

## RESEARCH ARTICLE

## Rosemary and CoQ<sub>10</sub> Alleviated the Detrimental Effects of Concomitant Administration of Acetaminophen and Carbamazepine by Accelerating Their Metabolism and Elimination

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Received date: Jun 27, 2022; Revised date: Aug 16, 2022; Accepted date: Sep 19, 2022

### Abstract

**BACKGROUND:** The interaction of carbamazepine (CBZ) and acetaminophen (APAP) could result in hepatic failure and mortality. This study was conducted to analyze the potential of rosemary ethanol extract (REE) or coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) to alleviate the interactions between CBZ and APAP.

**METHODS:** Forty-eight adult male rats were treated differently based on the assigned groups. Oxidative stress parameters, including malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST), and the expression levels of *CYP3A4*, *CYP2E1*, *IL-6*, *TNF-α*, and *IL-1B* in the liver were estimated. In addition, the histopathology of liver was examined and the plasma clearance rate of CBZ and APAP was estimated.

**RESULTS:** Combination of CBZ and APAP significantly elevated alanine aminotransferase (ALT) activity and hepatic MDA, and reduced the activities of GPx, GST, and GSH level in liver. The gene expression of *CYP3A4* and *CYP2E1*

was upregulated by CBZ and CoQ<sub>10</sub>, respectively. The expression of *IL-6* has decreased in the groups treated with CBZ alone or in combination with APAP. *TNF-α* expression was significantly downregulated in the groups treated with CBZ, APAP, REE, CoQ<sub>10</sub>, or combination CBZ and APAP. The liver from CBZ and APAP combination group showed centrilobular degeneration and necrosis. REE and CoQ<sub>10</sub> were able to alleviate most of these detrimental effects. The combined administration of CBZ and APAP extended the plasma clearance time of APAP and CBZ from 6 to 24 and from 9 to 24 hours, respectively.

**CONCLUSION:** REE and CoQ<sub>10</sub> alleviated the detrimental effects of the combination of CBZ and APAP through enhancing the cellular antioxidant milieu, induction of metabolizing enzymes, reduction of the plasma half-life of APAP and CBZ preventing their accumulation and potential interaction.

**KEYWORDS:** acetaminophen, antioxidants, carbamazepine, CoQ<sub>10</sub>, *CYP3A4*, *CYP2E1*, glutathione, lipid peroxidation, rosemary

*Indones Biomed J. 2022; 14(4): 349-57*

### Introduction

Drug-drug interaction (DDI) results when the effects of one of the drugs are altered by another. DDIs are usually classified into pharmacodynamic and pharmacokinetic interactions. In a previous study, about 1223 patient out of

18,820 were hospitalized due to adverse drug effects and one sixth of this number was attributed to DDI.(1)

Acetaminophen or acetyl-para-amino-phenol (APAP) is considered one of the most used analgesics. Hepatotoxicity from APAP overdose is the most common cause of drug-induced liver injuries in the USA and remains as a global issue.(2) APAP is primarily metabolized in the liver by the

sulphation and glucuronidation pathways and about 5% is metabolized by oxidation catalyzed by cytochrome P450s (CYP2E1, CYP3A4, and CYP1A2) forming N-acetyl p-benzoquinoneimine (NAPQI) toxic metabolite.(3) Administration of APAP was associated with glutathione (GSH) depletion, catalase activity reduction, and increase in lipid peroxidation and malondialdehyde (MDA).(4)

Carbamazepine (CBZ), one of the most administered drugs used for treatment of seizures, is metabolized primarily in the liver by CYP450 3A4.(5) Several drugs inhibit the metabolism of CBZ catalyzed by cytochrome P450, sometimes resulting in CBZ intoxication. Administration of CBZ for a long time causes an imbalance between oxidant and antioxidant systems, total protein and albumin reduction, and hepatotoxicity marked by elevations in the aminotransferases and total bilirubin.(6) CBZ induced oxidative stress, increased lipid peroxidation and reactive oxygen species (ROS) formation, an elevation in oxidized GSH levels, and a decrease in cellular reduced GSH.(7) Using APAP together with CBZ results in serious side effects and hepatotoxicity. In 2009, a 34-year-old man on CBZ treatment was reported to develop acute hepatic and renal toxicity after treated with a therapeutic dose of APAP.(8) Afterwards, no other reports were found on *pubmed.gov* regarding this problem. Hence it is necessary to investigate the possible interactions between CBZ and APAP.

Rosemary is known for its properties as antioxidant, mainly due to the presence of several phenolic compounds. The hydroalcoholic extract of rosemary leaves successfully alleviated the hepatotoxicity induced by APAP.(9) Carnosic acid, a major component in rosemary ethanol extract, has been reported to induce drug-metabolizing enzymes such as CYP3A4, CYP2B6, and UGT1A1, in human hepatocytes.(10) Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>, or ubiquinone) is formed in the body especially under stress but in insufficient quantities. CoQ<sub>10</sub> administration significantly attenuated the increase of nitrate and oxidative stress, and inflammation and it acts as a powerful antioxidant.(11) Therefore, this study was conducted to examine the potential of hepatoprotective agents such as rosemary or CoQ<sub>10</sub> to prevent interactions between these drugs, or to alleviate the expected side effects.

