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Aqueous Extract of Iranian Green Tea Prevents Lipid Peroxidation and Chronic Ethanol Liver Toxicity in Rat

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ABSTRACT

The aim of this study was to examine the protective effects of Iranian green tea aqueous extract (GTE) (*Camellia sinensis*) in chronic ethanol toxicity using rat model. Animals were divided into four groups and fed for 30 days by gavage technique: A (Control), B (Ethanol-15% (v/v)), C (Ethanol + GTE) and D (GTE-2.5% (w/v)). Aspartate aminotransferase (AST, 158%), alanine aminotransferase (ALT, 131%), alkaline phosphatase (ALP, 159%), γ-glutamyltranspeptidase (GGT, 89%) and malondialdehyde (MDA, 53%) values were significantly ($p<0.01$) increased in the ethanol group (B) as compared to the control (A). Treatment of animals with GTE, however, considerably ($p<0.05$) protected the liver damage by decreasing AST (32%), ALT (33%), ALP (26%), GGT (47%) and MDA (59%) values. GSH concentration was significantly decreased in the ethanol group as compared to the control (40%) but considerably recovered (58%) following GTE treatment. Furthermore, histopathological observations, consistent with biochemical findings, showed that GTE treatment significantly reduced portal inflammation and infiltration of mononuclear cells pronounced by ethanol.

Key words: Iranian green tea, antioxidant, liver damage, ethanol, lipid peroxidation

INTRODUCTION

Chronic ethanol consumption contributes to a number of disorders including hepatic and extra hepatic diseases (Lieber, 2000; Ansari and Jamil, 2011). Lipid peroxidation has an important role in the alcohol induced liver damage. Motivation of lipid peroxidation may arise from the formation of free radicals through a weakness of antioxidants leading to oxidative stress (Gheorghiu *et al*., 2004; Nordman *et al*., 1990; Montoliu *et al*., 1994; Diallo *et al*., 2009; Meera *et al*., 2008; Jun *et al*., 2007; Prasanna and Purnima, 2011; Shahriari *et al*., 2007). Free radicals play a central role in many pathways of alcohol-induced damage which can result in a

state called oxidative stress (Wu and Cederbaum, 2003). In several studies, therefore, ethanol is used as an experimental model for induction of lipid peroxidation, oxidative damage and hepatic disturbances (Barclay, 1993; Radosavljevic *et al*., 2009; Arteel, 2003). Proteins, lipids, carbohydrates and nucleic acids are major targets for the very reactive free radical molecules. As a consequence, cellular enzymes, structural proteins, membranes, simple and complex sugars, DNA and RNA are all susceptible to oxidative damage (Yu, 1994).

Chronic ethanol feeding have been related to a decrease in many antioxidant factors in the liver, including enzymes such as superoxide dismutase, catalase and glutathione peroxides (Polavarapu *et al*., 1998; Schisler and Singh, 1989) and non-enzymatic antioxidants like vitamins C, E and glutathione (Loguercio *et al*., 1996; Hagen *et al*., 1989). These changes have led to increased formation of lipid peroxidation products, such as Malondialdehyde (MDA) (Barclay, 1993). The harmful result of membrane peroxidation have guided investigations on the application and mechanisms of action of biologically relevant antioxidants, in particular naturally occurring ones, including green tea (Guo *et al*., 1996; Terao *et al*., 1994; Sano *et al*., 1995).

Green tea mainly contains polyphenols such as flavanols (catechins), flavandiols and phenolic acids. Polyphenols considered for about 25% of the solids in water extracts of green tea leaves (Graham, 1992). Natural antioxidants such as Green tea (*Camellia sinensis*) have hepatoprotective effect which considerably potentiates antioxidant capacity of plasma. Furthermore, it has been reported that tea antioxidants are more potent than antioxidants found in many fruits and vegetables which could neutralize highly reactive molecules including free radicals (Feng *et al*., 2001). Natural polyphenols such as catechins from various sources show antioxidant properties that may be useful in protection against the pro-oxidative states (Feng *et al*., 2001; McDonough, 2003). Furthermore, incorporation of green tea flavonoids in cell membrane has shown a decrease in the morphological and biochemical alteration of hepatocytes induced by toxic agents (Vargo and Buris, 1990).

