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*Original Article*

# Effect of epigallocatechin gallate on uncoupling protein 2 in acute liver injury

Mohammad H Jamal[1*], Hamad Ali[2], Ali Dashti[2], Jasim Al-Abbad[1], Husain Dashti[1], Chako Mathew[2], Waleed Al-Ali[3], Sami Asfar[1]

*[1]Department of Surgery, Faculty of Medicine, Health Sciences Center, Kuwait University, Kuwait; [2]Department of Medical Laboratory Sciences (MLS), Faculty of Allied Health Sciences, Health Sciences Center, Kuwait University, Kuwait; [3]Department of Pathology, Faculty of Medicine, Health Sciences Center, Kuwait University, Kuwait. [*]Equal contributors.*

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**Abstract:** Background: The aim of this study was to investigate the effect of epigallocatechin gallate (EGCG) on uncoupling protein 2 regulation in an acute liver injury-animal model. Methods: Twenty seven male Wistar rats were divided into three groups: control group (n = 9), TAA group (n = 9): acute liver injury was induced by the intraperitoneal injection of thioacetamide (200 mg/kg) and EGCG/TAA (n = 9 rats): Epigallocatechin gallate was given two weeks prior to the induction of acute liver injury by thioacetamide. The levels of uncoupling protein 2, CRP, TNF-α and interleukins (IL) 6 and 18 were analyzed in the liver using PCR analysis. Results: Q-PCR analysis showed that the genetic expression of UCP2, TNF-α and CRP in the EGCG/TAA group was the least in comparison to other groups ($P \leq 0.005$). The IL-6 and IL-18 were upregulated after induction of acute liver injury, but this upregulation was significantly less in the group that received epigallocatechin gallate (EGCG/TAA) compared to the TAA group. In addition, histological examination showed a reduction in hepatocyte injury in EGCG/TAA compared to the TAA group. Conclusion: Epigallocatechin gallate administration prior to induction of acute liver injury down-regulates uncoupling protein 2 expression and reduces IL-6, IL-18, TNF-α and CRP.

**Keywords:** Green tea, hepatitis, epigallocatechin gallate, uncoupling protein 2

## Introduction

Green tea (Camellia sinensis) is a widely consumed beverage known for its beneficial effects on health. It exhibits anti-inflammatory and anticarcinogenic effects, and is shown to protect against all stages of carcinogenesis including initiation, promotion and progression [1].

Green tea contains caffeine and polyphenolic compounds known as catechins. The ingredients contributing the most to the desired health effects of green tea are the catechins which contribute to 10% of the dry weight of green tea. The most abundant of these catechins, is Epigallocatechin-3-gallate (EGCG) which represents 50% of catechins found in green tea.

EGCG is a potent antioxidant and is shown to protect against hepatic reperfusion injury, liver fibrosis and alcoholic hepatitis. A green tea extract was also shown by Nakamoto et al to prevent liver dysfunction, increase plasma antioxidant and suppressing mitochondrial ROS production in non alcoholic steatohepatitis (NASH) rats [2].

We have previously shown the beneficial effects of green tea (the most active ingredient of which is EGCG) on thioacetamide-induced hepatitis in which a reduction in the AST/ALT ratio was observed as well as improvement in the hepatic architecture at the initial stages of thioacetamide toxicity [3].

Thioacetamide causes a major oxidative stress by the formation of a toxic metabolite (thioacetamide-s-oxide) which causes cell membrane injury by interfering with the movement of RNA from the nucleus to the cytoplasm [4]. It also initiates the process of lipid peroxidation (LPO) in the liver, which leads to hepatotoxicity and

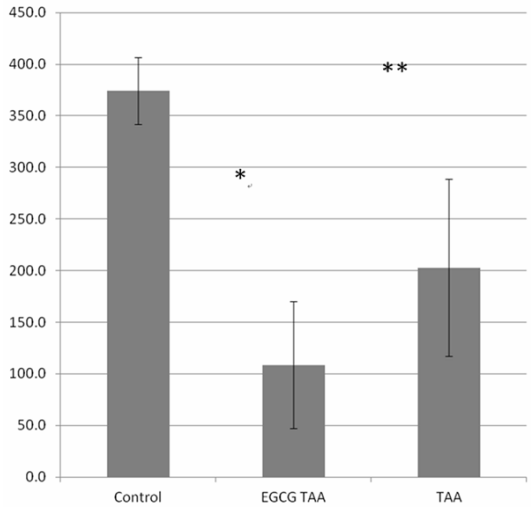


**Figure 1.** Functional study of Uncoupling Protein 2. *P* < 0.05 was considered to be statistically significant: *compared to control group; **compared to control or EGCG/TAA group.

cell death by oxidative stress which is known to increase the pro-oxidant to antioxidant ratio. The increase in lipid peroxy radicals damages the hepatocytes by increasing cell membrane permeability and decreasing its fluidity along with the inactivation of membrane proteins. The theory behind EGCG protection against thioacetamide-induced hepatotoxicity, is that it reduces the process of LPO through increasing the antioxidant to pro-oxidant ratio in favor of antioxidants [5].