## Methods

### Preparation of Rosemary Ethanol Extract (REE)

*Rosmarinus officinalis* L. (Lamiaceae) was collected from The Orman Botanical Garden, Giza, Egypt. Leaves were

dried in air and powdered using a blender. The obtained powder as much as 50 g was extracted with 1000 mL ethanol/H<sub>2</sub>O (7:3; v/v) at room temperature with stirring, followed by filtration. The filtered extract was concentrated with rotary evaporator under vacuum at 40°C, giving 19.0 g of ethanolic extract of *R. officinalis* leaves.

### Experimental Design

Adult male Sprague-Dawely rats weighing 230±10 g were obtained from the animal house of Egyptian Drug Authority (EDA), Cairo, Egypt, and were kept at standard conditions (22±1°C and 12 h natural light/dark cycle). They were supplied with standard laboratory chow and water *ad libitum*. The rats were then randomly divided into 8 groups (n=6, each) and were treated orally as follow: 1) Control group received a daily dose of 5% tween 80 (ADWIC, Cairo, Egypt) in saline orally for 14 days; 2) CBZ group received a daily dose of 108 mg/kg CBZ for 14 days (12); 3) APAP group received a daily dose of 360 mg/kg APAP for 10 days (13); 4) CBZ+APAP group received CBZ daily for 14 days and APAP for 10 days from day-5 to day-14; 5) REE group received 100 mg/kg REE for 10 days from day-5 to day-14 (9); 6) CoQ<sub>10</sub> group received 6 mg/kg CoQ<sub>10</sub> for 10 days from day-5 to day-14 (14); 7) CBZ+APAP+REE group received CBZ daily for 14 days, then starting from day-5 to day-14, animals were administered with APAP and REE with an hour interval; 8) CBZ+APAP+CoQ<sub>10</sub> group received CBZ daily for 14 days, then starting from day-5 to day-14, animals were administered with APAP and CoQ<sub>10</sub> with an hour interval. The animals were treated in according to the guidelines of laboratory animal care of the National Institute of Health (No. 86-23, revised 1985). The study design was approved by the Research Ethics Committee of Faculty of Science, Ain Shams University (No. 11A/10/16).

### Samples Collection

Animals were sacrificed and blood samples were collected in non-heparinized tubes and allowed to clot at room temperature. Then, the blood was centrifuged at 5000 rpm for 10 min. Serum aliquots were stored at -80°C for further biochemical analyses. The liver was divided into three parts; the first portion was fixed in 10% formalin for histological examination, the second part was stored at -80°C for estimation of oxidative stress parameters, and the third part was quickly immersed in polymerase chain reaction (PCR) lysis buffer for gene expression of cytochrome P450-3A4 (CYP3A4), P450-2E1 (CYP2E1), tumor necrosis factor alpha (TNF-α), interleukins (IL-6 and IL-1B).

### Measurement of Body Weight Changes and Relative Liver Weight

Body weights were measured at the beginning and at the end of the experiment, difference in weight and the percentage of weight change were then calculated. Meanwhile, the liver weights were measured at the end of the experiment to calculate the relative liver weight.

### Biochemical Analysis

Serum alanine aminotransferase (ALT) was measured using Spectrum kit from The Egyptian Company for Biotechnology (Cairo, Egypt) according to standard instruction. MDA was formed from the breakdown of polyunsaturated fatty acids and serves as an index for lipid peroxidation. MDA reacts with thiobarbituric acid in acidic medium to give a pink color that was measured at 520 nm and expressed as nmol/mg protein. The level of MDA was measured as described previously.(15) The presence of superoxide dismutase (SOD) in the reaction medium would inhibit the pyrogallol autoxidation at alkaline pH 9.5 by scavenging the produced  $O_2^-$  with a resultant increase in the absorbance at 420 nm. The results were expressed as unit per milligram protein (Unit/mg protein). SOD activity was measured based on previous study.(16) The decomposition of  $H_2O_2$  catalyzed by catalase using ethanol and Triton X-100 and the change in absorbance at 240 was recorded. Hence the measurement of catalase activity was conducted as described in previous literature.(17)

The measurement of the glutathione peroxidase (GPx) activity was done according to previous study. (18) The amount of GSH consumed per unit time was a measure of the catalytic activity of GPx and expressed as

mg/min/mg protein. GSH level was determined using the method described elsewhere.(19) Meanwhile, the activity of glutathione S-transferase (GST) was measured following the instruction in previous literature (20), which measured the conjugation of 1-chloro-2,3-dinitrobenzene (CDNB) with GSH and increase in absorbance at 340 nm. The rate of the increase was directly proportional to GST activity in the sample. The method was depend on the reduction of Elman's reagent after precipitation of protein thiols with a trichloroacetic acid, and the absorbance was measured at 412 nm.

