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Ameliorative impact of epigallocatechin-3-gallate against methionine induced toxicity

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Abstract: Hyperhomocysteinemia (HHcy) is associated with a variety of pathological processes. Epigallocatechin-3-gallate (EGCG), a plentiful catechin found in green tea, is a powerful natural antioxidant with a multitude of biological advantage The objective of this study was to determine whether EGCG could counteract liver function alterations brought on by methionine (Met). Met was infused into drinking water (1g/kg body weight) for 35 days to induce HHcy in adult male mice. In addition to Met, EGCG (5mg/kg body weight) was given orally every day. While IL-10 levels in serum of the Met group considerably decreased as compared to the control group, VCAM-1 and ICAM-1 serum levels were found to be greater in the Met group than in the control group. By improving the adhesion molecules VCAM-1 and ICAM-1 and increasing the anti-inflammatory cytokine IL-10, EGCG substantially reduces inflammation. The ALT, AST, and bilirubin levels in the serum considerably elevated whereas albumin and total protein levels dropped significantly in the HHcy mice treated with Met. EGCG improved albumin, total protein, ALT, AST, and total bilirubin, which helped to correct liver function markers. In conclusion, HHcy induction by Met cause liver parameter alterations and inflammation. The liver's biochemical changes brought on by HHcy can be significantly prevented by EGCG.

keywords: Methionine, liver injury, inflmmation, hyperhomocysteinemia

1.Introduction

Homocysteine (Hcy) is a sulfhydryl-containing amino acid produced during the metabolism of methionine (Met) [25, 40]. It is either degraded by the re-methylation pathway or converted to cysteine by the trans-sulfuration pathway [36]. The increased level of Hcy is known as hyperhomocysteinemia (HHcy). The main causes of HHcy are enzymes dysfunction, cofactors involved in homocysteine biosynthesis, and excessive methionine intake [20]. Hcy contains a reactive sulfhydryl group, thus can undergo rapid auto-oxidation in the presence of oxygen and metal ions, producing superoxide anion and hydrogen peroxide, resulting in oxidative stress and apoptosis [12]. Additionally, Hcy also upregulates the expression of NADPH oxidase, resulting in an increase in superoxide anion generation [19].

HHcy activates transcription factors in the liver, leading to increase the expression of HMG-CoA reductase and biosynthesis of cholesterol [49]. Additionally, [11] demonstrated that, Hcy is high in patient with nonalcoholic fatty liver. Moreover, [45] showed that HHcy induce liver toxicity and fibrosis by increase the expression of TIMP-1 and type I procollagen and [51] also found that HHcy induce liver steatosis in male mice by upregulate the expression of CD 36 which has effect on the activation of aryl hydrocarbon.

Epigallocatechin gallate (EGCG), the most prevalent antioxidant polyphenol flavonoid catechin in green tea leaves, exhibits outstanding physiological activity and exhibits anti-inflammatory, anti-cancer, antioxidant, and antiviral actions. [37]. Moreover, [44] demonstrated that, EGCG can attenuate liver fibrosis by reducing the collagen formation in liver. EGCG consists of four rings resulting from esterification of EGC with gallic acid (Fig.1) [9]. Furthermore, EGCG, which has eight hydroxyl groups and a gallate moiety at

C-3, is a better electron donor than the other catechins, making it the best free radical scavenger [17]. The capacity of EGCG to bind metal ions such as iron and copper, which are catalysts in free radical formation, contributes to its antioxidant effect. Thus, EGCG may trap peroxyl radicals, breaking the free radical chain reaction [38, 34]. This study aimed to investigate the protective effect of EGCG on HHcy induced liver toxicity.