The goal of this study was to examine the hepatoprotective effects of Iranian green tea aqueous extract (*Camellia sinensis*), as a source of water-soluble antioxidants, in prevention of lipid peroxidation, oxidative stress and chronic ethanol toxicity in a rat model.

MATERIALS AND METHODS

Chemicals: Ethanol was purchased from Merck Company (Germany). Other chemicals used were prepared with the highest purity grade. The assays of aspartate amino transferase (AST), alanine amino transferase (ALT), Alkalin phosphatase (ALP) and γ-glutamyltranspeptidase (GGT) activity were performed using Ellitech diagnostic kits (Sees, France).

Total extract: Tea leaves were collected from Komoleh, Langrood, Gilan Provine, North of Iran in summer, 2008, with kind help of Gorgan Plant-Essence Pharmaceutical Co. Aqueous extract of green tea was then prepared according to the method of Wei *et al*. (1999).

Animals: Male Wistar rats weighing 180-220 g were housed environmentally (25±2°C) at aired humidity controlled room and maintained on standard laboratory diet on a 12 h light/dark cycle. All animal experiments were performed according to the ethical guidelines suggested by the animal ethics committee of the Tehran University of Medical Sciences, Faculty of Pharmacy, Iran.

Treatment: 24 Animals were divided into four groups and fed for 30 days by gavage technique: Group A (control), Group B (ethanol-treated), Group C (ethanol + GTE) and Group D (GTE).

Animals of group A received deionized water, 1 mL/100 g b.wt./day, as vehicle. Animals of group B were fed with 15% (v/v) ethanol at a single dose of 1 mL/100 g body wt. per day; animals of group C were fed with ethanol (15% (v/v)) and after 30 min with GTE (2.5% (w/v)) and group D were fed with 2.5% (w/v) GTE at a single dose of 1 mL/100 g b.wt./day. Body weight of all Animals was recorded daily during the whole experimental period.

Preparation of serum and estimation of liver enzyme activity: After the treatment period was over, the animals of all groups were anaesthetized by ketamine/xylazine and blood samples were obtained from heart. The blood serum was separated by centrifugation at 2000 g for 20 min and kept at -70°C until use. The assays of aspartate amino transferase (AST), alanine amino transferase (ALT), Alkalen phosphatase (ALP) and γ-glutamyltranspeptidase (GGT) activity were performed using Ellitech diagnostic kits (Sees, France).

Tissue extracts preparation: The abdomen was opened, liver was quickly removed and cold saline perfused and part of the right lobe was placed in ice-cold Tris-HCl buffer (pH 7.4) container. The liver slice was cut into small pieces on ice and immediately homogenized using ice-cold phosphate buffer (pH 8) for the estimation of Malondialdehyde (MDA) and glutathione (GSH and GSSG).

Estimation of Lipid peroxidation: The quantitative measurement of lipid peroxidation was determined according to the thiobarbituric acid (TBA) test (Esterbauer and Cheeseman, 1990). The amount of MDA formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as nmol MDA/mg protein.

Estimation of GSH and GSSG: Liver tissue homogenates were acidified with 50% (W/V) trichloroacetic acid and centrifuged at 8000 g for 20 min. The supernatant was divided into two equal tubes. To the first tube, in order to analyze GSH, 2 mL of 0.4 M Tris buffer, pH 8.9 and 0.5 mL of 0.1 M 5, 5-dithiobis nitrobenzoicacid (DTNB) were added, vortexed and the absorbance read at 412 nm after 5 min. For GSSG determination, 1 mL of 5% $NaBH_4$ slowly was added to the second tube and incubated for 1 h at 45°C. Then, 0.5 mL of 0.3 M Na_2HPO_4 was added and the pH adjusted to 7.2 by 2N HCl. Finally, 0.5 mL of 0.1 M DTNB was added, vortexed and the absorbance read at 412 nm after 5 min. The amounts of GSH from the first tube deducted from the amount of GSH measured in the second tube which gives the extra amount of GSH in the first tube. Dividing the result by 2 gives the equivalent of GSSG. Results were expressed as μmol GSH/mg protein.

Estimation of protein: Protein content in the homogenate of liver tissue was estimated by the Bradford dye-binding assay with bovine serum albumin as standard (Bradford, 1976).