### cDNA Synthesis

The total extracted RNA (0.5-2  $\mu$ g) from tissue was used for cDNA conversion using high-capacity cDNA reverse transcription kit (Cat. #K1621, Fermentas, Waltham, MA, USA). For the synthesis of cDNA from RNA, murine leukemia virus reverse transcriptase was used. Meanwhile, to inhibit the RNase activity, the human placental ribonuclease inhibitor was used. Deoxynucleotide triphosphate were used for extension of primers, random hexamers: primers for reverse transcription of RNA (Stratagene, San Diego, CA, USA), diethyl pyrocarbonate (DEPC)-treated water, and thermal cycler (Biometra, Jena, Germany) were used.

### Real-time qPCR Analysis

The real-time qPCR analysis was done using SYBR Green I technology. In the qPCR assay, specific primer sets (Table 1) were used for *CYP3A4*, *CYP2E1*, *TNF- $\alpha$* , *IL-1B*, and *IL-6* expression. GAPDH was used as a housekeeping gene. All samples were run in duplicate.

**Table 1. The sequence of primers of the investigated genes.**

| Gene Symbol                    | Primers Sequence                 | GenBank Accession No. |
|--------------------------------|----------------------------------|-----------------------|
| <i>CYP3A4</i>                  | 5'-GCTCTTGATGCATGGTTAAAGATTG-3'  | AH005338.2            |
|                                | 5'-ATCACAGACCTTGCCAACTCCTT-3'    |                       |
| <i>CYP2E1</i>                  | 5'-CTGATTGGCTGCGCACCTGC-3'       | AF061442.1            |
|                                | 5'-GAACAGGTCGGCCAAAGTCAC-3'      |                       |
| <i>TNF-<math>\alpha</math></i> | 5'-GCCTCACCACCTATGCCTTA-3'       | AB052685              |
|                                | 5'-CCTGTGGAGGCTCAAACAT-3'        |                       |
| <i>IL-1B</i>                   | 5'-GCTCTTCTGGCTACCGTCAC-3'       | AB048790              |
|                                | 5'-GGAACAGCGGAAAGTACTCG-3'       |                       |
| <i>IL-6</i>                    | 5'-CCTGCTGTCTGTGGCCC-3'          | AB025230              |
|                                | 5'-CTGCCAGTGAGCGGTCC-3'          |                       |
| <i>GAPDH</i>                   | 5'-CCCCTTCATTGACCTCAACTACATGG-3' | NM_017008.4           |
|                                | 5'-GCCTGCTTCACCACCTTCTTGATGTC    |                       |

For each sample, 1  $\mu\text{L}$  for forward primer, 1  $\mu\text{L}$  for reverse primer, 12.5  $\mu\text{L}$  for Syber green mix, 5  $\mu\text{L}$  for cDNA template, and 5.5  $\mu\text{L}$  for RNase free water were added. The running conditions for RT-PCR were as follows; one cycle at 50°C, 40 cycles of denaturation at 95°C for 15 s, annealing at 55-60°C for 60 s, followed by extension at 72°C for 60 s. The target gene expression was assessed relative to the reference gene and the respiratory coefficient (RQ) was calculated using the  $2^{-\Delta\Delta C_t}$  method.

### Histopathological Examination

Samples were taken from the liver and fixed in 10% formal saline for 24 h. Samples were washed in isotonic saline then dehydrated in serial dilutions of ethanol. Specimens were cleared in xylene and embedded in paraffin at 56°C in oven for 24 h. Paraffin bee wax tissue blocks were prepared for sectioning at 4  $\mu\text{m}$  thickness, deparaffinized and stained by hematoxylin & eosin (HE) stain for routine examination by the light microscope.

### Determination of CBZ and APAP in Blood

The CBZ was determined as previously described (21), by using a High-Performance Liquid Chromatographic (HPLC) system (Dionex, Sunnyvale, CA, USA). For the calibration curve, a series of CBZ was prepared in 100  $\mu\text{L}$  plasma (0.5, 1, 2, 5, 10 and 20  $\mu\text{g}/\text{mL}$ ), then 100  $\mu\text{L}$  of chlorzoxazone in acetonitrile was added to all tubes as an internal standard and after deproteinization, vortex-mixed for 30 s, and centrifuged for 10 min at 4,500 rpm. Twenty-five  $\mu\text{L}$  of the clear supernatant was then injected into the HPLC system. For the samples, 100  $\mu\text{L}$  of Chlo in acetonitrile were added to all tubes.

