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The Possible Ameliorative Mechanisms of Curcumin and/or Coenzyme Q10 Against Hyperthyroidism Induced Liver Damage in Rats

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ABSTRACT

This investigation was designed to study the ameliorative mechanisms of curcumin (CUR) and /or coenzyme Q10 (CO Q10) in comparison to the antithyroid drug, carbamizole, against hyperthyroidism induced liver damage. Hyperthyroidism was induced in rats through injection of L-thyroxine (LT4, 0.3mg/kg) subcutaneously for 12 consecutive days. CUR (100mg/Kg) and /or CO Q10 (50mg/Kg) or carbamizole (30mg/kg) were administered to hyperthyroidism rats for 21 consecutive days simultaneously with LT4 administration. The results revealed that oral intake of CUR and /or CO Q10 or carbamizole to hyperthyroidism rats, significantly reduced the serum levels of thyroxine (T4) and triiodothyronine (T3) and boosted the level of thyroid–stimulating hormone (TSH) with respect to hyperthyroidism rats. The used agents also reduced the hepatic increase in malondialdehyde (MDA) and increased the levels of catalase and glutathione (GSH) in hyperthyroidism rats. In addition, treatment with CUR and /or CO Q10 or carbamizole efficiently attenuated the hepatic DNA damage, the hepatic increase in caspase-3, the serum elevation in tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) as well as the alteration in serum liver function indices namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin in hyperthyroidism rats with respect to untreated ones. Liver histopathological and ultrastructural studies were also done to confirm the biochemical investigation.
Conclusion: The current work demonstrated that CUR and /or CO Q10 were effective in mitigating hyperthyroidism induced liver damage. Carbimazole was the least efficient in reversing liver damage, however the combination of CUR and CO Q10 was the beneficial one in restoring hyperthyroidism induced liver dysfunction.
Keywords: Curcumin, Coenzyme Q10, Hyperthyroidism, DNA Damage, Caspase-3

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INTRODUCTION

Research continues to develop safe new agents for treatment of human diseases, among these are medicinal drugs. Hyperthyroidism is a disease that has attracted various interventions towards its treatment. The disease results in diverse disarrangement, most of which are life threatening. This form of thyrotoxicosis is caused by thyroid gland abnormalities, resulting in massive thyroid hormone generation [1]. The disease leads to forth diverse complications, including alteration of the basal metabolic rate, osteoporosis and diabetes mellitus [2-4]. In addition, hyperthyroidism can cause oxidative damage and death of liver cells [5-6]. Evidence has documented that triiodothyronine (T3) acts directly on the mitochondria of the hepatocytes, resulting in their apoptosis [7]. Also, it has found that the increase in metabolism during hyperthyroidism is a hazard factor of increased oxidative stress in the liver, which is represented by increases in levels of free radicals and lipid peroxide [8]. Other findings have established that the ratio of prooxidants to antioxidants increases during hyperthyroidism, causing oxidation of many biomolecules and DNA damage [9]. Some authors also have observed that pro-inflammatory proteins (interleukin 6, tumor necrosis factor- α and C-reactive protein) have been increased during hyperthyroidism [10]. Anti-hydroid drugs are widely used in treatment of hyperthyroidism, however these drugs have reported to exhibit

many side effects, including agranulocytosis, cholestatic hepatitis and liver failure [11].

The use of natural products in the pharmaceutical industry has been growing, as they have several therapeutic benefits [12]. Kim et al [13] have pointed out that various medicinal plants, herbs and other crude drug substances have been used as anti-inflammatory agents or antioxidants, hence gaining popularity in the treatment of different diseases including hyperthyroidism.

Curcumin is a yellow colored polyphenol, extracted from Curcuma longa rhizomes. Curcumin exhibits a number of pharmacological benefits, including immunomodulatory, antioxidant, anti-inflammatory, antitumor, immunoprotective and hepatoprotective properties [14-16].

Coenzyme Q10 (CO Q10) is one of electron carrier components in mitochondrial electron transport chain [17]. CO Q10 is a powerful cellular antioxidant which can scavenge oxygen radicals and suppress lipid peroxidation of cellular membrane lipids [18]. Furthermore, CO Q10 has hepatoprotective and anti-inflammatory properties. It inhibits the generation of inflammatory mediators such as tumor necrosis factor-α [18-19]. Previous investigations have studied the prophylactic potential of CO Q10 in oxidative and inflammatory models of tissue injury [18, 20].

In view of the previous studies, the aim of the current investigation was to study the prophylactic mechanisms of curcumin and/or CO Q10 in comparison to the currently available drug, carbimazole, against hyperthyroidism induced rat liver damage as one of its complications.

MATERIALS AND METHODS

Chemicals

L-Thyroxine (T4), curcumin and coenzyme Q10 were bought from Sigma Chemicals Company, St. Louis, USA)

Animals

Sixty male Wistar rats (180-200g) were utilized in this research. The rats were sourced from King Fahad Medical Research Center, King Abdulaziz University. The rats were caged at 20-22°C and 60% humidity with 24 cycles of light and dark. The animals were given standard chow and tap water ad libitum and left for seven days to acclimatize before the experimental duration. The handling of the rats was done according to the guide supplied by the Animal Care and Use Committee of the King Abdulaziz University, Faculty of Science.

Induction of Hyperthyroidism

Hyperthyroidism in the rats was induced by daily subcutaneous injection of L-thyroxine 4 (LT4, 0.3 mg/kg) for twelve days [4].

Experimental Design

Rats were classified into five groups (n=10) as follows:

Group 1: Control rats.

Group 2: Hyperthyroidism animals.

Group 3: Hyperthyroidism rats were treated orally with CUR (100 mg/ kg) [21] daily for 21 days simultaneously with LT4 injection.

