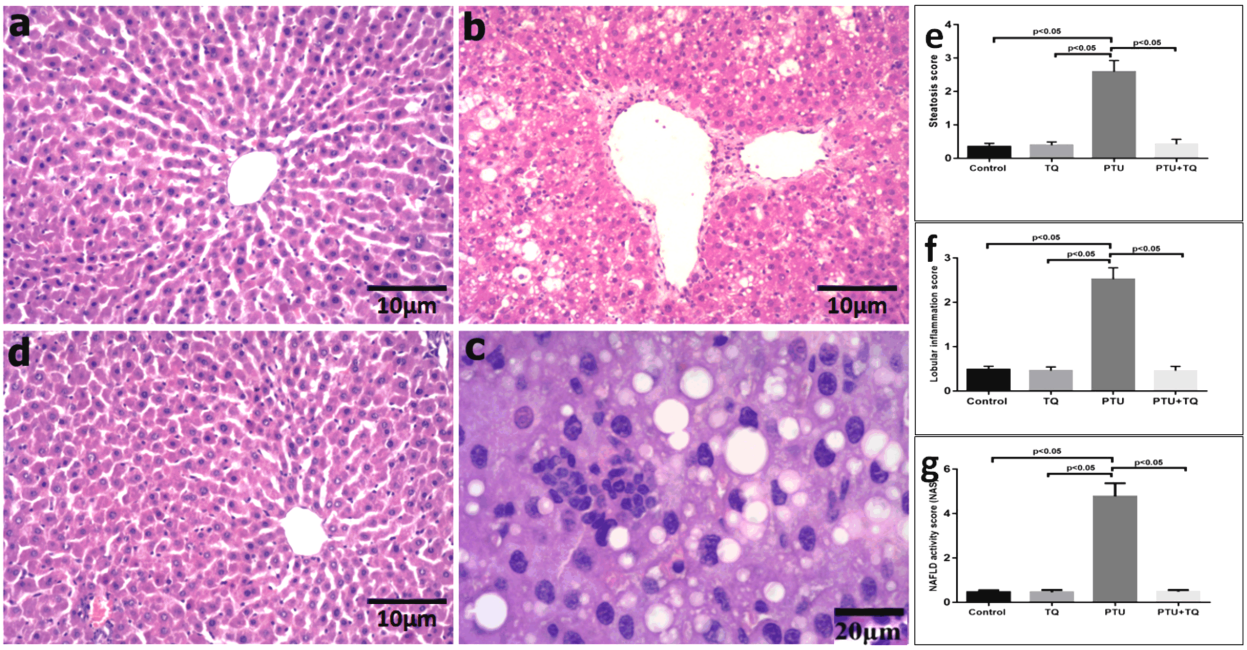

Figure 3 – ***Representative photomicrographs of HE staining in various groups. Fatty degeneration was the main histopathological manifestation seen in the PTU livers (b), along with occasional focal inflammatory reaction (c). Complete clearance of steatosis and steatohepatitis is observed in livers treated with PTU+TQ (d), which appear similar to those of the control (a). The statistical validation of the morphological data is presented on the right panel (e–g). HE staining: (a, b and d) ×200; (c) ×400. Scale bars: (a, b and d) 10 µm; (c) 20 µm. HE: Hematoxylin and Eosin; TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil; NAFLD: Non-alcoholic fatty liver disease.***