Meanwhile, APAP was determined as previously described (22), using HPLC system (Waters, Milford, MA, USA). For the calibration curve, a series of APAP (2, 5, 10, 20, 40, 80 and 100  $\mu\text{g}/\text{mL}$ ) in 80  $\mu\text{L}$  plasma was prepared, then 160  $\mu\text{L}$  of theophylline (100  $\mu\text{g}/\text{mL}$ ) in 6% perchloric acid was added to all tubes as an internal standard and after deproteinization, vortex-mixed for 1 min, and centrifuged for 15 min at 4,000 rpm. Twenty  $\mu\text{L}$  of the clear supernatant was then injected into the HPLC system. For the samples, 160  $\mu\text{L}$  of theophylline in (100  $\mu\text{g}/\text{mL}$ ) in 6% perchloric acid was added to all tubes.

### Statistical Analysis

The distribution of data was examined using Kolmogorov-Smirnov test, and the results were expressed as mean $\pm$ SEM. The statistical analyses were made using One-Way ANOVA followed by Tukey-HSD test for multiple comparisons using GraphPad InStat version 3.1 (GraphPad, San Diego, CA, USA). Differences were considered significant at  $p < 0.05$ .

## Results

### Body Weight Changes, Relative Liver Weight, and Liver Function Results

Most of the treatments did not significantly affect the body weight change. The reductions reported after REE and CoQ<sub>10</sub> did not achieve a statistical significance. The last two groups treated with CBZ+APAP+REE and CBZ+APAP+CoQ<sub>10</sub> showed a significant reduction in the body weight change when compared to the group treated with CBZ+APAP (Table 2). Although all treatments (except

**Table 2. Effect of CBZ, APAP, REE, and CoQ<sub>10</sub> on body weight changes, relative weight of liver, and ALT activity.**

| Groups                     | Body Weight Change (%)        | Relative Liver Weight (%)    | ALT (U/L)                   |
|----------------------------|-------------------------------|------------------------------|-----------------------------|
| Control                    | 11.87 $\pm$ 1.39              | 2.79 $\pm$ 0.10              | 28.0 $\pm$ 0.9              |
| CBZ                        | 10.88 $\pm$ 1.03              | 3.78 $\pm$ 0.15 <sup>a</sup> | 51.7 $\pm$ 2.6 <sup>a</sup> |
| APAP                       | 15.02 $\pm$ 0.66              | 3.12 $\pm$ 0.10              | 56.8 $\pm$ 3.9 <sup>a</sup> |
| REE                        | 8.03 $\pm$ 0.66               | 2.72 $\pm$ 0.08              | 27.2 $\pm$ 0.7              |
| CoQ <sub>10</sub>          | 7.72 $\pm$ 0.72               | 3.24 $\pm$ 0.18              | 20.5 $\pm$ 0.4              |
| CBZ+APAP                   | 13.66 $\pm$ 0.80              | 3.69 $\pm$ 0.15 <sup>a</sup> | 46.2 $\pm$ 3.2 <sup>a</sup> |
| CBZ+APAP+REE               | 5.08 $\pm$ 0.81 <sup>b</sup>  | 3.59 $\pm$ 0.16              | 23.2 $\pm$ 1.8 <sup>b</sup> |
| CBZ+APAP+CoQ <sub>10</sub> | -1.52 $\pm$ 2.01 <sup>b</sup> | 3.49 $\pm$ 0.11              | 22.2 $\pm$ 1.1 <sup>b</sup> |

Data are expressed as mean $\pm$ SEM (n=6). <sup>a</sup> $p < 0.05$  compared to control group, <sup>b</sup> $p < 0.05$  compared to CBZ+APAP group.

REE alone) caused hepatomegaly, the relative weight of liver was significantly increased only in the groups treated with CBZ and CBZ+APAP compared to the control group (Table 2).

The enzymatic activity of ALT significantly increased in the groups treated with CBZ and APAP alone or in combination compared to the control group indicating hepatotoxicity. This elevation in the activity level was abolished in the groups treated with CBZ+APAP when administered with REE or CoQ<sub>10</sub> (Table 2).

### Oxidative Stress Parameters Results

MDA was measured as an indicator of lipid peroxidation. CoQ<sub>10</sub> significantly decreased the hepatic MDA level, while treatment with CBZ+APAP significantly elevated the MDA level compared to the control animals. On treating CBZ+APAP groups with REE or CoQ<sub>10</sub>, a significant reduction in hepatic MDA was reported compared to the CBZ+APAP group. The hepatic SOD activity significantly decreased by APAP compared to the control group. The catalase activity in liver has not been affected by any treatment in the present study. The combined treatment with CBZ+APAP resulted in a significant reduction in GPx activity in liver compared to the control group. GST was the most responsive enzyme. GST activity in liver was significantly reduced by CBZ, APAP, and CBZ+APAP to varying degrees compared to the control group. When animals treated with CBZ+APAP administered with REE or CoQ<sub>10</sub>, the reduction in GST activity was aggravated. The hepatic GSH content significantly decreased after administration of CBZ+APAP. Rats treated with REE or CoQ<sub>10</sub> along with CBZ+APAP showed a significant elevation in GSH level compared to CBZ+APAP group (Table 3).