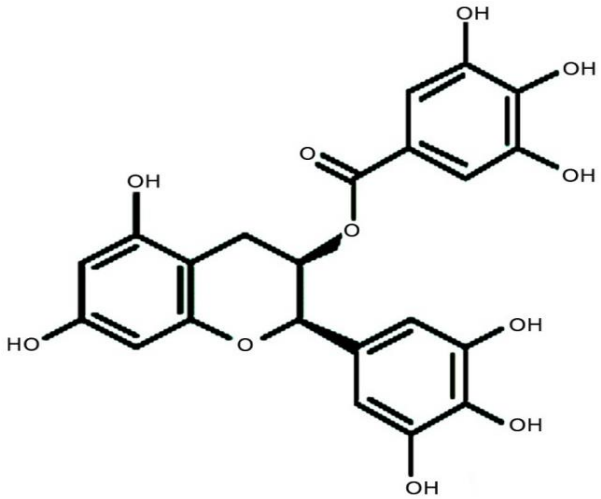

Fig.1: Chemical structure of epigallocatechin-3-gallate [50]

2. Materials and methods

Chemicals

L-methionine (Met) and epigallocatechin-3-gallate (EGCG) were purchased from Sigma (St. Louis, MO, USA).

Animals

Male albino mice weighing 20 ± 5 g, obtained from (VACERA, Cairo, Egypt) and housed in the animal house, Department of Zoology, Faculty of Science, Mansoura University. Mice were given two weeks to get used to the typical lab settings. They experiment were performed at a constant temperature of 25 to 30 °C, with a 12-hour light/ 12-hour dark cycle and free access to standard commercial mice chow diet and water. All experiments and animal's care were performed in accordance with the Institutional Animal Ethics committee (IAEC) of Mansoura University, Egypt (approval number Sci-Z-M-2021-27).

Experimental design and animal treatment

Post acclimatization, mice were randomized into 4 groups of 6 animals each using as follow.

Group 1: Control mice fed a regular diet. According to [13], group 2 mice received EGCG (5 mg/kg body weight) orally through a stomach tube for 35 days. Group 3: In accordance with [13, 2], mice received Met dissolved in drinking water (1g/kg body weight) every day for 5 weeks. Group 4: For 5 weeks, mice got EGCG and Met every day concurrently. To modify the Met dosage, the animals were weighed. Additionally, body weights were monitored throughout a 5-week period.

Sample collection

Mice were fasted overnight at the end of experiment. The next day, mice underwent intraperitoneal ketamine/xylazine anaesthesia (0.1 mL/100g body weight) [6]. Blood were collected then centrifuged to obtain sera and kept at – 20 ºC until further analysis.

Biochemical investigation

The serum levels of IL-10, VCAM-1 and ICAM-1 were determined quantitively using sandwich enzyme immunoassay technique in accordance with instruction procedure of the kit (Catalog #: ELM-IL10, ELM-VCAM1, ELM-ICAM1) purchased from RayBiotech (Norcross, GA, USA). ALT, AST, albumin, total protein, and total bilirubin were determined serum in accordance with the colorimetric method of the kits (Catalog #: AT 10 34 (45), AB 10 10, TP 20 20, and BR 1111 respectively).

Statistical analysis

GraphPad Prism 8.0 was used for all statistical analysis. The mean and standard error of the mean (SEM) are used to represent all data. One-way analysis of variance (ANOVA) was used to perform statistical comparisons, and then Tukey was used as a post-hoc test.

3. Results

The mean bodyweight of all experimental groups was determined weekly during experimental period and illustrated in (Fig.2). EGCG supplementation daily for 35 days did not affect the body weight gain. On the other hand, Met administration daily for 35 days resulted in a decrease in the body weight as compared to control group. In contrast, concurrent treatment with EGCG and Met

showed comparable body weight with control group.

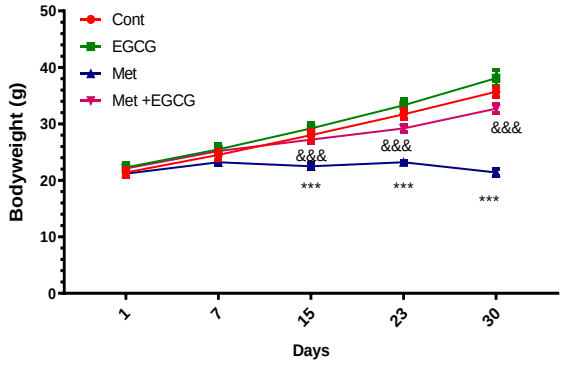

Fig. 2. Effect of Met on bodyweight (g).