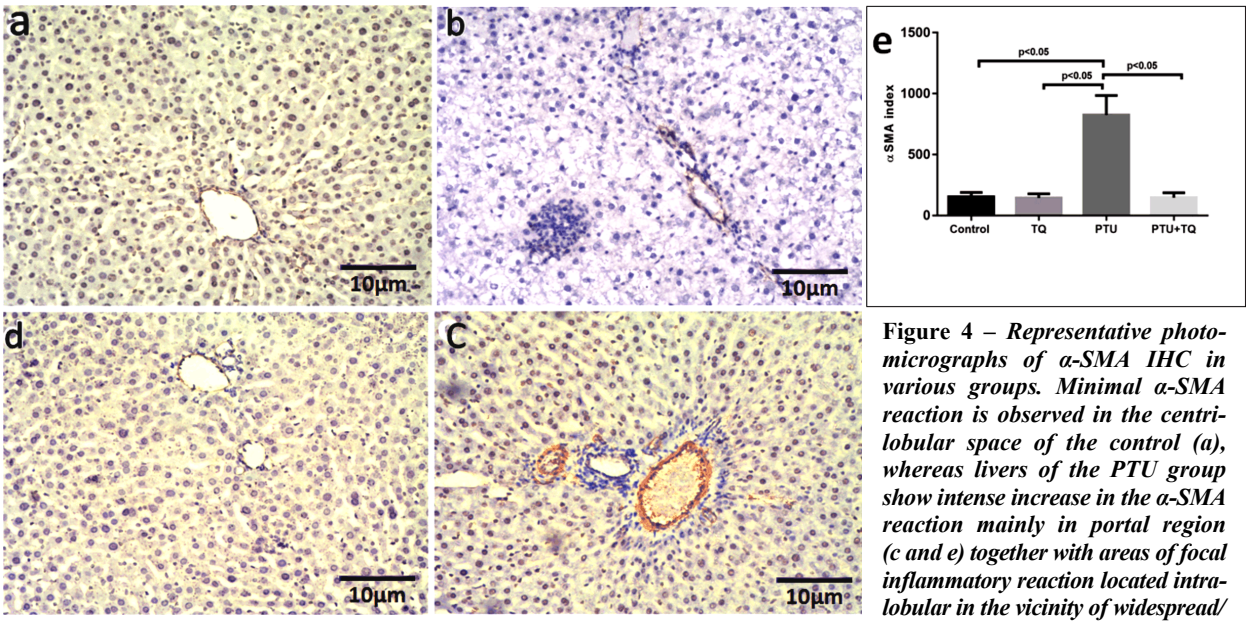

Figure 4 – ***Representative photomicrographs of α-SMA IHC in various groups. Minimal α-SMA reaction is observed in the centrilobular space of the control (a), whereas livers of the PTU group show intense increase in the α-SMA reaction mainly in portal region (c and e) together with areas of focal inflammatory reaction located intralobular in the vicinity of widespread/diffuse fatty degeneration of hepatocytes (b). On the other hand, administrating TQ after PTU decreases the α-SMA reaction (d and e). Anti-α-SMA antibody immunomarking: (a–d) ×200. Scale bar: 10 µm. α-SMA: Alpha-smooth muscle actin; IHC: Immunohistochemistry; TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil.***