In this study we aim to examine the effect of EGCG at the molecular level by examining its effect on the uncoupling protein 2 (UCP2), which regulates the mitochondrial production of oxygen free radicals.

## Methods

Twenty seven male Wistar rats (11-12 weeks of age, wt = 200-250 g) locally bred in the animal facility, Health Sciences Center, Kuwait University. Rats were housed (4-5 animals per cage) in standard plastic cages with wood chip bedding. The animals were kept in well-ventilated rooms with adjustable light-dark cycle and temperature regulation systems. The rooms and animal cages were cleaned daily, along with the bedding for all animals to maintain sanitary conditions. All animals were provided with rat chow and water on a daily basis. They were also inspected for any possible signs of inflammation, respiratory or gastrointestinal infection. If such signs develop, the animal was excluded from the study.

The animals were divided into three groups each containing nine rats; control group, TAA group (acute liver injury), EGCG/TAA group. The control group received standard laboratory diet for two weeks prior to starting the experiment and afterwards. The TAA group received standard laboratory diet for two weeks prior to induction of acute liver toxicity with intraperitoneal (i.p) Thioacetamide 200 mg/kg. The EGCG/TAA received EGCG 20 mg/kg daily dissolved in normal saline solution by gavage feeding for two weeks in addition to standard laborartory diet prior to induction of acute liver toxicity with i.p, Thioacetamide 200 mg/kg.

Animals from each group were sacrificed at 72 hours; their Livers were collected and immediately frozen in liquid nitrogen and stored at -80C for subsequent analyses. The levels of UCP2, C reactive protein (CRP), Tumor necrosis factor Alpha (TNF-α), interleukins (IL-6, IL-18) were assessed in the liver using PCR analysis. In addition enzyme linked immunosorbent assay (ELISA) was used to measure the levels of UCP2 in the liver. Sections for the liver in all groups were stained with standard hematoxylin and eosin staining (H&E) to study the hepatocytes architecture.

*RT-PCR analysis*

Total RNA was extracted from the liver of mice using the Trizol reagent (Sigma, US) based on the manufacturer's instructions. One microgram of total RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, US) according to manufacturer's protocol. Quantitative real-time PCR was performed with a 7500 Realtime PCR system (Applied Biosystem, US) using 5x HOT FIREPOL Eva Green Qpcr Mix Plus (ROX) (Solis BioDyne, Estonia). The cDNA product was amplified in a total volume of 20 µl with 10 pmol for each primer. All the primers were designed to anneal and amplify only mRNA. The sequence primers used are: UCP2 (forward) 5'-GCAGTTCTACACCAAGGGCT-3', UCP2 (reverse) 5'-GGAAGCGGACCTTTACCACA-3'; IL6 (forward) 5'-CACTTCACAAGTCGGAGGCT-3', IL6 (reverse) 5'-TCTGACAGTGCATCATCGCT-3'; IL18 (forward) 5'-ACCGCAGTAATACGGAGCAT-3' IL18 (reverse) 5'-TCTGGGATTCGTTGGCTGTT-3'; TNF-α (forward) 5'-CATCCGTTCTCTACCCAG-

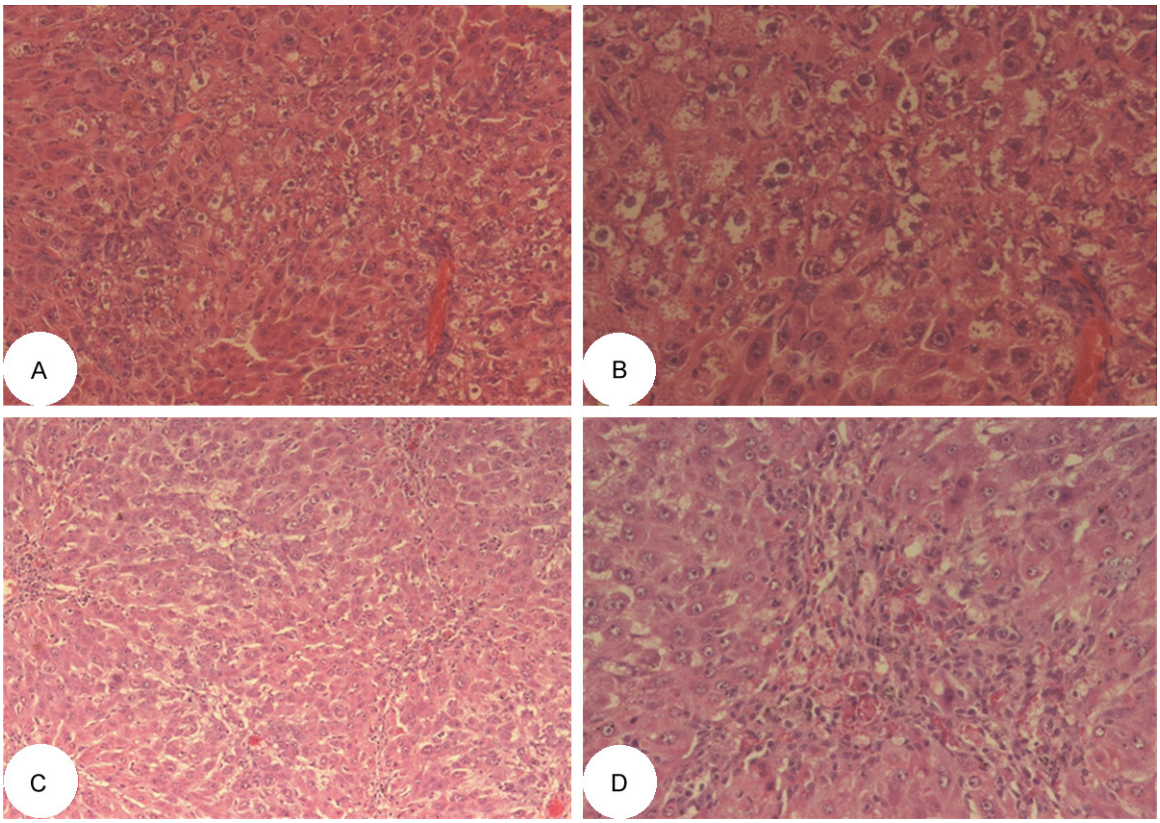


**Figure 2.** At 72 hours: Haematoxylin and eosin stained paraffin sections showing the hepatic structure of the TAA group (A, B) and EGCG/TAA group (C, D). Note the early recovery from thioacetamide induced changes in the EGCG/TAA group (C, D) as compared to the TAA group.

CC-3', TNF-α (reverse) 5'-AATTCTGAGCCCGG-AGTTGG-3'; CRP (forward) 5'-GCAGTAGGTGG-GCCTGAAAT-3', CRP (reverse) 5'-CCCGTCAA-GCCAAAGCTCTA-3'; GAPDH (forward) 5'-CTC-AGTTGCTGAGGAGTCCC-3', GAPDH (reverse) 5'-ATTCGAGAGAAGGGAGGGCT-3'. Expression level of all primers was normalized to GAPDH. A negative control for PCR consisted of omitting cDNA in the reaction tube.

*ELISA analysis*

Samples were homogenized with cellular phosphate-buffered saline (PBS) and their protein concentrations measured. Assay for UCP2 was performed by ELISA in the liver homogenate in all the groups by commercially available kit (R&D Systems, USA) according to the manufacturer's instructions.

*Statistical analysis*

Student's t-test and Anova tests were used to obtain *P* values between groups. Results with *P* values of less than 0.05 were considered statistically significant.

## Results

Elisa analysis revealed that in the EGCG/TAA group, the levels of UCP2 were the lowest when compared to other groups ($P \leq 0.005$) (**Figure 1**). H&E staining showed a reduction in hepatocyte injury in EGCG/TAA group when compared to the TAA group (**Figure 2**).

Q-PCR analysis showed that in acute liver injury group (TAA) there was an upregulation of UCP2 and CRP compared to the control group. In the ECGC treated group (EGCG/TAA) there was a significant reduction in the genetic expression of UCP2 and CRP compared to the TAA and control groups (**Figure 3**). The IL-6 and IL-18 were upregulated after induction of acute liver injury, but this upregulation was significantly less in the group that received EGCG (EGCG/TAA) compared to the TAA group (**Figure 3**). For TNF-α both EGCG/TAA treated group and TAA group

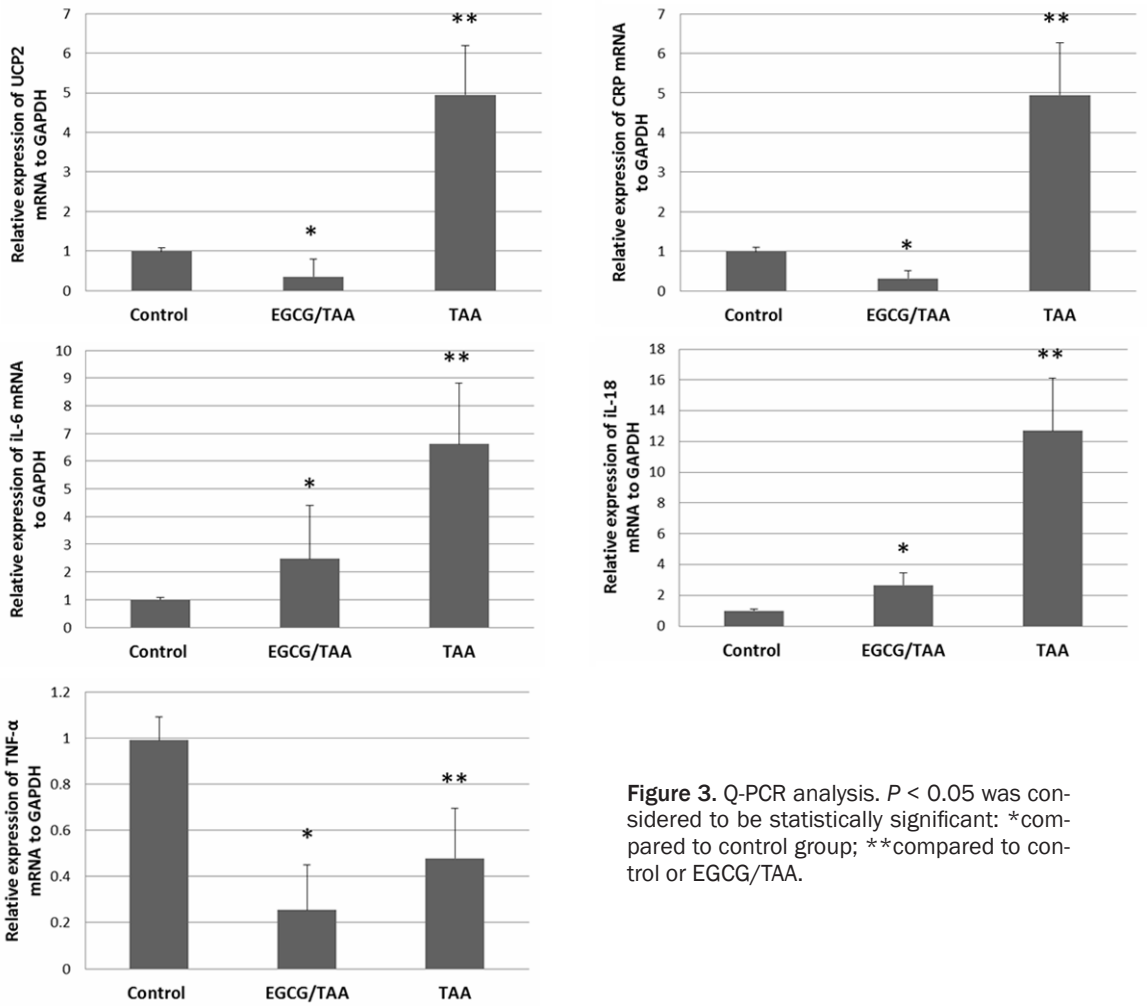


**Figure 3.** Q-PCR analysis. $P < 0.05$ was considered to be statistically significant: *compared to control group; **compared to control or EGCG/TAA.

showed down-regulation in gene expression in comparison to the control group with the down-regulation more significant in the EGCG/TAA treated group (**Figure 3**). All results shown are statistically significant (P < 0.05, Anova signal factor).

## Discussion

The uncoupling proteins are a family of mitochondrial inner membrane proteins that function to induce nonshivering thermogenesis by uncoupling the mitochondrial electron transport from ATP synthesis [6]. UCP2 was studied in non-alcoholic steatohepatitis and in fatty liver disease, as it was shown to play a role in fatty acid metabolism. Jiang et al examined the UCP2 expression in rats fed with high fat diet for 12 weeks and found that in rats developing NASH, the expression of UCP2 was 3.5 fold higher than in rats without NASH [7]. The uncoupling proteins were shown to be increased in obese ob/ob and db/db deficient mice, which are genetically obese due to leptin hormone resistance and are prone to fatty liver disease [8]. It was also shown that livers of lean mice contain lower levels of UCP2 than livers of mice with fatty liver disease and in mice fed with ETOH [9]. This evidence indicates a rule for UCP2 in the pathology of liver diseases but may not necessarily indicate that a reduction in UCP2 is associated with improved outcomes; however UCP2 is not well studied in other models of hepatitis.

In a previous study from our group we have shown that ingestion of green tea before inducing acute liver injury by TAA halted the acute injury to the hepatocytes as shown by fewer rises in liver enzymes and normalization of the hepatocytes morphology. In the current study, we aimed at finding at what cellular level does

green tea and its active ingredients induce these anti-inflammatory effects. We chose EGCG the active ingredient of green tea to study the effects on UCP2 expression and upregulation.

In this study we showed in an acute liver injury animal model that administration of EGCG before induction of acute liver injury will reduce UCP2 gene expression. It seems that the beneficial effects of EGCG ingestion involves not only its inherent nature as a potent antioxidant but also involves reducing the UCP2 expression which leads to significant reduction in the pro inflammatory markers and hence decreasing the magnitude of thioacetamide-induced liver injury.

Fulup et al [10] raised the question as to whether reducing the expression of UCP2 is beneficial in protecting against acute liver injury. They used ob/ob mice that were deficient for UCP2 and a group of ob/ob mice that were not deficient in UCP2. They challenged these animals with anti-*fas* antibody (Jo2) which induces death receptor mediated hepatocellular injury and fulminant liver failure in mice. They found that all the UCP2 deficient mice survived 24 hours after treatment with Jo2 but only one animal survived in the group that expressed UCP2. They reported worse acute liver injury in the latter group and found that UCP2 deficiency was protective against fas-mediated hepatocytes death. In addition they found that the baseline apoptosis was lower in the UCP2-deficient ob/ob mice. Despite these positive results, when they measured the degree of lipid peroxidation, they found that UCP2 deficiency did not confer any advantage. They contributed this finding to the reduction of kupfer cells in ob/ob mice, making the inhibition of UCP2 less effective in reducing lipid peroxidation. It is known that in normal livers the UCP2 is mostly expressed in kupfer cells, whereas in fatty livers the UCP2 is present in higher than normal concentration in the hepatocytes [11].

Shang et al used transgenic mice with targeted hepatocytes expression of UCP2 to study the rule of UCP2 in liver disease. They reported altered metabolic parameters in the mitochrondria of the transgenic mice, with increased state-4 respiration and lower ATP levels. After induction of liver injury with LPS and galactosamine (GalN), they found worse liver injury in the transgenic mice expressing UCP2 in their hepatocytes when compared to normal mice. More importantly, they pretreated a group of transgenic mice with genipin which is a UCP2 inhibitor, and found a reduction in apoptosis induced by LPS/GalN [12]. This is in accord with our findings whereby reducing UCP2 expression by EGCG had beneficial effects in the acute liver injury animal model. This was evident in the histology of the livers in this study, whereby the group that was pretreated with EGCG, had the lowest UCP2 expression and the least severe liver injury on H&E staining. We also observed lower proinflammatory IL-6 and IL-18 level in the group pretreated with EGCG.

To our knowledge this is the first report on the reduction of UCP2 by EGCG administration in the setting of experimental acute liver injury. However we acknowledge that these are only preliminary results and further testing is necessary to confirm our findings. Studies are underway in our lab to further elucidate the underlying molecular changes and the different caveats of UCP2, pro-inflammatory markers and EGCG effect. In conclusion, we found that EGCG is beneficial in thioacetamide-induced acute liver injury, by reducing the expression of UCP2 and proinflammmatory markers which could partly explain its protective mechanism of action.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Mohammad H Jamal, Department of Surgery, Kuwait University, PO. BOX 24923, SAFAT 13110, Kuwait City, Kuwait. Tel: +965-24636213; Fax: +965-25319597; E-mail: mjamal@HSC.EDU.KW

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