Group 4: Hyperthyroidism rats were treated orally with CO Q10 (50 mg/kg) [18] daily for 21 days simultaneously with LT4 injection.

Group 5: Hyperthyroidism rats were treated orally with both CUR and CO Q10 for 21 consecutive days simultaneously with LT4 injection

Group 6: Hyperthyroidism rats were treated orally with carbamizole (30 mg/kg) [22] for 21 consecutive days simultaneously with LT4 injection

After 21 days, the rats were starved for about 14 hours, after which blood specimens were gathered from all rat groups, and placed in sterilized tubes for clotting and serum isolation for biochemical analysis. Rats were then dissected under anesthesia and the liver specimens were harvested for biochemical estimation of different parameters.

Biochemical serum analysis:

Estimation of hyperthyroidism and liver damage biomarkers:

The thyroid functional indices namely T3 and T4 and TSH as well as the liver functional tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and albumin were estimated using automated biochemical analyzer (ci16200, Abbott, USA).

Determination of serum immuno-inflammatory mediators

C-reactive Protein was evaluated with latex-enhanced immunonephelometry placed on Behring BN II Nephelometer (Dade Behring). Inflammatory cytokines, IL-6 and TNF-α, concentrations were estimated utilizing enzyme-linked immunosorbent assay (ELISA) kits following the instruction of manufacturer. Vascular endothelial growth factor (VEGF) was measured by ELISA assay kit (R&D Systems, UK)

in accordance to the instruction of manufacturer.

Determination of oxidative stress and antioxidant biomarkers in liver:
Malondialdehyde (MDA) as an index of membrane lipid peroxides was estimated utilizing thiobarbituric acid reagent [23]. Reduced glutathione (GSH) as antioxidant index was evaluated by the method adopted by Bentler et al [24]. Catalase (CAT) activity was estimated through an experiment involving the decomposition of hydrogen peroxide [25].

Determination of liver caspase 3 activity
Caspase 3 activity was measured as an apoptosis index, utilizing the method developed by Vaculova and Zhivotovsky [26].

Determination of DNA fragmentation by comet assay
The extent of DNA fragmentation was carried out by single-cell gel electrophoresis (comet assay) for individual cells [27]. The DNA fragmentation was estimated by measuring the percent tail DNA (% tail DNA) and olive tail movement utilizing the software.

Histopathological and ultrastructural studies
Small portions of live were fixed in 10 % formaldehyde and then immersed into paraffin. The liver specimen was sectioned (5–6-µm) and stained with hematoxylin-eosin for light microscope observation. Ultrastructural alterations in the liver tissue were measured by Transmission Electron Microscope (TEM). Liver tissue specimens were post-fixed in 1 % osmium tetaroxide (OsO4) and dehydrated by ascending grades of ethanol. The specimens were then Ultrathin sectioned (50–60 nm) by an ultramicrotome and stained with uranyl acetate and lead citrate. Sections were examined utilizing TEM (JEOLJEM-1011).

Statistical Analysis:
The data of this work were calculated as the mean ± SD. Significant variations between mean values were carried out utilizing one-way analysis of variance (ANOVA) and Bonferroni as a post-ANOVA test. The variations between data were statistically significant at $p \leq 0.05$.

RESULTS

Levels of serum hyperthyroidism indices
The effects of CUR and /or CO Q10 in comparison to carbimazole on the levels of serum thyroid function markers are shown in Table 1. The results revealed that rats treated with LT4 showed high serum levels of T4 and T3 with a concomitant reduction in TSH level compared to control rats ($P \leq 0.001$). Treatment with CUR and /or CO Q10 or carbimazole significantly modulated the alterations in the thyroid hormones with respect to hyperthyroidism untreated rats ($P \leq 0.001$). Carbimazole as well as the combination of CUR and CO Q10 were more effective in reversing the levels of these hormones.

Levels of liver oxidative stress markers
The effects of CUR and /or CO Q10 or carbimazole on the levels of hepatic oxidative stress (MDA) and antioxidant indices (catalase and GSH) are depicted in Table 2. In relation to control rats, hyperthyroidism rats showed a marked increase in MDA and decreases in catalase and GSH ($P \leq 0.001$). However, hyperthyroidism rats treated with CUR and /or CO Q10 or carbimazole demonstrated marked reduction in MDA and elevation in catalase and GSH compared to hyperthyroidism untreated rats. Importantly, the modulations in the levels of these markers were pronounced in hyperthyroidism rats ingested with the combination of CUR and CO Q10 compared to the other treated groups.

Levels of hepatic DNA damage and apoptosis indices
The potential efficacies of CUR and /or CO Q10 or carbimazole in ameliorating the hyperthyroidism induced hepatic DNA fragmentation are demonstrated in Table 3 and Figure 1. DNA fragmentation was evaluated by measuring the percentage of DNA tail and olive tail moment. In hyperthyroidism rats, a pronounced increase in DNA tail percentage as well as in olive tail moment were observed in relation to control rats ($P \leq 0.001$). In contrary, treatment with the different used agents significantly resulted in depletion in DNA tail percentage as well as in olive tail moments. The result also revealed that an increases in the hepatic apoptosis enzyme, caspase 3, in hyperthyroidism rats (Table 3). Administration of CUR and /or CO Q10 or carbimazole to hyperthyroidism rats, effectively decreased caspase-3 level with respect to control ones. The amelioration of these parameters was more pronounced in hepatic of hyperthyroidism rats supplemented with CUR simultaneously with CO Q10.