### Genes Assessment of Pro-inflammatory Cytokines and Drug Metabolizing Enzymes

In this study, we measured the genes of pro-inflammatory cytokines, including *IL-1B*, *IL-6*, and *TNF-α*, as well as drug metabolizing enzymes, namely *CYP3A4* and *CYP2E1*. The gene expression of *IL-1B* has not been affected by any treatment compared to control or CBZ+APAP groups. The *CYP3A4* expression was elevated by CBZ only. The *CYP2E1* expression was significantly upregulated only in the group treated with CBZ+APAP+CoQ<sub>10</sub> compared to CBZ+APAP group. The expression of *IL-6* has decreased in the groups treated with CBZ alone or in combination with APAP compared to the control group. Only REE was able to restore its normal expression when given with CBZ+APAP. *TNF-α* expression was significantly downregulated in the groups treated with CBZ, APAP, REE, CoQ<sub>10</sub>, or CBZ+APAP compared to the control group (Table 4).

### Histological Examination of Liver Tissue

The control (Figure 1A), REE (Figure 1E), CoQ<sub>10</sub> (Figure 1F), and CBZ+APAP+REE (Figure 1G) groups did not show any histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the hepatic parenchyma were recorded. The CBZ group (Figure 1B) showed severe dilatation in the central vein associated with ballooning degeneration in the surrounding adjacent hepatocytes. In the APAP-treated animals (Figure 1C), there were focal inflammatory cells aggregation in the hepatic parenchyma with severe congestion in the central vein. The liver from group treated with CBZ+APAP (Figure 1D) showed centrolobular ballooning degeneration and necrosis in the hepatocytes. The group treated with CBZ+APAP+CoQ<sub>10</sub> showed severe dilatation and congestion in the central vein (Figure 1H).

**Table 3. Effect of CBZ, APAP, REE, and CoQ<sub>10</sub> on hepatic oxidative stress parameters.**

| Groups                     | MDA<br>(nmol/mg<br>protein) | GSH<br>(mmol/mg<br>protein) | GST<br>(mmol/min/mg<br>protein) | SOD<br>(U/mg protein) | Catalase<br>(U/mg protein) | GPx<br>(mg/min/mg<br>protein) |
|----------------------------|-----------------------------|-----------------------------|---------------------------------|-----------------------|----------------------------|-------------------------------|
| Control                    | 63.51±3.81                  | 0.14±0.01                   | 390±32                          | 312±28                | 1.73±0.09                  | 0.13±0.01                     |
| CBZ                        | 51.05±6.46                  | 0.15±0.01                   | 297±6 <sup>a</sup>              | 334±26                | 1.87±0.08                  | 0.14±0.01                     |
| APAP                       | 69.57±7.11                  | 0.16±0.02                   | 241±16 <sup>a</sup>             | 150±10 <sup>a</sup>   | 1.98±0.17                  | 0.14±0.01                     |
| REE                        | 55.77±7.09                  | 0.12±0.02                   | 331±5                           | 332± 8                | 1.35±0.04                  | 0.10±0.01                     |
| CoQ <sub>10</sub>          | 46.37±2.93 <sup>a</sup>     | 0.18±0.01                   | 328±14                          | 214±15                | 1.52±0.02                  | 0.10±0.01                     |
| CBZ+APAP                   | 92.98±4.19 <sup>a</sup>     | 0.08±0.01 <sup>a</sup>      | 149±7 <sup>a</sup>              | 291±21                | 1.92±0.14                  | 0.08±0.01 <sup>a</sup>        |
| CBZ+APAP+REE               | 36.93±0.70 <sup>b</sup>     | 0.15±0.01 <sup>b</sup>      | 37±4 <sup>b</sup>               | 232±23                | 1.81±0.08                  | 0.10±0.01                     |
| CBZ+APAP+CoQ <sub>10</sub> | 43.37±9.99 <sup>b</sup>     | 0.14±0.01 <sup>b</sup>      | 40±8 <sup>b</sup>               | 276±11                | 1.62±0.10                  | 0.11±0.01                     |

Data are expressed as mean±SEM (n=6). <sup>a</sup>p<0.05 compared to control group, <sup>b</sup>p<0.05 compared to CBZ+APAP group.