Histopathological evaluation: After 30 days, left lobules of liver were placed in cold acetone and fixed by 10% neutral buffered formalin, embedded in paraffin and stained with periodic acid-Schiff reagent and hematoxylin-eosin, respectively, to evaluate any histological changes.

Statistical analysis: The data presented as Mean±SEM. The difference between groups was evaluated by ANOVA which followed by Tukey multiple comparisons test and p-values less than 0.05 were considered significant.

RESULTS

Effect of chronic ethanol administration on serum AST, ALT and ALP activities: The results on activities of the serum enzymes, AST, ALT and ALP, following chronic ethanol administration (30 days) are presented in Table 1. The findings show that there was a significant increase in the activities of AST (158%, $p<0.01$), ALT (131%, $p<0.01$) and ALP (159%, $p<0.01$) in the ethanol group (B) over the control (A). The increased in enzyme activities was significantly decreased (AST 32%, $p<0.05$; ALT 33%, $p<0.05$; ALP 26%, $p<0.05$) by GTE treatment (C).

Effect of chronic ethanol administration on GGT activity: Activity of GGT was increased significantly (89%, $p<0.01$) in the ethanol group (B). This effect, however, was decreased remarkably (47%, $p<0.05$) by GTE treatment (C) (Table 1).

Effect of chronic ethanol administration on GSH and GSSG content in liver: Table 2 demonstrates the total GSH content of the liver homogenates. Administration of GTE (D) was found to increase significantly the GSH content (91%, $p = 0.026$) compared to control (A), while it was decreased (40%) in the ethanol group (B) group. GTE treatment following ethanol (C) was able to reload GSH content by 58%. Furthermore, GSSG formation was reduced (21%) in GTE administered group (D) compared to control (A), whereas it was increased (197%, $p<0.01$) in the ethanol group (B). GTE treatment following ethanol (C) was decreased GSSG content by 39%, $p<0.05$.

Effect of chronic ethanol administration on MDA production in liver: MDA formation, an indicator of lipid peroxidation, was expressed as nmol mg^{-1} protein. MDA production was increased significantly ($p<0.01$) by about 53% in ethanol group (B) as compared to the control (A). GTE treatment (C) significantly ($p<0.05$) prevented MDA production (59%, Table 2).

Table 1: Effect of ethanol (EtOH) [15% (v/v)/100 g b.wt./day] and GTE (2.5% at a dose of 1 mL/100 g b.wt./day) for 30 days on serum AST, ALT, ALP and GGT activities in rat

Groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	GGT (U L^{-1})
Control	68.39±6.76	22.67±2.62	85.34±6.15	3.12±0.14
EtOH	176.68±11.62**	52.33±3.71**	221.14±14.77**	5.90±0.56*
GTE	26.47±3.67	20.21±2.13	79.80±4.3	2.40±0.11
EtOH plus GTE	119.76±9.02* #	35.15±2.32* #	164.50±11.97** #	3.11±0.17 #

All data are given as Mean±SEM (n = 6). **p<0.01 and *p<0.05, significantly different when compared with control. # p<0.05, significantly different from EtOH group

Table 2: Effect of ethanol (EtOH) [15% (v/v)/100 g^{-1} b.wt./ day] and GTE (2.5% at a dose of 1 mL 100 g^{-1} b.wt. day^{-1}) for 30 days on total GSH, GSSG and MDA level in rat liver tissue homogenate

Groups	GSH (nmol mg^{-1} protein)	GSSG (nmol mg^{-1} protein)	MDA (nmol mg^{-1} protein)
Control	12.13±2.07	0.34±0.06	2.35±0.18
EtOH	7.31±1.07**	1.01±0.05**	3.60±0.22**
GTE	23.12±3.01**	0.27±0.04	1.06±0.14**
EtOH plus GTE	11.55±1.89 #	0.61±0.1* #	1.46±0.15* #

All data are given as Mean±SEM (n = 6). **p<0.01 and *p<0.05, significantly different when compared with control. # p<0.05, significantly different from EtOH group