Levels of serum inflammatory markers

Table 4 illustrates the impacts of CUR and /or CO Q10 or carbimazole on the levels of serum inflammatory molecules. Significant increment in TNF-α, IL-6 and CRP was observed in hyperthyroidism rats with respect to control ones ($P \leq 0.001$) and treatment with the used agents significantly reduced the serum levels of these markers versus hyperthyroidism rats ($P \leq 0.001$). The combination of CUR and CO Q10 was synergistically the potential one in restoring the levels of theses inflammatory molecules. The results also demonstrated that an increase in the level of angiogenic factor (VEGF) in hyperthyroidism rats with respect to control rats ($P \leq 0.001$, Table 4). Administration of CUR and /or CO Q10 or carbimazole, significantly attenuated the deviation in this factor versus hyperthyroidism rats.

Levels of serum hepatic function parameters

The levels of serum hepatic functional indices, namely ALT, AST, ALP and albumin in different experimental rat groups are represented in Table 5. With relation to control rats, hyperthyroidism rats showed marked elevation in serum ALT, AST and ALP accompanied with a decrease in albumin level. Treatment of hyperthyroidism rats with CUR and /or CO Q10 or carbimazole significantly reversed the levels of serum hepatic function parameters The combination of CUR and CO Q10 was the successful one in normalizing these markers.

Histopathological liver observation

The impacts of CUR and /or CO Q10 or carbimazole on hepatic histomorphologic pictures of hyperthyroidism rats are shown in Figure 2. Liver section of control rat showed normal appearance of the hepatocytes, regular hepatic cords and central vein. Liver section of hyperthyroidism rat showed degeneration of some hepatocytes, cytoplasmic vacuolization of other cells with fatty changes, apoptotic cell death with pyknotic nuclei and infiltration of immune inflammatory cell. Hyperthyroidism rats treated with CUR or the combination of CUR showed normal liver futures. Treatment with CO Q10 or carbimazole displayed improvement, however fatty changes and cytoplasmic vacuolization of hepatocytes with infiltration of inflammatory cells are still observed.

Ultrastructure observation of liver

The influences of CUR and /or CO Q10 or carbimazole on hepatic ultrastructure changes of hyperthyroidism rats, utilizing TEM are shown in Figure 3. Liver of control rats revealed normal hepatocytes with normal nucleus and organelles, including Golgi, mitochondria and rough endoplasmic reticulum. Liver sections in liver of hyperthyroidism rats showed many ultrastructure changes, including irregular nuclear membrane in some hepatocytes and shrunken of nucleus in others with condensed chromatin undergoing apoptosis, swelling of mitochondria and hepatic cytoplasmic vacuolation with fat droplets. Liver sections of hyperthyroidism rats treated with CUR or the combination of CUR and CO Q10 displayed normal hepatic ultrastructure feature. Liver section of hyperthyroidism rat treated with CO Q10 showed nucleus with few chromatin and increased number of lysosomes. Liver section of hyperthyroidism rat treated with carbimazole, showed inflammatory cells in the hepatic sinusoids.

Table 1. Effect of curcumin, and /or coenzyme Q10 or carbimazole on the levels of serum thyroid hormones in hyperthyroidism rats

Parameters	Control	HT	HT+ CUR	HT+ CO Q10	HT + CUR and CO Q10	HT +Carbimazole
TSH (ng/ml)	1.95 ±0.13	0.53 ±0.02^{a}	1.40 ±0.04 ^{a*}	1.14 ±0.076 $^{a*\#}$	1.32 ±0.06 ^{a*}	1.60 ±0.04 $^{a*\#\#}$
T4 (µg/dl)	6.48 ±0.26	28.5 ±0.91 ^{a}	7.45 ±0.26*	8.35 ±1.04$^{b*\#\#}$	6.32 ±0.31*	6.67 ±0.41*
T3 (ng/ml)	0.46 ±0.02	1.60 ±0.08 ^{a}	0.73 ±0.04 $^{a*\#}$	0.98 ±0.06 $^{a*\#\#}$	0.60 ±0.04 ^{b*}	0.65 ±0.05 ^{a*}

HT, hyperthyroidism; CUR, curcumin; CO Q10, Coenzyme Q10. Values are calculated as mean ± S.D. (n=10). $^{a}P \leq 0.001$, $^{b}P \leq 0.01$ in comparable to control group, $^{*}P \leq 0.001$ with respect to HT group, $^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.01$ in comparable to HT+ combination (CUR and CO Q10) group.

Table 2. Effects of curcumin and /or coenzyme Q10 or carbimazole on liver oxidative stress and antioxidant indices of hyperthyroidism rats.

Parameters	Control	HT	HT + CUR	HT + CO Q10	HT + CUR and CO Q10	HT + Carbimazole
MDA (nmol/mg protein)	1.48 ±0.08	3.54 ±0.15^{a}	1.86 ±0.06 $^{b*\#}$	1.90 ±0.08 $^{b*\#}$	1.67 ±0.05 ^{c*}	2.23 ±0.11$^{a*\#\#}$
CAT (U/mg protein)	38.52 ±2.10	21.32 ±1.12 ^{a}	34.32 ±1.06^{c*}	29.27 ±1.07 $^{b*\#}$	35.3 ±1.02^{c*}	25.8 ±1.7$^{a*\#\#}$
GSH (nmol/mg protein)	24.75 ±1.62	11.75 ±0.66 ^{a}	25.80 ±1.72*	22.01 ±0.31 $^{c*\#}$	25.67 ±2.13*	18.77 ±0.31$^{b*\#\#}$
Olive tail moment	0.25 ±0.04	11.06 ±0.44^{a}	4.67 ±0.46 $^{a*\#\#}$	5.50 ±0.17$^{a*\#\#}$	3.43 ±0.177^{a*}	7.45 ±0.21$^{a*\#\#}$
Caspase-3 (nmol/mg protein)	8.65 ±0.26	29.00 ±1.42^{a}	13.82 ±0.25$^{a*\#}$	16.12 ±0.61$^{a*\#\#}$	12.5 ±0.57^{a*}	17.2 ±0.70$^{a*\#\#}$

HT, hyperthyroidism; CUR, curcumin; CO Q10, Coenzyme Q10. Data are calculated as mean ± S.D. (n=10). $^{a}P \le 0.001$, $^{b}P \le 0.01$, $^{c}P \le 0.05$ in comparable to control group, $^{*}P \le 0.001$ in comparable to HT group, $^{\#}P \le 0.05$, $^{\#\#}P \le 0.01$ versus HT+ combination (CUR and CO Q10) group.