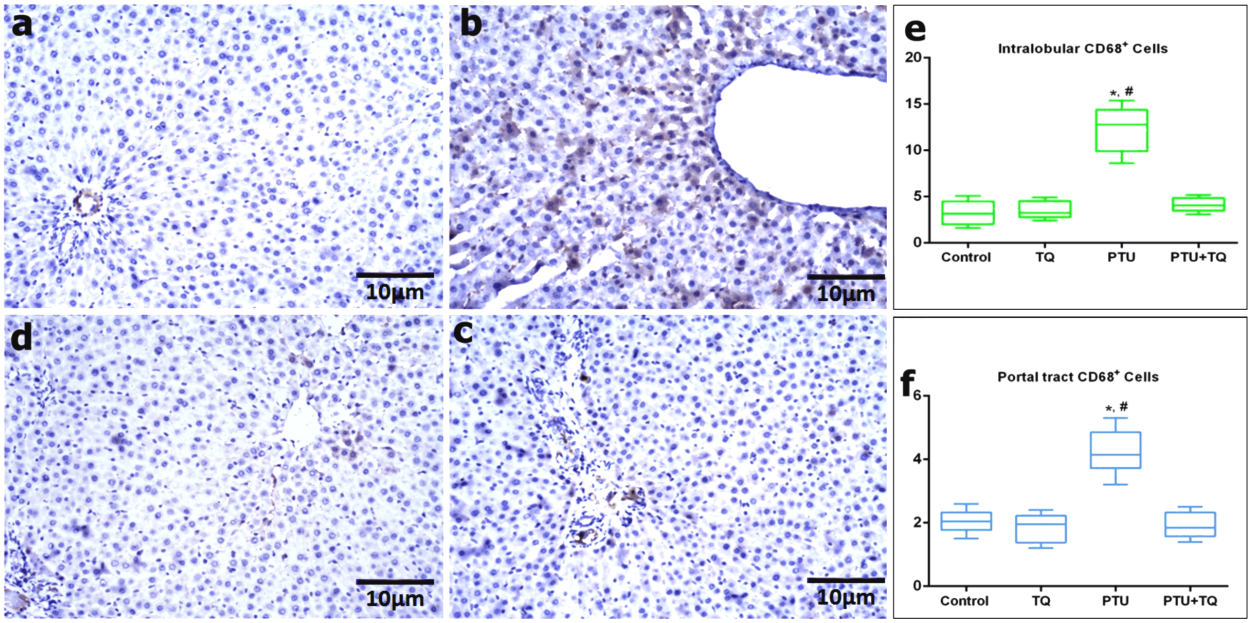

Figure 5 – ***Representative photomicrographs of CD68 IHC in various groups. Obvious increase in the number of CD68$^+$ cells is demarcated in the PTU group both at lobular (b and e) as well as portal (c and f) levels. On the other hand, for the PTU+TQ group, the CD68$^+$ cells (d) were scarcely seen similar to those in the control group (a). *Significant* versus *the control, $^{\#}$significant* versus *the PTU+TQ group. Anti-CD68 antibody immunomarking: (a–d) ×200. Scale bar: 10 µm. CD68: Cluster of differentiation 68; IHC: Immunohistochemistry; TQ: Thymoquinone; PTU: 6-Propyl-2-thiouracil.***

Discussions

NAFLD is one of the most common causes of chronic liver disease from which millions of people suffer [21]. It comprises of a wide spectrum of lesions with their mainstay pathology is lipid deposition inside the hepatocytes [22]. In the current study, the general architecture of livers with hypothyroidism had not been much affected; nonetheless, they displayed NAFLD in the form of steatosis, as well as NASH adding to the intralobular inflammatory reaction together with the classical steatotic lesions. Although previous studies suggest that hypothyroidism may play a crucial role in the development of NAFLD, others proposed that this role is debatable. Some studies displayed that no significant association between NAFLD and hypothyroidism [23]. On the other hand, most of previous researches showed that hypothyroidism, even subclinical, is related to NAFLD in a dose-dependent manner [24]. Chung *et al.* added that hypothyroidism is independently associated with NAFLD regardless the common metabolic risk factors [25].

α-SMA IHC staining was used to detect activated hepatic stellate cells (HSCs) [26]. In addition, the presence of collagen IV beneath the sinusoidal endothelium provides an IHC marker of basement membrane formation, which is an integral feature of capillarization of sinusoids that is a significant pathology found in advanced fibrosis [14]. We believe that activated HSCs are a key player in NAFLD induced by hypothyroidism. This was supported by our finding of the intense α-SMA reaction and significant increase in its index in livers with hypothyroidism.

On contrary, previous *in vitro* studies using the rat HSCs, showed that T3 upregulates transforming growth factor-beta-induced collagen I gene expression, and suppresses metalloproteinase-2 secretion, which may indicate that low T3 levels can support resolution of fibrosis [27].