**Table 4. Effect of CBZ, APAP, REE, and CoQ<sub>10</sub> on PCR analysis of drug metabolizing enzymes and pro-inflammatory cytokines.**

| Groups                     | CYP3A4                 | CYP2E1                 | TNF- $\alpha$          | IL-1B     | IL-6                   |
|----------------------------|------------------------|------------------------|------------------------|-----------|------------------------|
| Control                    | 1.00±0.02              | 1.04±0.14              | 1.01±0.07              | 1.07±0.18 | 1.00±0.04              |
| CBZ                        | 1.92±0.03 <sup>a</sup> | 0.95±0.03              | 0.42±0.05 <sup>a</sup> | 1.04±0.17 | 0.80±0.01 <sup>a</sup> |
| APAP                       | 0.85±0.08              | 1.01±0.11              | 0.58±0.04 <sup>a</sup> | 1.26±0.17 | 1.03±0.05              |
| REE                        | 0.96±0.04              | 0.92±0.05              | 0.56±0.08 <sup>a</sup> | 0.58±0.02 | 0.92±0.03              |
| CoQ <sub>10</sub>          | 0.98±0.03              | 0.83±0.05              | 0.55±0.07 <sup>a</sup> | 0.79±0.06 | 0.86±0.03              |
| CBZ+APAP                   | 1.05±0.01              | 0.93±0.05              | 0.39±0.01 <sup>a</sup> | 0.85±0.10 | 0.78±0.02 <sup>a</sup> |
| CBZ+APAP+REE               | 1.11±0.06              | 1.01±0.14              | 0.59±0.04              | 0.79±0.02 | 1.00±0.02              |
| CBZ+APAP+CoQ <sub>10</sub> | 1.22±0.09              | 1.54±0.14 <sup>b</sup> | 0.32±0.03              | 0.67±0.16 | 0.79±0.02              |

Data are expressed as mean±SEM (n=3). <sup>a</sup>*p*<0.05 compared to control group, <sup>b</sup>*p*<0.05 compared to CBZ+APAP group.

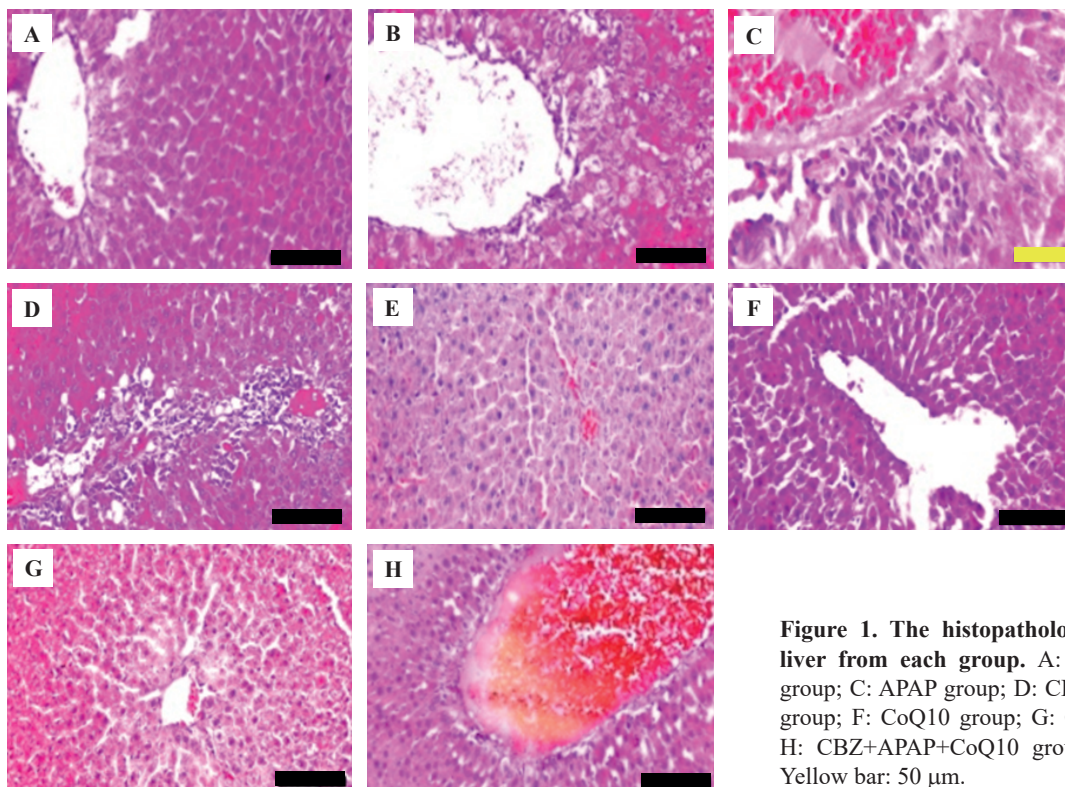
**Pharmacokinetics of APAP and CBZ**

The HPLC results (Figure 2A) showed that APAP was eliminated after 6 hours. The combined administration of CBZ with APAP extended the elimination time of APAP from the body to 24 hours. Adding REE to the combined treatment of CBZ+APAP helped in almost restoring the normal elimination of APAP and reduced it to 9 hours. The plasma clearance time of CBZ when administered alone was 9 hours (Figure 2B). Similarly, this time was extended to 24 when APAP was administered in combination with CBZ. In a repeated scenario, REE shortened this time to 9 hours

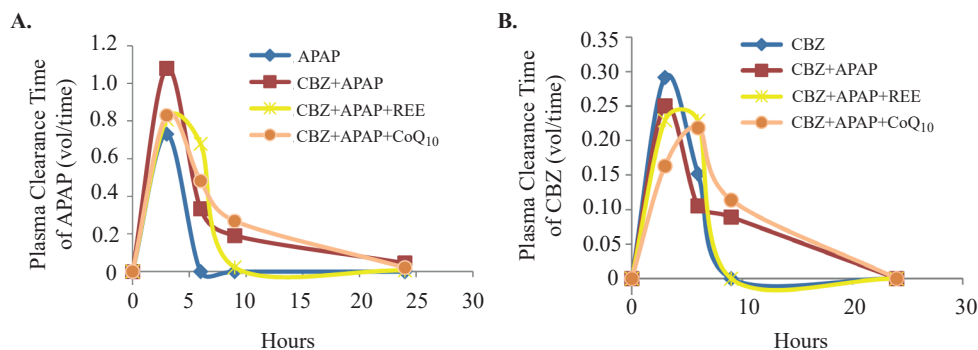
when given along with CBZ+APAP. CoQ<sub>10</sub> was devoid of any significant effect on the pharmacokinetic elimination of either APAP or CBZ when administered along with CBZ+APAP.

**Discussion**

The main goal of the present study was to investigate the potential of REE and CoQ<sub>10</sub> to reduce the toxicity occurred due to the simultaneous administration of CBZ and APAP.



**Figure 1. The histopathological alterations in the liver from each group.** A: Control group; B: CBZ group; C: APAP group; D: CBZ+APAP group; E: REE group; F: CoQ<sub>10</sub> group; G: CBZ+APAP+REE group; H: CBZ+APAP+CoQ<sub>10</sub> group. Black bar: 200 µm; Yellow bar: 50 µm.



**Figure 2.** The effect of different treatments on the plasma clearance time as determined by HPLC. A: Plasma clearance time of APAP; B: Plasma clearance time of CBZ.

APAP is the most used analgesic and antipyretic all over the world by both adults and children.(23) The use of APAP is intensified during the last few years due to COVID pandemic. Although APAP is safe, the overdose or slow metabolism and hence accumulation can cause severe hepatotoxicity. APAP is the main cause of liver failure in the USA.(24) The metabolism of a therapeutic dose of APAP mainly occurs in the liver through glucuronidation and sulfation. Overdose or accumulated APAP will be metabolized into NAPQI resulting in depletion of GSH and antioxidants, oxidative stress, lipid peroxidation, and inflammation.(3,4)

Administration of CBZ alone could lead to oxidative stress. The co-administration of CBZ and APAP at therapeutic levels was reported to cause acute hepatic toxicity.(8) REE and CoQ<sub>10</sub> insignificantly reduced the body weight gain by 30 and 35%, respectively but when combined with CBZ+APAP, they caused significant reductions (~57 and 113% compared to the control), and (~63 and 111% compared to the CBZ+APAP group). These reductions were not associated with any sign of toxicity; morphological, biochemical, or histopathological. Therefore, we think these reductions could be attributed to the ability of rosemary and CoQ<sub>10</sub> to reduce calorie intake, induce satiety centers, and/or accelerate the metabolic rate. These results are in agreement with a previous investigation.(25) CBZ alone or combined with APAP resulted in hepatomegaly and increased the relative liver weight by 35 and 32%, respectively. This was manifested by the elevation of ALT activity caused by CBZ or APAP alone or in combination CBZ+APAP indicating hepatotoxicity. Similar results were previously reported.(6,26) This hepatotoxicity was also typified by the histopathological examination where the groups treated with CBZ, APAP, or CBZ+APAP showed severe congestion in the central vein associated with ballooning degeneration in the surrounding adjacent hepatocytes with focal aggregation of inflammatory cells in the hepatic parenchyma with some

necrotic cells. These histopathological deteriorations were reported in rats after treatment with CBZ (6) or APAP (27). On treating rats with CBZ+APAP in addition to REE or CoQ<sub>10</sub>, the ALT activity was reduced by ~50% indicating the potential of both REE and CoQ<sub>10</sub> to protect against the hepatotoxicity caused by CBZ and APAP possibly through preventing the accumulation of NAPQI. Similar effects were reported in rats for REE (28) and CoQ<sub>10</sub> (14) against hepatotoxicity induced by APAP.

The combined administration of CBZ+APAP resulted in depletion of GSH probably due to the accumulation of NAPQI. CBZ+APAP also resulted in the reduction of GPx and GST activities in liver and elevated the MDA level. GSH is an essential coenzyme needed by GPx and GST to remove hydrogen peroxide, lipid peroxides, and electrophiles.(29) Therefore, GSH reduction would reduce the activity of these GSH-dependent enzymes. This would leave the cell vulnerable to oxidative stress and would lead to lipid peroxidation and elevation of MDA.(30) REE and CoQ<sub>10</sub> administered to the CBZ+APAP groups, were able to modulate the lipid peroxidation and reduce the MDA by 60 and 53%, respectively, and elevate the GSH level by 88 and 75%, respectively, as in line with the result of previous study.(31) Administration of REE and CoQ<sub>10</sub> to the CBZ+APAP groups significantly aggravated the reduction of GST activity in liver. The hepatic SOD activity in the current study was significantly reduced only by APAP which also caused a significant reduction in GST activity in agreement with a previous study.(32) GST was also reduced by CBZ alone. GST is responsible for the conjugation of electrophiles, and it is also dependent on GSH. Interestingly, when GSH is depleted or when the oxidative milieu prevails in the cell, GST could directly react with free radicals or electrophiles protecting the cell from oxidative damage and exhausted during the process.(33) However, this could not explain the aggravation resulted

after administration of REE or CoQ<sub>10</sub> which requires further investigations.

In the present study, only CBZ when given alone induced the gene expression of *CYP3A4*. CBZ is mainly metabolized in the liver by CYP3A4 to carbamazepine-10,11-epoxide<sup>12</sup>. The latter is further metabolized to its trans-diol form by epoxide hydrolase.<sup>(34)</sup> CBZ induces its own metabolism by induction of its metabolizing enzymes.<sup>(35)</sup> APAP is metabolized into NAPQI by CYP3A4 and CYP2E1.<sup>(3)</sup> CoQ<sub>10</sub> given to the CBZ+APAP was the only treatment that induced the expression of *CYP2E1*. The assumption that APAP would inhibit the metabolism and elimination of carbamazepine through inhibition of CYP3A4 was not verified at the genomic level in the current study. The possibility of epigenetic or proteomic effects could not be ruled out but this possibility was not investigated in the current study. The therapeutic dose of APAP in this study did not induce the expression of *CYP2E1*. The protective effects of CoQ<sub>10</sub> could be at least partially attributed to its induction of the metabolism of APAP by CYP2E1 preventing its accumulation and interaction with CBZ.

The main action of APAP is its inhibitory effect on arachidonic acid metabolism and formation of prostaglandins. However, contradictory reports have shown that APAP could be deacetylated in the liver forming p-aminophenol that causes toxicity, and it also acts as a pro-inflammatory mediator in rats.<sup>(36)</sup> We have measured the expression of some pro-inflammatory cytokines. None of the treatments has affected the expression of *IL-1B*. CBZ, APAP, REE, CoQ<sub>10</sub>, and CBZ+APAP have all reduced the expression of *TNF-α*. CBZ and CBZ+APAP have reduced the expression of *IL-6*. *TNF-α* and *IL-6* are pro-inflammatory and apoptotic cytokines involved in many inflammatory and autoimmune diseases.<sup>(37)</sup> CBZ and APAP were reported to reduce *TNF-α* and *IL-6* levels before.<sup>(38)</sup> The anti-inflammatory effects of REE and CoQ<sub>10</sub> were also reported where they reduced the levels of many pro-inflammatory cytokines including *TNF-α* and *IL-6*.<sup>(39)</sup>

Since a mutual effect on the pharmacokinetics and elimination of APAP and CBZ is expected, we have measured the plasma clearance rate of these two drugs and investigated the effect of concomitant administration of REE and CoQ<sub>10</sub>. APAP was eliminated after 6 hours but this time was extended by the combined administration of CBZ with APAP to 24 hours. Only REE was able to restore the normal elimination rate of APAP and reduced it to 9 hours. APAP is highly absorbed and not widely bound to plasma proteins and has a half-life 4-8 hours.<sup>(23)</sup> The half-life of CBZ was 9 hours. The reported plasma half-life in

literature varies from 9-36.8 hours depending on the dosing frequency and duration.<sup>(40)</sup> Similarly, the half-life time of CBZ was prolonged to 24 when APAP was administered in combination with CBZ. Again, REE shortened this time to 9 hours when given along with CBZ+APAP. This effect of REE merits further investigations to explore the possible mechanisms. The potential protective effect of REE against hepatotoxicity in the present study could be attributed to its ability to reduce the prolonged plasma half-life of APAP and CBZ.

## Conclusion

CBZ+APAP caused hepatotoxicity, lipid peroxidation, oxidative stress, and disturbed the tissue architecture of liver. They reduced the pro-inflammatory cytokines. REE and CoQ<sub>10</sub> were able to alleviate most of these detrimental effects through enhancing the cellular antioxidant milieu, induction of metabolizing enzymes, reduction of the plasma half-life of APAP and CBZ preventing their accumulation and potential interaction. In many instances, REE offers superior protection to that of CoQ<sub>10</sub>.

## Authors Contribution

WME was involved in concepting and planning the research. MMH, NIZ, and SAN performed the data acquisition/collection. MMH, NIZ and WME calculated the experimental data and performed the analysis. MMH and WME drafted the manuscript and designed the figures. NIZ aided in interpreting the results. All authors took part in critical revision of the manuscript.

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