Data are shown as mean ± SEM (*n* = 6).

***, $^{\&\&\&}$ Significant at *P < 0.001*.

*** Comparisons versus the control group.

$^{\&\&\&}$ Comparisons versus the methionine group.

Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate.

Obtained results showed an insignificant change in serum levels of VCAM-1, ICAM, and IL-10 of mice treated with EGCG (5 g/kg/day) alone for 5 weeks, as compared to control results In contrast, Met supplementation in drinking water resulted in significantly increase (*P<0.001*) in VCAM-1, ICAM-1 and decrease in (*P<0.001*) IL-10 as compared to control mice group. Whereas mice treated with Met + EGCG for 5 weeks, showed a very highly significant (*P<0.001*) decrease in serum levels of VCAM-1, ICAM-1 and increase (*P<0.01*) in IL-10 as compared to the Met-treated mice (Fig. 3,4, 5).

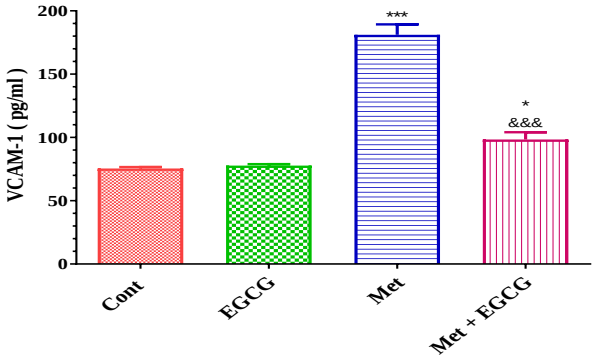

Fig. 3. Serum levels of VCAM-1 (pg/ml) in all experimental groups. Data are shown as mean ± SEM (*n* = 6). ***, $^{\&\&\&}$ Significant at *P < 0.001* and *significant at *P < 0.05*. ***,* Comparisons versus the control group. $^{\&\&\&}$ Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

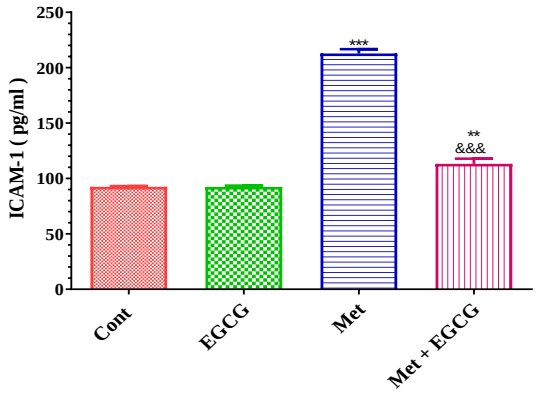

Fig. 4. Serum levels of ICAM-1 (pg/ml) in all experimental groups. Data are shown as mean ± SEM (*n* = 6). ***, $^{\&\&\&}$ Significant at *P < 0.001* and **significant at *P < 0.01*. ***,** Comparisons versus the control group. $^{\&\&\&}$ Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

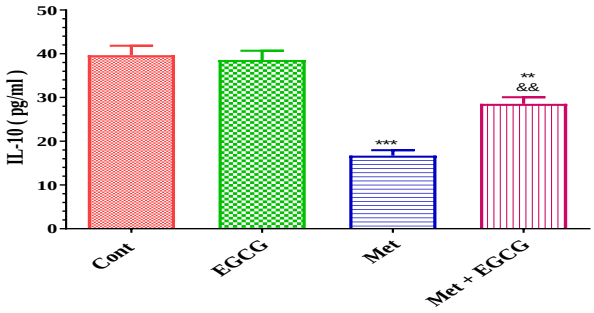

Fig. 5. Serum levels of IL-10 (pg/ml) in all experimental groups. Data are shown as mean ± SEM (*n* = 6). $^{\&\&\&}$ Significant at *P < 0.001* and **,$^{\&\&}$ significant at *P < 0.01*. ***,** Comparisons versus the control group. $^{\&\&}$ Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

The levels of ALT, AST and total bilirubin in serum were illustrated in (Fig.6,7,8). Treatment of mice with EGCG (5 mg/kg/day) alone for 5 weeks showed an insignificant effect on the levels of ALT, AST and total bilirubin in the serum when compared to the control group. In Met-treated group, there was a highly significant (*P<0.001)* increase in ALT, AST and total bilirubin levels in serum as compared

with control group. In group treated with Met + EGCG for 5 weeks, there was a very highly significant (*P<0.001; P<0.01*) decrease in ALT, AST and total bilirubin levels in serum as compared with Met group.

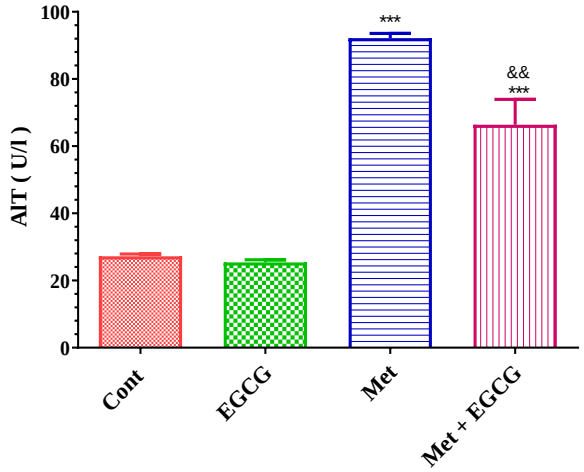

Fig. 6. Levels of ALT (U/l) in serum of all experimental groups. Data are shown as mean ± SEM (*n* = 6). ***, &&& Significant at *P < 0.001* and && significant at *P < 0.01*. *** Comparisons versus the control group. && Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

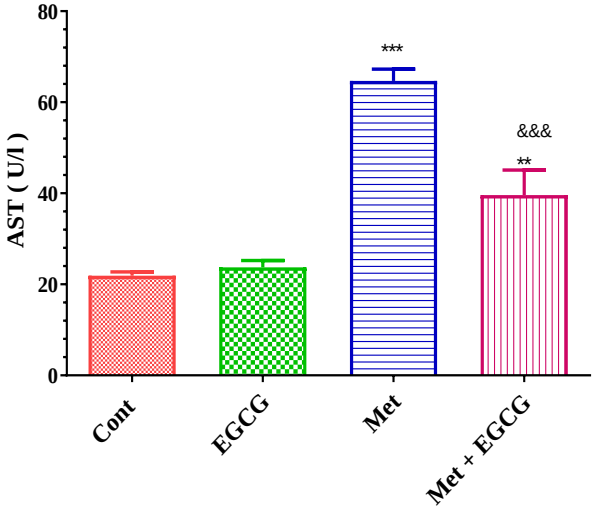

Fig. 7. Levels of AST (U/l) in serum of all experimental groups. Data are shown as mean ± SEM (*n* = 6). ***, &&& Significant at *P < 0.001* and ** significant at *P < 0.01*. ***,** Comparisons versus the control group. &&& Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

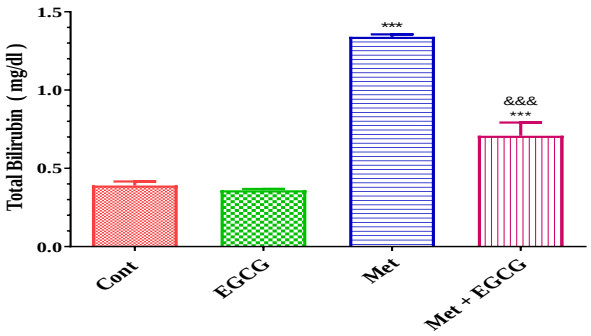

Fig. 8. Levels of total bilirubin (mg/dl) in serum of all experimental groups. Data are shown as mean ± SEM (*n* = 6). ***, &&& Significant at *P < 0.001*. *** Comparisons versus the control group. &&& Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

The levels of albumin and toral protein in serum were illustrated in (Fig. 9,10). Treatment of mice with EGCG (5 mg/kg/day) alone for 5 weeks had no significant effect in albumin and total protein levels as compared to control group. In HHcy, the levels of albumin and toral protein were significantly decrease (*P<0.001*) as compared to control group (Fig 5). In contrast, in mice treated with EGCG and Met together, the levels of albumin and toral protein were significantly improved (*P<0.01; P<0.001*) as compared with Met group.

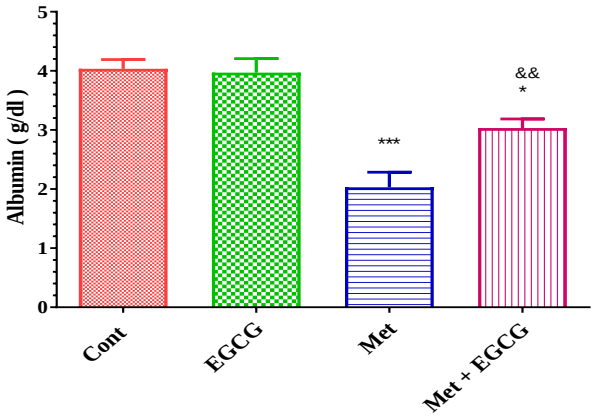

Fig. 9. Levels of albumin (g/dl) in serum of all experimental groups. Data are shown as mean ± SEM (*n* = 6). *** Significant at *P < 0.001*, && significant at *P < 0.01* and , * significant at *P < 0.05*. ***,* Comparisons versus the control group. &&, Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

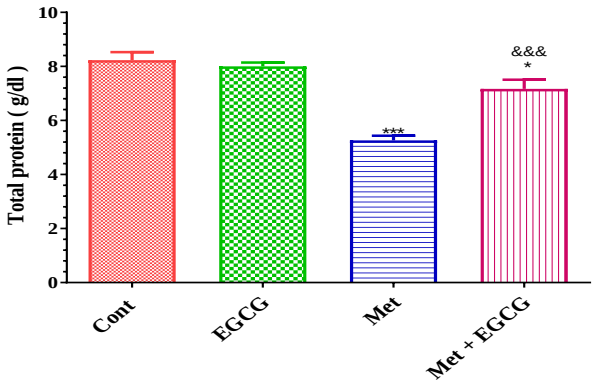

Fig. 10. Levels of total protein (g/dl) in serum of all experimental groups. Data are shown as mean ± SEM (n = 6). ***, &&& Significant at $P < 0.001$, and * significant at $P < 0.05$. ***,* Comparisons versus the control group. &&& Comparisons versus the methionine group. Cont control, EGCG epigallocatechin-3-gallate, Met methionine, Met + EGCG methionine + epigallocatechin-3-gallate

4. Discussion

Hyperhomocysteinemia (HHcy) has been identified as a biomarker of cardiovascular disease, and liver fibrosis [43]. Experimental animal models are important strategies for examining the pathophysiological processes underlying these diseases. Liver regarded as important sites for all metabolic process of the body. Hepatoxicity is associated with the exposure of liver to toxic chemicals. Thus, liver injury is associated with deterioration of it is function [47]. In this regard, we induce HHcy by Met administration in drinking water for 35 days. Administration of Met has been reported to induce HHcy [21]. According to several evidence, Hcy has a major effect in inducing hepatoxicity [16]. The liver is an important organ of most of the body's Hcy and dietary methionine metabolism and production [32].

Recently, [4] provided that the use of natural polyphenols form medicinal plants has a potential effect in the treatment of hepatotoxicity for a long time.

The present study revealed that, when compared to the control group, body weight gain was considerably reduced in mice treated with Met. These findings align with [30]. Also, [12] reported that mice treated with Met (1g/kg) for 30 days showed a significant decrease in their body weight. These findings could be related to methionine's ability to increase metabolic rate, decrease fat deposition, increase lipolysis, and increase energy expenditure [39].

Hcy act as a marker of inflammation [28]. Moreover, [33] reported that chronic HHcy were able to develop liver fibrosis, elevated oxidative stress, and inflammation. [48] reported that auto-oxidation of Hcy lead to the formation of superoxide anion and induction of hepatic NADPH oxidase which associated with cell injury, inflammation and cell death. Our results demonstrated that HHcy elevated the serum levels of VCAM-1 and ICAM-1 in serum. Previously, [5] and [8] reported that Hcy stimulate the expression of VCAM and ICAM-1. Moreover, large number of pro-inflammatory markers are produced during inflammation, these cytokines may induce the expression of adhesion molecules[18].

showed that expression of VCAM-1 and ICAM-1 were positively correlated with liver function. Additionally, [24] [22] reported that induction of homocysteine in rat model is associated with the increase of TNF-α. [23] showed that TNF-α significantly increase the expression of ICAM-1 and VCAM-1. It is essential to halt the damaging consequences of the inflammatory cascade that cytokines trigger. The inflammatory response was significantly reduced as a result of the oral EGCG therapy. These findings support those of [31] suggesting that EGCG reduce the expression of VCAM-1 by inhibiting IL-1β. Also, [26] demonstrated that EGCG intake inhibit the induction of ICAM-1 by inhibiting TNF-α. In a prior study, pancreas damage in rats the levels of TNF-, IL-1, and IL-6 were significantly lowered by treatment with EGCG [14]. In another study, EGCG treatment modulate the gene expression of NF-kB, TNF-α and IL-1β in liver injury induced by bile duct ligation [41]. More recently endothelial inflammation in human endothelial cells can be protected by EGCG which attenuates the expression of NF-kB and other inflammatory modulators including acute phase protein, IL-6, and VCAM-1[3]. In another study, in serum of mice exposed to arsenic, EGCG decreased the levels of IL-1β, IL-6 and TNF-α [52].

ALT, AST, albumin are a good marker for screening liver disease[29]. These enzymes are released into the blood stream as a result of

liver cell destruction. An increase in serum AST levels indicates liver injuries, including infarction and muscle damage, that are similar to viral hepatitis. A useful biomarker of hepatic damage is ALT, which is specific to the liver and mediates the conversion of alanine to pyruvate and glutamate. Increased levels of these enzymes are a sign of cellular infiltration and liver cell membrane dysfunction[10].

In the precent study, there was a significant increase in AST and ALT activities in HHcy mice. [1] suggested that HHcy cause endoplasmic reticulum stress and alteration in the levels of ALS and AST in blood. Also, [46] demonstrated that, injection of Hcy in neonatal rats, resulting in increases the levels of ALT,AST, total bilirubin with changes in the levels of albumin. On the other hand, oral treatment of EGCG normalized the levels of theses markers in serum as compared to control group. According to Li *et al.* (2016) [27], EGCG enhanced the levels of theses liver biomarkers in serum, showing its hepato-ameliorative effect. In recent study, In a recent study, EGCG reduced liver damage as seen by an improvement in ALT and AST activities [42]. Moreover, [15] reported that, increased level of Hcy may associated with the deceased level of albumin. Increased Hcy levels and the risk of (micro) albuminuria are linked through an unknown pathophysiological route. Some evidence suggest that the disturbance of albumin levels may be related Hcy induced oxidative stress[35]. [7] suggested that, EGCG treatment modulates the levels of albumin. **In conclusion,** EGCG ameliorate liver toxicity in HHcy model. These findings point to the possible moderating effects of EGCG in the treatment of diseases with high Hcy levels.

5. References

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