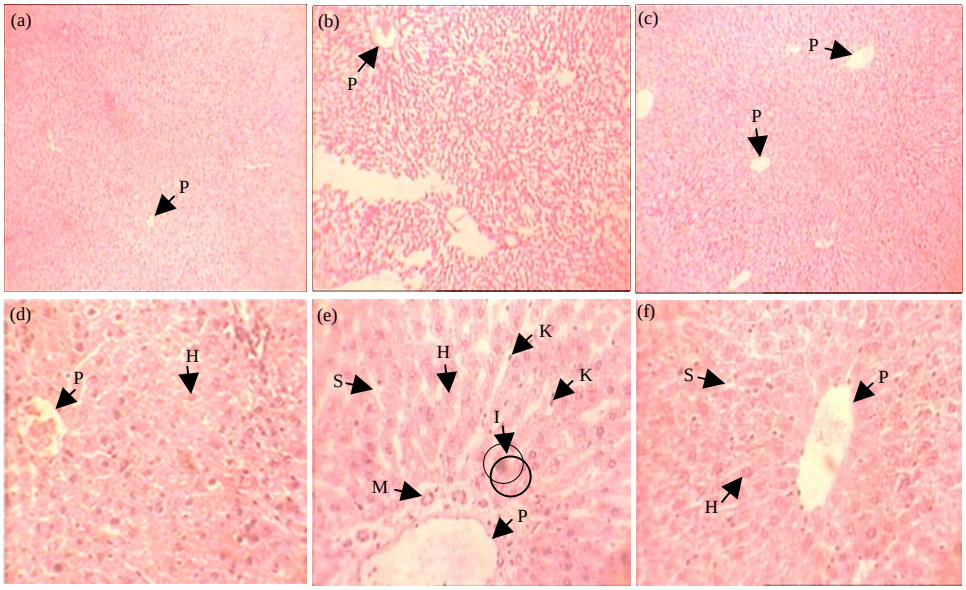

Fig. 1(a-f): Histopathology of rat liver sections (a-c: x400 magnifications, d-f: x100 magnifications-h and e). A, D (control group), no pathologic changes are present. Normal structure of liver was shown, i.e., normal central vein with radiating hepatocyte and normal limited plate in a classic lobule. (B and E) (ethanol group, 15% v/v per 100 g b.wt./day), histopathological changes are present, i.e., inflammation and necrosis, degeneration and hyperplasia of kupffer cell with infiltration of mononuclear cell and disruptor of radiated hepatocyte, limited plate of lobule and dilation of sinusoids. C and F (ethanol 15% and GTE 2.5% at a dose of 1 mL/100 g b.wt./day), mild histopathological changes are present, i.e. a mild structure changes of liver with presence of mild inflammation and decreased mononuclear cell and kupffer cell hyperplasia. P: Portal vein, H: Hepatocyte, K: Kupffer cell, S: Sinusoid, M: Mononuclear cell, I: Inflammation

Histopathological changes: Photomicrographs of rat livers following ethanol administration with or without GTE are presented in Fig. 1. With ethanol only, Fig. 1b-f show some histopathological changes of liver, compared to control group (Fig. 1a). These changes include necrosis and degradation, disruptor of radiated hepatocyte, fatty infiltration, hyperplasia of kupffer cells, infiltration of mononuclear cells and inflammation. GTE treatment, however, reduced these histopathological changes and decreased the number of inflammatory cells (Fig. 1c, f). Hyperplasia of kupffer cells, necrosis and degradation were also decreased by GTE treatment (Fig. 1c, f).

DISCUSSION

The result of this study shows that GTE have hepatoprotective effect against ethanol liver toxicity using rat experimental model. Hepatocytes exposure to ethanol disturbs the membrane structure and functions which is illustrated by the increased secretion of aminotransferases (Akrishnan and Menon, 2001; Kew, 2000). GTE administration blunted ethanol-induced increase in activities of marker enzymes of hepatocellular injury including AST, ALT, ALP and GGT

(Table 1). The effect could be due to prevention of intracellular leakage via membrane stability. Hepatoprotection is often evaluated by histopathological examination and monitoring of biochemical parameters such as ALT, AST, ALP and GGT which are among the most sensitive markers of hepatocellular damage (Rajagopal *et al*., 2003; Natanzi *et al*., 2010; Iyawe *et al*., 2006; Ghazi-Khansari *et al*., 2005). Similar pattern applies to the alkaline phosphatase which is located in the cytoplasm and is released into the circulation following damage to the structural integrity of hepatic cells (Radosavljevic *et al*., 2009; Arteel, 2003; