

Green Tea Attenuates Experimental Hepatitis in Context of Oxidative Stress

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ARTICLE INFO

Article history:

Received on: 12/05/2013

Revised on: 19/10/2013

Accepted on: 07/12/2013

Available online: 31/12/2013

Key words:

Experimental hepatitis;
paraoxonase; coenzyme Q10;
HPLC; oxidative stress; green
tea.

ABSTRACT

Food derived antioxidants have a strong potential for long term use as chemo-preventive agents in disease states involving oxidative stress such as tea. This study was done to clarify the potential effect of green tea extract in protecting the liver from lipopolysaccharide (LPS)-induced hepatitis in D-galactosamine (D-GalN) sensitized rats. Forty male albino rats weighing 180 - 200 g were divided into four groups; group I: healthy rats received vehicle only, group II: healthy rats received green tea (GT) extract orally for thirty days, Group III: healthy rats received vehicle only before induction of hepatitis. Group IV: healthy rats received green tea extract orally for thirty days before induction of hepatitis. After the experimental period, serum liver enzymes and liver oxidant /antioxidant profile were determined. Liver coenzymeQ10 (CoQ10) was estimated by HPLC method using C18 column and UV detector at 275 nm. The current date appeared the effective role of green tea extract in protecting the liver against the injury induced by D-GalN/LPS which may be attributed to polyphenolic compounds that scavenge a wide range of free radicals and increasing the antioxidant enzymes.

INTRODUCTION

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from liver disease (Fyiad *et al.*, 2012). Among the numerous models of experimental hepatitis, D-Galactosamine (D-GalN) induced liver damage is similar to human viral hepatitis in its morphological and functional features (Shivashengari *et al.*, 2006). Lipopolysaccharide (LPS) a major component of the outer membrane of Gram-negative bacteria is an endotoxin that is thought to contribute significantly to hepatic failure (Lee *et al.*, 2010). Galactosamine-induced dismutase; is generally attributed to the formation of the highly reactive hydroxyl radical (OH \cdot) which leads to severe oxidative damage of the liver cells' components like lipids, proteins and DNA (Mckillop and Schrum, 2005). In addition, D-galactosamine (D-GalN) together with lipopolysaccharide (LPS) can lead to pronounced secretion by Kupffer cells of pro-inflammatory mediators, which have been shown to be early and important mediators of liver injury (Osman *et al.*, 2007). Food derived antioxidants have a strong potential for long term use as chemo-preventive agents in disease

states involving oxidative stress such as tea which is a food source rich in phenolic compounds that has many important functions including antioxidant, antimutagenic & antiinflammatory activities (Othman *et al.*, 2007). Paraoxonases (PONs) are a family of three enzymes termed PON1, PON2 and PON3, (Aviram *et al.*, 2004), the best known among the paraoxonases is PON1 which degrades oxidized phospholipids in low-density lipoproteins (LDL) and HDL and, as such, plays a role in the organism's antioxidant system (Mackness and Durrington, 1995). Alterations in circulating PON1 levels are associated with a variety of diseases involving oxidative stress (Mackness *et al.*, 2006 and Marsillach *et al.*, 2008). The liver plays a key role in the synthesis of PON1 (Leviev *et al.*, 1997) and chronic liver diseases are associated with increased oxidative stress and lipid peroxidation, also a reduction of serum PON1 (Marsillach *et al.*, 2009). Coenzyme Q10 (CoQ10) is an essential component of oxidative phosphorylation at mitochondrial level, and also functions to stabilize cell membranes as well as acting as a potent antioxidant antihypertensive, anti-atherogenic, neuro-protective & bioenergetic (Littarru, 1993). Indeed, it affects the function of all cells in the body, making it essential for the health of all tissues and organs (Cheng *et al.*, 2010). From this point of view, we aimed to evaluate the protective role of green tea against experimental hepatitis with emphasis on CoQ10 and paraoxenase status.

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MATERIALS AND METHODS

Materials

Chemicals

D-galactosamine Hydrochloride (DGalN), lipopolysaccharide (LPS) and CoQ10 standard for HPLC were purchased from Sigma-Aldrich Company, St. Louis, MO, USA. Green tea was purchased from local market.

Animals

Male albino rats weighing 180 - 200 g were obtained from the animal house of National Research Center, Giza, Egypt. The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of the National Research Centre (NRC).

Methods

Plant extraction

Water extract of the green tea was prepared by adding 200 ml deionized water at 90°C into a glass containing 2 g of green tea (Majchrzak *et al.*, 2004).

Induction of hepatitis

LPS was dissolved in normal saline 0.9% w/v. The solution containing (42 µg/180 g body weight), pH was adjusted at 7.4 and intraperitoneally injected in rats and after 2 h, DGalN dissolved in normal saline (0.9% w/v). The solution containing (105 mg/180 g body weight), pH was adjusted at 7.4 and intraperitoneally injected in rats; blood samples were withdrawn after 22 h to check induction of hepatitis (He *et al.*, 2001).

Experimental design

Forty male albino rats weighing 180 - 200 g were divided into four groups (10 rats in each group). Group I (control group): healthy rats received vehicle only. Group II (green tea group): healthy rats received green tea extract (36 mg/rat twice a day) orally for thirty days (Majchrzak *et al.*, 2004). Group III (hepatitis group): healthy rats received vehicle only before induction of hepatitis. Group IV (treated group): healthy rats received green tea extract (36 mg/rat twice a day) orally for thirty days before induction of hepatitis.

Blood sampling

After 22 h of DGalN administration (He *et al.*, 2001), fasting blood samples were withdrawn from the retro-orbital plexus vein of each animal, under light anesthesia by diethyl ether. Blood samples were clotted and serum was separated by centrifugation at 3000 rpm for 15 min, then divided into aliquots and stored at -20°C for biochemical assays.

Biochemical analysis

Serum liver enzymes alanine amino-transferase (ALT) and aspartate amino-transferase (AST) were measured according to the method of Reitman and Frankel (1957), alkaline

phosphatase (ALP) was determined according to the method of Kind and King (1954).

Preparation of tissue homogenate

Liver was removed quickly and placed in iced normal saline, perfused with normal saline solution to remove blood cells, blotted on filter paper and frozen at -80°C. The frozen tissues were cut into small pieces and homogenized in 5 ml cold buffer (0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄ per 500 ml deionized water (pH 7.4) per gram tissue, then centrifuged at 4000 rpm for 15 min at 4°C and the supernatant was removed for oxidant/antioxidant parameters estimation (Manna *et al.*, 2005).

Liver malondialdehyde (MDA) was measured according to the method of Uchiyama and Mihara (1978). Also, liver superoxide dismutase (SOD) was measured according to the method of Nishikimi *et al.* (1972). Advanced oxidation protein products (AOPP) as a marker of oxidative stress was measured by ELISA kit as described by Deschamps-Latscha *et al.* (2005).

Determination of liver coenzyme Q10

Liver CoQ10 was estimated according to the method described by Hussein *et al.* (2013) by high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat. pump, G131A model). Homogenate samples were treated with 2 ml ethanol and CoQ10 was extracted with 5ml hexane, after vigorous shaking, 4 ml of hexane layer were dried under nitrogen gas and the residue was dissolved in 400 µl ethanol.

HPLC condition

20 µl from the solution were injected in HPLC; separation was achieved on reversed phase column (C18, 25 × 0.46 cm i.d. 5 µm). The mobile phase consisted of ethanol/methanol 70/30 (v/v) and was delivered at a flow rate of 2 ml/min. UV detection was performed at 275 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve.

Determination of liver paraoxonase activity

The arylesterase activity of paraoxonase was measured spectrophotometrically in supernatants using phenylacetate (Sigma) as a substrate (Higashino *et al.*, 1972). In this assay, arylesterase/paraoxonase catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The rate of phenol formation is measured by monitoring the increase in absorbance at 270 nm at 25 °C. The working reagent consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM CaCl₂ and 4 mM phenyl acetate, as the substrate. Samples diluted 1:3 in buffer were added, and the change in absorbance was recorded following a 20-s lag time. Absorbance at 270 nm was taken every 15 s for 120 s using a UV-VIS Recording Spectrophotometer (Shimadzu Corporation, Australia).

Statistical analysis

Statistical analysis of the results were carried out using the standard computer program SPSS (Version 12). Normally distributed results were compared using student's test. Differences among groups were evaluated using one way ANOVA. Results were expressed as mean \pm SE. P values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Viral infection, alcohol and drug toxicity may elicit an interaction with the liver cells that may lead to hepatic damage (Chang *et al.*, 2005). In this study, we demonstrated that, DGa1N / LPS significantly increased serum liver enzymes (ALT, AST and ALP) levels (table:1) which could be taken as an index value of liver damage. The elevation of liver enzymes induced by DGa1N / LPS may be attributed to the disturbance of plasma membrane permeability causing leakage of liver enzymes from the cell, which leads to elevation in serum enzymes levels (Mitra *et al.*, 2000), thus, DGa1N-induced liver damage and excessive production of free radicals resulting from oxidative stress can damage macromolecules as lipids and proteins (the main components of cell membrane) (Najmi *et al.*, 2005).

Table. 1: Effect of green tea administration on AST , ALT and ALP levels in different studied groups.

Groups		AST (U/L)	ALT (U/L)	ALP (U/L)
Control	Range	81-85	21-24	86-92
	Mean \pm SE	83 \pm 0.88	23 \pm 0.47	89 \pm 0.99
Green tea	Range	81-84	20-25	89-92
	Mean \pm SE	83 \pm 0.55	22 \pm 1	90 \pm 0.65
	P ^a value	N.S.	N.S.	N.S.
	% change ^a	0%	-4.3%	1.1%
Hepatitis	Range	149-154	48-50	268-286
	Mean \pm SE	152 \pm 1	49 \pm 0.42	277 \pm 3.4
	P ^a value	0.000*	0.000*	0.000*
	% change ^a	83.2%	113%	211%
Treated	Range	91-97	22-27	111-131
	Mean \pm SE	94 \pm 1.1	25 \pm 1.07	121 \pm 3.9
	P ^a value	0.000*	N.S.	0.000*
	P ^b value	0.000*	0.000*	0.000*
	% change ^a	13.2%	8.6%	35.9%
	% change ^b	-38.2%	-48.9%	-56.3%

Significant P*value <0.05, N.S. not significant.

P^a value vs.control, P^b value vs.hepatitis,

% change^a :Percent of change from control group,

% change^b :Percent of change from hepatitis group

Our results indicated that DGa1N / LPS significantly increased the mean value levels of both liver MDA and AOPP (the main markers of lipids and proteins oxidation) concomitant with a reduction in liver antioxidant enzymes (SOD, CoQ10 and PON1) (Tab. 2, Fig. 1, 2).

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. Hepatotoxicity could also be explained by the impaired antioxidant enzyme activities in the liver. Indeed, the antioxidant enzymes SOD, coenzyme Q10 and paraoxonase limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury by means of their

being free radical scavengers (Halliwell and Gutteridge, 2001). These enzymes work together to eliminate active oxygen species.

Table. 2: Effect of green tea administration on MDA , SOD and AOPP in different studied groups.

Groups		MDA (n mol/ g tissue)	SOD (U/ g tissue)	AOPP (ng / g tissue)
Control	Range	32.2- 35.6	433.4-	52.6- 55.4
	Mean \pm SE	33.9 \pm 0.65	447.2	54 \pm 0.55
440.3 \pm 2.6				
Green tea	Range	32.2- 34.7	508 - 515	52-55
	Mean \pm SE	33.5 \pm 0.50	511 \pm 1.3	54 \pm 0.55
	P ^a value	N.S.	0.000*	N.S.
	% change ^a	-1.17%	16%	0%
Hepatitis	Range	52- 55.2	141- 155	62-64
	Mean \pm SE	53.6 \pm 0.62	148 \pm 2.5	63 \pm 0.37
	P ^a value	0.000*	0.000*	0.000*
	% change ^a	58.1%	-66%	16.6%
Treated	Range	37.3-40.3	315-326.9	56.5-58.4
	Mean \pm SE	38.8 \pm 0.59	321 \pm 2.3	57.4 \pm 0.37
	P ^a value	0.000*	0.000*	0.000*
	P ^b value	0.000*	0.000*	0.000*
	% change ^a	14.4%	-27%	6.3%
	% change ^b	-27.6%	116.8%	-8.8%

Significant P*value <0.05, N.S. not significant.

P^a value vs.control, P^b value vs.hepatitis,

% change^a :Percent of change from control group,

% change^b :Percent of change from hepatitis group

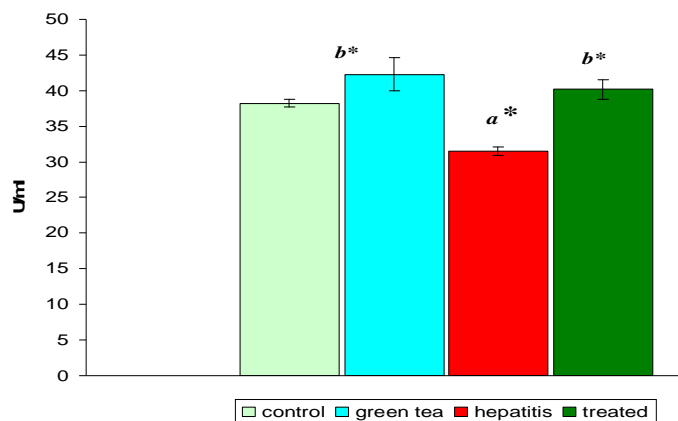


Fig. 1: Liver paraoxonase1 activity in different studied groups.

a* significant compared to control

b* significant compared to hepatitis

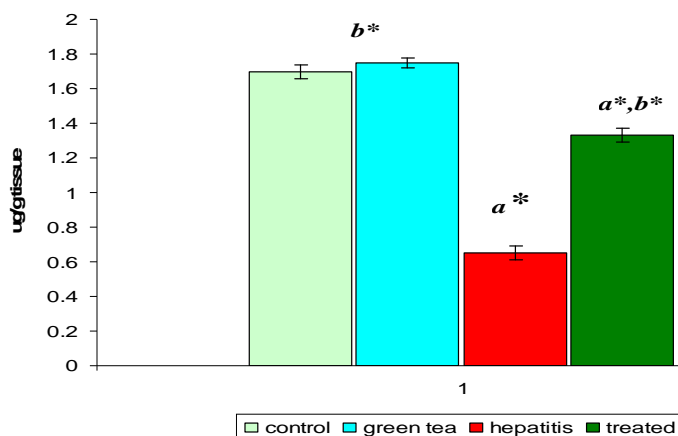


Fig.2: Liver Co Q10 in different studied groups

a* significant compared to control

b* significant compared to hepatitis

The health benefits of green tea have been extensively studied in the past few decades. Nowadays, tea is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health (Mandel *et al.*, 2006 and Chung *et al.*, 2009). Results of the current study revealed that GT extract reversed the elevation of liver enzymes, lipid peroxidation and AOPP in addition to attenuating the reduction of SOD, CoQ10 and paraoxonase levels.

The mechanism of hepatoprotection of GT extract may be attributed to polyphenolic compounds (e.g. epicatechins) that scavenge a wide range of free radicals including the most active hydroxyl radical, which initiate lipid peroxidation (Valcic *et al.*, 2000). Therefore, it may decrease the concentration of lipid free radicals (Skryzdelewska *et al.*, 2002). It was reported previously that phenolic compounds chelate metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides (Azram *et al.*, 2004). Also, GT attenuates the lipid peroxidation and liver antioxidant enzymes (SOD, CoQ10 and paraoxonase) which in turn restore the integrity of the cell membrane and improve the disturbance in permeability leading to the attenuating of liver enzymes elevation (Heikal *et al.*, 2012).

CONCLUSION

Green tea extract effectively attenuated liver injury induced by DGA1N/LPS. The Activity of green tea extract may be related to the presence of catechins which possess the ability to prevent free oxygen radicals formation through inhibiting the activity of enzymes participating in their generation, and to scavenge the free radicals as well as to chelate transition metal ions which enhanced radical reactions, in addition to the elevation of antioxidant enzymes SOD, PON1 and coenzyme Q 10.

ACKNOWLEDGEMENT

The authors are thankful to the National Research Center (NRC) for the unlimited support to carry out this work.

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How to cite this article:

Jihan Hussein, Eman Refaat, Safaa Morsy, Dalia Medhat and Fatma Oraby. Green Tea Attenuates Experimental Hepatitis in Context of Oxidative Stress. *J App Pharm Sci*, 2013; 3 (12): 124-128.