In the current study, we demonstrated an obvious increase in the number of CD68$^+$ cells in livers with hypothyroidism, which may indicate a key role played by resident hepatic macrophages in steatosis and NASH induced by hypothyroidism. Hepatic macrophages are conventionally nominated Kupffer cells, in order to distinguish the tissue-resident macrophages from infiltrating cells [28]. Although there is no single marker that can definitely distinguish these cells, CD68 has been proposed as a marker, yet non-exclusive, for human Kupffer cells [29, 30].

Liver injury activates Kupffer cell, leading to pro-inflammatory cytokine release, infiltration of monocytes and hepatocyte injury, with subsequent activation of HSCs and fibrogenesis [31]. Although most of them in normal and diseased liver are CD68$^+$ [28], liver macrophages can be divided into two subsets: CD68$^+$ embryonically derived Kupffer cells and bone marrow-derived cells, which exhibit specific cell functions [16]. McGuinness *et al.* demonstrated that CD68$^+$ cells can be both expressed in the portal and lobular regions of normal and diseased livers that is positively correlated with messenger RNA (mRNA) of proinflammatory cytokines [30]. However, our data indicated that in NASH associated with hypothyroidism, the CD68$^+$ cells were located preferentially intralobular. In the current study, resolution of hypothyroid hepatic steatosis and NASH was observed in livers after treatment with TQ. The significantly increased steatosis, lobular inflammation and NAFLD activity scores induced by hypothyroidism were corrected after TQ administration. Interestingly, administrating TQ after induction of hypothyroidism restored α-SMA reaction to the control level. Favorably, these CD68$^+$ cells were scarcely seen after TQ treatment in hypothyroid livers that displayed a comparable density to that detected in the control group.

Liver continuously produces enormous amount of ROS, especially hydrogen peroxide that subsequently induce oxidative stress [5], this is evidenced in our study by increased plasma activity of MDA in PTU-induced hypothyroidism rats with subsequent decease in its level with TQ administration. These findings are supported by Yilmaz *et al.* [32], who reported an increase in plasma activity of hepatic, muscular and cardiac MDA level in hypothyroid rats. Our findings are also in agreement with the previous studied of Bhanja & Chainy [33] and Sahoo *et al.* [34], who displayed a hepatotoxic effect of PTU and in the same time a hepatoprotective one of TQ.

CAT is one of the hepatoprotective mechanisms against the harmful effect of hydrogen peroxide. It is evidenced that CAT is regulated by thyroid hormones Subudhi & Chainy [35]. The effect of PTU on CAT gene expression was contradictory; we found increased activity of CAT gene in PTU-induced hypothyroid rats. Our finding is in great agreement with Chattopadhyay *et al.* [36] study, who reported an increased activity of CAT in the heart of hypothyroid rat, which was suggested to be a reflex mechanism against increased hypothyroidism-induced oxidative stress. On the other hand, our finding is against the published work of Bunker *et al.* [20] that demonstrated a significant decrease in CAT gene expression by five-fold in rats treated with PTU for 90 days, this might be due to the longer experiment time (12 weeks compared to six weeks in our experiment). This study also demonstrated a significant upregulation of CAT gene expression in liver of the PTU rats treated with TQ, which is in a good agreement with Mollazadeh & Hosseinzadeh [37] and Tabassum *et al.* [38] works. These results may support our assumption that the antioxidant and radical scavenging effect of TQ can alleviate the oxidative stress mediated effect of hypothyroidism on the liver.

Conclusions

The current study provides a satisfactory evidence, at least at structural level, that experimental hypothyroidism is associated with NAFLD, which encompasses an inflammatory reaction promoted by interplay of the resident hepatic macrophages, as well as the activated HSCs. In addition, TQ efficiently restores the normal liver histology with upregulation of the antioxidant CAT gene. The next step would be further researches particularly at epigenetic/molecular level to unveil more mechanistic actions of the pathogenesis of NAFLD associated with hypothyroidism.

Conflict of interests

The authors have no conflict of interests.

Acknowledgments

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