Table 3. Effects of curcumin and/or coenzyme Q10 or carbimazole on liver DNA damage and caspase -3 of hyperthyroidism rats.

Parameters	Control	HT	HT + CUR	HT + CO Q10	HT + CUR and CO Q10	HT + Carbimazole
DNA tail %	0.59 ±0.07	28.9 ± 1.4^{a}	11.9 ±0.59 $^{a*\#\#}$	13.8 ±0.48$^{a*\#\#}$	8.6 ±0.33^{a*}	18.8 ±0.38$^{a*\#\#}$

HT, hyperthyroidism; CUR, curcumin; CO Q10, Coenzyme Q10. Data are calculated as mean ± S.D. (n=10). $^{a}P \le 0.001$ in comparable to control group, $^{*}P \le 0.001$ in comparable to HT group, $^{\#}P \le 0.05$, $^{\#\#}P \le 0.01$ in comparable to HT+ combination (CUR and CO Q10) group.

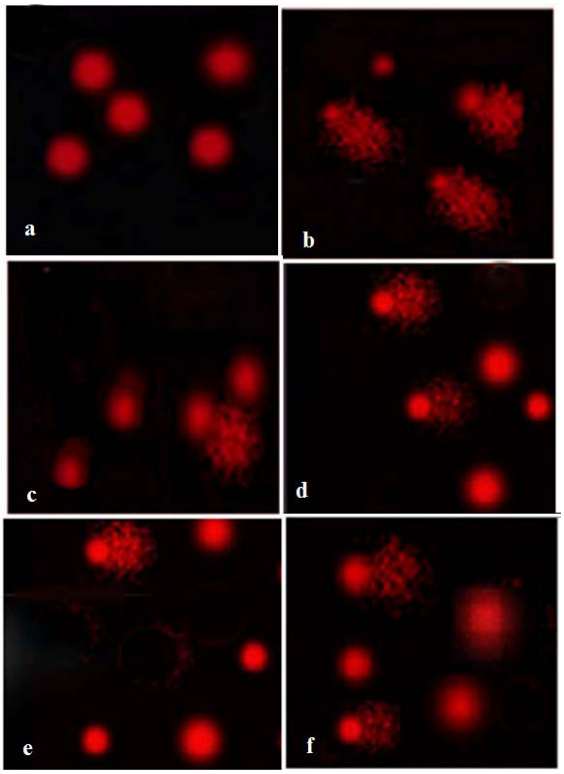

Fig 1. Effect of curcumin, and/or coenzyme Q10 or carbimazole on liver DNA fragmentation in hyperthyroidism rat groups. Comet assay showing the degree of DNA fragmentation. (a)Control, (b)hyperthyroidism +curcumin, (d)hyperthyroidism + coenzyme Q10, (e)hyperthyroidism +combination (curcumin

and coenzyme Q10) and (f) hyperthyroidism + carbimazole.

Table 4. Effects of curcumin and /or coenzyme Q10 or carbimazole on serum inflammatory indices of hyperthyroidism rats

Parameters	Control	HT	HT + CUR	HT + CO Q10	HT + CUR and CO Q10	HT + Carbimazole
TNF-α (pg/ml)	24.95 ±1.7	123.35 ±8.35[a]	68.20 ±5.02 [a*#]	76.90 ±1.54 [a*##]	55.75 ±3.06 [a*]	94.20 ±3.40 [a*##]
IL-6 (pg/ml)	14.77 ±0.79	86.87 ±3.14 [a]	36.75 ±2.12 [a*#]	46.97 ±3.44 [a*##]	31.57 ±1.46 [a*]	45.50 ±1.78 [a*##]
CRP (mg/L)	2.45 ±0.21	6.17 ±0.30 [a]	3.17 ±0.17 [b*##]	3.26 ±0.18 [b*##]	2.35 ±0.31[*]	3.73 ±0.15 [a*##]
VEGF (pg/ml)	21.12 ±1.2	37.44 ±0.84 [a]	22.32 ±1.20[*]	25.7 ±1.57[c*#]	21.9 ±0.99[*]	28.7 ±1.20[b*##]

HT, hyperthyroidism; CUR, curcumin; CO Q10, Coenzyme Q10. Data are calculated as mean ± S.D. (n=10). $^{a}P \le 0.001$, $^{b}P \le 0.01$, $^{c}P \le 0.05$ in comparable to control group, $^{*}P \le 0.001$ in comparable to HT group, $^{\#}P \le 0.05$, $^{\#\#}P \le 0.01$ in comparable to HT+ combination (CUR and CO Q10) group.

Table 5. Effects of curcumin and /or coenzyme Q10 in comparison to Carbimazole on liver function indices of hyperthyroidism rats

Prameters	Control	HT	HT + CUR	HT + CO Q10	HT + CUR and CO Q10	HT + Carbimazole
ALT (U/L)	22.50 ±1.3	80.0 ±5.88[a]	32.50 ±2.10 [a*#]	38.70 ±3.70 [a*##]	24.30 ±2.4[*]	44.25 ±1.70 [a*##]
AST (U/L)	36.0 ±2.6	184.7 ±8.95 [a]	53.0 ±2.90 [a*##]	73.7 ±2.87 [a*##]	35.7 ±2.63[*]	90.5 ±7.32 [a*##]
ALP (U/L)	53.4 ±2.16	129.75 ±7.13 [a]	60.75 ±3.86[c*#]	79.5 ±2.64 [b*##]	54.7 ±4.9[*]	94.65 ±3.60 [a*##]
Albumin (g/dl)	5.02 ±0.45	2.37 ±0.23 [a]	3.8 ±0.8[c*#]	3.65 ±0.12 [b*#]	4.9 ±0.18[*]	2.7 ±0.15 [a*##]

HT, hyperthyroidism; CUR, curcumin; CO Q10, Coenzyme Q10. Data are calculated as mean ± S.D. (n=10). $^{a}P \le 0.001$, $^{b}P \le 0.01$, $^{c}P \le 0.05$ in comparable to control group, $^{*}P \le 0.001$ in comparable to HT group, $^{\#}P \le 0.05$, $^{\#\#}P \le 0.01$ in comparable to HT+ combination (CUR and CO Q10) group.

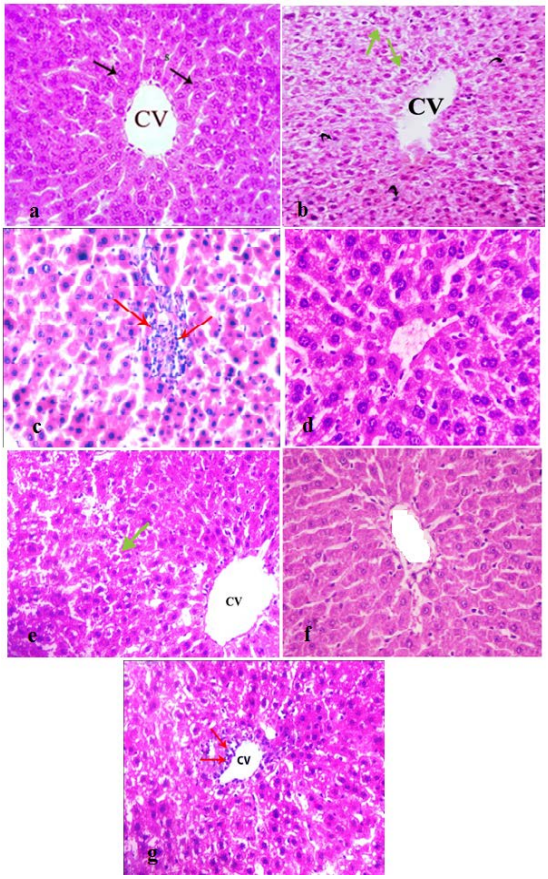

Fig 2. Photomicrograph of liver sections of different experimental hyperthyroidism rat groups: (a) Section in liver of control rat, showing normal appearance of the hepatocytes (arrows) and central vein(CV). (b&c) Sections in liver of hyperthyroidism rat group, (b) showing degenerated hepatocytes with cytoplasmic

vacuolization and fatty changes (green arrows) as well as apoptotic cells with pyknotic nuclei (curved arrows) and (c) showing inflammatory cells infiltration (red arrows). (d) Section in liver of hyperthyroidism rat treated with curcumin group showing normal structure and no histopathological alterations. (e) Section in liver of hyperthyroidism rat group treated with COQ10, showing improvement in the hepatic tissue; moderate fatty changes and cytoplasmic vacuolization of hepatocytes (green arrow) with restoration of lobular architecture. (f) Section in liver of hyperthyroidism rat group treated with the combination of curcumin and COQ10, showing regular hepatic cords, central vein and hepatocytes. (g) Section in liver of hyperthyroidism rat group treated with carbimazole, showing improvement of the liver, however fatty changes and cytoplasmic vacuolization of hepatocytes with infiltration of inflammatory cells are still observed (red arrows) (H & E x 400).

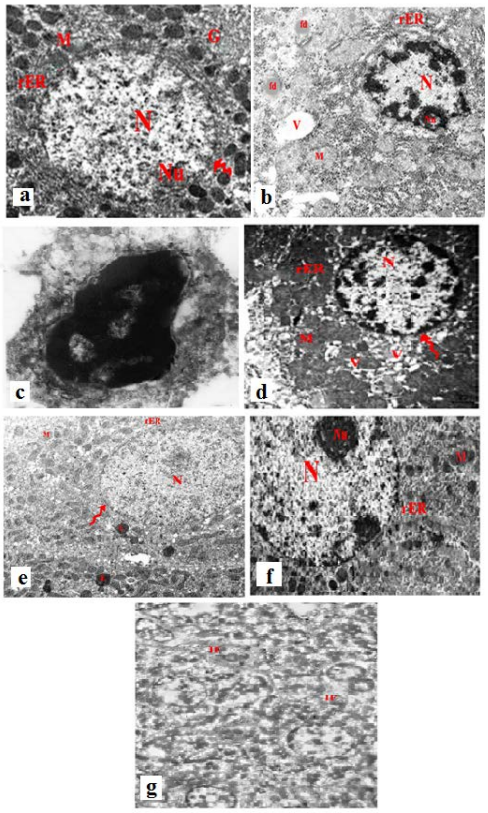

Fig 3. Transmission electron micrograph of livers in different hyperthyroidism rat groups. (a) liver of control rats, showing nucleus (N), nucleolus (NU), nuclear envelop (zigzag arrow), Golgi (G), mitochondria(M) and rough endoplasmic reticulum (rER) with few fat droplets and well-distributed cytoplasm. The nucleus was normal with other organelles (TEM mag. × 8000). (b & c) liver sections of hyperthyroidism rat, (b) showing the nucleus (N) with irregular nuclear membrane, dilated mitochondria (M), some fat droplets (fd) and many vacuoles (v) appeared in the cytoplasm; (c) showing hepatocyte with shrunken nucleus and condensed chromatin undergoing apoptosis (TEM mag. × 8000). (d) liver section of hyperthyroidism rat treated with curcumin, showing hepatocyte exhibited euchromatic nucleus with regular outline (N), many mitochondria (M) separated by rER, two electron lucent vacuoles (V) of variable sizes with the smaller on near the cell surface (TEM mag. =8000x). (e) Liver section of hyperthyroidism rat treated with COQ10, showing nucleus with few chromatin and increased number of lysosomes (L) (TEM mag. =5000x g) (f) Liver section of hyperthyroidism rat treated with the combination of curcumin and COQ10, showing binucleated cell with normal nucleus, mitochondria and rough endoplasmic reticulum with dense chromatin (TEM mag.=5000×). (g) Liver section of hyperthyroidism rat treated with carbimazole, showing inflammatory cells (IF) in the hepatic sinusoids.

DISCUSSION

The thyroid gland can become overactive due to hyperthyroidism, a condition that results in several health problems including an alteration in the basal metabolic rate and compromised functionality of some body organs such as liver [6]. In order to explore some of the remedies to the condition of hyperthyroidism and its complication, the current investigation is designed to study the prophylactic mechanism of CUR and/or CO Q10 in comparison to the currently available drug, carbimazole, against hyperthyroidism caused rat liver damage.

The results of the present investigation revealed that treatment of rats with LT4 showed high serum levels of T4 and T3 and a reduction in TSH level compared to control rats. Increases in T3, T4 with a decrease in TSH levels in rats indicated the establishment of hyperthyroidism state in LT4 treated rats. Treatment with CUR and /or CO Q10 or carbimazole significantly modulated the alterations in the thyroid hormones with respect to hyperthyroidism untreated rats ($P \leq 0.001$). Carbimazole and the combination of CUR and CO Q10 were effective in reversing the levels of these hormones.

Oxidative stress is one of the adverse impacts of hyperthyroidism induced liver damage [28]. Parallel with former investigation, the current investigation illustrated that hyperthyroidism

induced a state of oxidative stress in rat livers as represented by a marked elevation in hepatic MDA (marker of oxidation of cellular membrane lipids) and decreases in catalase and GSH (antioxidant indices) when compared with control rats [29]. The hyperthyroid oxidative stress was coupled with oxidative liver DNA fragmentation as shown by increases in the tail DNA percentage as well as in olive tail moment in hyperthyroidism rats compared with control animals. Increase in lipid peroxidation and the depletion in antioxidants in liver of hyperthyroidism rats may be due to overproduction of free radicals in response to high level of thyroid hormones. It has reported that elevation in T3 causes calorigenesis due to an increase in O2 consumption rate in most tissues including liver, leading to overproduction of free radicals such as superoxide (O·-2) and H2O2 radicals by liver mitochondria [8]. Also, former studies showed that the development of hyperthyroidism is associated with increase in the activities of enzymes involved in the production of oxygen and nitrogen reactive species, namely NADPH oxidase, xanthine oxidase and nitric oxide synthase [8,30]. Over generation of such species leads to lipid peroxidation, depletion in antioxidants and DNA fragmentation [8, 31].

Free reactive elements can bind to DNA, leading to alteration of pyrimidine and purine bases as well as the DNA strands [32]. Beside, MDA, a byproduct of lipid oxidation, is a mutagen and can react with DNA, forming adduct with its nucleosides [33]. Some investigations have found that hyperthyroidism causes marked increase in the circulating MDA, a depletion in antioxidant parameters and oxidative DNA fragmentation [34-35].

Treatment of hyperthyroidism rats with CUR and /or CO Q10 or carbimazole, significantly modulated the hepatic increase in MDA as well as the depletion in catalase and GSH compared to hyperthyroidism untreated rats. Also these agents could modulate the increases in DNA fragmentation parameters. The modulations in the levels of these markers were pronounced in hyperthyroidism rats ingested the combination of CUR and CO Q10 compared to the other treated groups, however carbimazole, the currently available antithyroid drug was the least effective one in ameliorating hyperthyroid liver oxidative stress and DNA damage. These results may indicate that the combination of CUR and CO Q10 can act synergistically as a powerful antioxidant agent. Similarly the antioxidant potential actions of CUR and CO Q10 were Also documented [14, 18].

Apoptosis is considered a key incident after DNA oxidative fragmentation [36]. The result of the present work revealed a significant elevation in the activity of apoptotic enzyme, caspase 3, in the livers of hyperthyroidism rats, proposing that apoptosis might relate to hyperthyroid DNA fragmentation. A study has indicated that hyperthyroid can cause apoptosis by enhancing the generation of many death receptors and their ligands, including TNF-α, FasL, causing activation of caspases which have the major role in apoptotic cell death [7]. Administration of CUR and /or CO Q10 or carbimazole to hyperthyroid rats, obviously depleted the hepatic caspase-3 activity versus untreated rats. Ingestion of CUR simultaneously with CO Q10 was the most effective in attenuating the elevation in apoptosis enzyme. This result may indicate the anti-apoptosis properties of the used agents. The anti-apoptotic benefits of both CUR and CO Q10 were proved by former investigations in rat models [20, 37].

This work also revealed significant rising in serum proinflammatory molecules, namely TNF-α, IL-6, and CRP, in hyperthyroidism rats in comparable to control ones. Some authors have found that hyperthyroidism is a hormonal stimulus for the nuclear factor kappa (NFκB) activation which involved in the production of many inflammatory cytokines including TNF-α, IL-6 [38]. Also, some authors declared that thyroid calorigenesis can boost the release of free radicals by hepatic Kupffer cells, which in turn increase the expression of genes involved in inflammatory cytokine production, including TNF-α and its release into the blood circulation [39]. The over production of TNF-α promotes the generation of IL-6, the principle stimulator of CRP biosynthesis, causing inflammatory liver injury [40]. This result may imply that generation of the inflammatory mediators is another mechanism by which hyperthyroidism can cause liver damage. Intake of CUR and /or CO Q10 or carbimazole along with LT4 injection, significantly attenuated hyperthyroidism caused over expression of TNF- α, IL-6 and CRP, proposing that their hepatoprotective efficacy may be correlated to their anti-inflammatory and immunomodulatory useful effectiveness. The anti-inflammatory beneficial effects of CUR and CO Q10 were confirmed [14, 18].

The existing research detected that a remarkable rising I n the angiogenic VEGF in the

serum of hyperthyroidism rats with respect to control ones. Our result is supported by former authors who found that elevation in serum VEGF concentration in Graves' disease patients [41]. Angiogenesis, in terms of promoting vasculogenesis, is an important event of hyperthyroidism [42]. VEGF is generated by thyroid follicles due to long term stimulation of thyroid-stimulating hormone (TSH) receptor (TSHR). VEGF activates its receptors on endothelial cells in thyroid, causing thyroid gland hypervascularization [42]. It has suggested that increased in VEGF expression in hyperthyroidism might be important for thyrocyte proliferation which may contribute to goiter development [43]. Treatment of hyperthyroidism rats with CUR and /or CO Q10 or carbimazole, markedly attenuated the increase in the serum VEGF level, suggesting their potential anti-angiogenic impact. CUR alone as well as its combination with CO Q10 were efficient in reversing VEGF level. The antiangiogenic impact of both CUR and CO Q10 is coped with other studies [44-45].

The damaging impact of hyperthyroidism on rat livers presented in this study was confirmed by elevation in the serum liver function indices namely ALT, AST and ALP, implying cellular leakage and loss of liver plasma membrane function. Also the decrease in serum albumin of hyperthyroidism rats may indicate the alteration of hepatic protein metabolism. A report has demonstrated that protein is greatly influenced by the building of reactive elements, leading to the formation of derivatives from carbonyl protein. These reactive elements can induce oxidative deterioration of amino acid side chains [46]. In line with other authors, the present liver damage promoted in rats in response to hyperthyroidism is confirmed by histopathological alterations on the level of hepatic architecture as well as by ultrastructural changes on the level of individual hepatocytes [47]. Intake of CUR and /or CO Q10 or carbimazole, significantly ameliorated the deviation in liver function indices (ALT, AST, ALP and albumin) as well as in histomorphology and ultrastructure of liver, indicating their hepatoprotective potential impact. The combination of CUR and CO Q10 was the efficient in reversing the hepatic function biomarkers to normal levels as well as the histology and ultrastructure of hepatic pictures. The hepatoprotective beneficial efficacies of both CUR and CO Q10 were proved [16, 20].

Conclusion

The present work has demonstrated that hyperthyroidism state (thyrotoxicosis) has many adverse effects on rat livers, including oxidative stress, oxidative DNA fragmentation, apoptosis, inflammation and alterations in the serum liver function markers as well as in histology and ultrastructure of liver tissue. Prophylactic treatment with CUR and /or CO Q10 or carbimazole ameliorated thyrotoxicosis induced hepatocyte damage. The protective mechanisms of these agents may be due to their antioxidant, antiapoptosis, anti-inflammatory and antiangiogenic properties. In comparison to carbimazole and other agents, treatment with the combination of CUR and CO Q10 was synergistically the potential one in attenuating thyrotoxicosis caused liver dysfunction. So this investigation may candidate the use of this combination as a promising hepatoprotective agent against thyrotoxicosis liver damage.

REFERENCES

1. M.A. Gulcelik, N.E Gulcelik , S. Dinc , B. Kuru , M. Camlibel and H. Alagol *, J. Surg. Oncol*, 2006. 94: p. 35–39.
2. P. Tahiliani and A. Kar, *Phytomedicine*, 2003. 10: p. 665–668.
3. T. Majima, Y. Komatsu, K. Doi, C. Takagi, M. Shigemoto, A. Fukao, T. Morimoto, J. Corners and K. Nakao, *J. Bone Miner. Metab*, 2006. 24: p. 105–113.
4. S. Panda and A. Kar , *Clin. Exp. Pharmacol. Physiol*, 2007. 34: p.1217–1219.
5. K. Das, G.B. Chainy, *Biochim. Biophys. Acta*, 2001. 1537:p.1–13.
6. A. Kumar, R.A. Sinha, M. Tiwari, R. Singh, T. Koji, N. Manhas, L. Rastogi, L. Pal, A. Shrivastava, R.P. Sahu , M.M. Godbole . *J. Hepatol* , 2007. 46(5): p. 888-898.
7. G. Upadhyay, R. Singh , A. Kumar, S. Kumar, A. Kapoor and M.M. Godbole , Hepatology , 2004. 39:p.1120–1130.
8. V. Ferna´ndez, X. Barrientos, K. Kipreos, A. Valenzuela and L.A. Videla, *Endocrinol,* 1985. 117: p. 496–501.
9. U. Subudhi, D. Das, B. Paital, S.Bhanja and G.B. Chainy, *Chem. Biol. Interact*, 2008. 173:p.105–114.
10. M.J. Chuang and H.C. Lam, *Metabolism*, 2008. 57(10) p. 1380-1383.
11. S.L. Andersen and J.O.P. Laurberg, *J. Clin. Endocrinol. Metab*, 2016. 101 (4), 1606–1614.
12. G. Schmeda-Hirschmann and E. Yesilada , *J. Ethnopharmacol*, 2005. 100:p. 61–66.

13. S.M. Kim, S.C. Kim, I.K. Chung, W.H. Cheon and S.K. Ku, *Evid. Based Complement Alternat. Med* , 2012. 2012 : p. 578497.
14. 1R.M. Srivastava , S. Singh , S.K. Dubey, K. Misra and A. Khar, *Int. Immunopharmacol*, 2011. 11: p. 331–341.
15. H.L. Son, N.T.H. Trang, D.T. Sinh and M.N.T. Anh, *IJPSR*, 2013. 4: p. 4542- 4548.
16. A.M. Elmansi, A.A. El-Karef , M.M. Shishtawy and L.A. Eissa , *Ann. Hepatol,* 2017. 16(4): p. 607-618.
17. F.L.Crane , *J. Am. Coll. Nutr*, 2001. 20: p. 591–598.
18. S. A. Mirmalek , A.Gholamrezaei Boushehrinejad , H. Yavari , B. Kardeh , Y. Parsa , S.A. Salimi-Tabatabaee , S. Yadollah-Damavandi , T. Parsa and E. Shahverdi , *Oxid. Med. Cell Longev,* 2016. 2016 : p. 5818479.
19. S. Esfahani , E. Esmaeilzadeh, F. Bagheri , Y. Emami and M. Farjam, *Hepat. Mon,* 2013. 13(8): p. e13685.
20. A.A. Fouad and I. Jresat , *Environ .Toxicol. Pharmacol*, 2012. 33(2): p. 158-167.
21. P. Sankar , A.G. Telang , A. Manimaran and J.K. Malik , *Toxicol. Environ. Chem*, 2010. 92: p. 909–917.
22. A.J. Al-Naely and D.T. Shattna, *Journal of Global Pharma Technology (JGPT),* 2017. 09(9): p.174-181
23. J.A. Buege and S.D. Aust, *Methods Enzymol*, 1978. 52: p. 302-315.
24. E. Bentler, O. Duran and K.B. Mikus, *J. Lab. Clin. Med*, 1963. 61 :p. 882.
25. H. Aebi, *Methods Enzymol*, 1984. 105:p. 121–126.
26. A. Vaculova and B. Zhivotovsky, *Methods Enzymol*, 2008. 442:p. 157–181.
27. Singh NP, McCoy MT, Tice RR, Schneider EL, *Exp Cell Res*, 1988.175:p 184–191.
28. C. Europa, B.V. Vanessa, F.C. Margarita, O.B. Rocio, Volodymyr Lushchak (Ed.), 2012.ISBN: 978-953-51-0552-7.
29. N. Kumar, A. Kar and S. Panda, *Cell Biochem. Funct*, 2014. 32(6):p.538-546.
30. K. Huh, T.H. Kwon, J. S. Kim and J.M. Park, *Arch. Pharm. Res*, 1998. 21:p. 236– 240.
31. V.I. Lushchak, *Comp. Biochem. Physiol. C Toxicol. Pharmacol,* 2011. 153:p. 175-190.
32. G.R. Martinez , A.P. Loureiro , S.A. Marques , S. Miyamoto , L.F. Yamaguchi , J. Onuk , E.A. Almeida , C.C. Garcia , L.F. Barbosa , M.H. Medeiros and P. Di Mascio, Mutat Res 2003. 544: p.115–127.
33. L.J. Niedernhofer, J.S. Daniels, C.A. Rouzer, R.E. Greene , L.J. Marnett, *J. Biol. Chem,* 2003. 278:p. 31426–31433.
34. J. Bednarek, H. Wysocki and J. Sowinski, *Free Radic. Res*, 2004. 38:p. 659–664
35. A. Zambrano, V. García-Carpizo, M.E. Gallardo , R. Villamuera , M.A. Gómez-Ferrería , A. Pascual , N. Buisine, L.M. Sachs, R. Garesse and A. Aranda, *J. Cell Biol,* 2014. 204(1):p.129-146.
36. V. Sharma, R.K. Shukla, N. Saxena, D. Parmar, M. Das and A. Dhawan, Toxicol. Lett, 2009. 185: p.211–218
37. 37- L. Saha , A. Chakrabarti , S. Kumari , A. Bhatia and D. Banerjee, *Indian J. Exp. Biol*, 2016. 54(2): p.133-141.
38. V. Fernandez, G. Tapia, P. Varela, P. Romanque, D. Cartier-Ugarte and L.A. Videla, *Comp. Biochem. Physiol. C Toxicol. Pharmacol*, 2006. 142 : p 231-239.
39. 39- L.J. Huffman, J.D. Prugh, K. Brumbaugh , M. Ding, *Inhal. Toxicol*, 2002. 14(11): p. 1161-1174.
40. A. Kerner, O. Avizohar, R. Sella, P. Bartha P, O. Zinder, W. Markiewicz, L. Yishai, G.J. Brook and D. Aronson, *Arterioscler, Thromb, Vasc, Biol,* 2005. 25: p.93-197
41. J.D. Ramsden, *J. Endocrinol*, 2000. 166: p. 475–480.
42. D. Kajdaniuk, B. Marek, H. Borgiel-Marek and B. Kos-Kudła, *Endokrynol. Pol*, 2011. 62: p. 444–455
43. P. Malkomes, E. Oppermann , W.O.Bechstein and K. Holzer , *Exp. Clin. Endocrinol. Diabetes,* 2013. 121:p. 6–13. 57.
44. P. Sachdanandam, *Biofactors*, 2008. 32(1-4):p.151-159.
45. Y.F. Huang, X.X. Zhu, Z.S. Ding, G.Y. *Zhongguo Zhong Yao Za Zhi*, 2015. 40(2): p.324-329.
46. A. Salvi, P. Carrupt, J. Tillement and B. Testa, *Biochem. Pharmacol*, 2001. 61(10):p.1237-1242
47. H. Luo , X.Li, S. Xiao and X. Zhang, *Int. J. Clin. Exp. Med*, 2017.10(8):p.11903-11911.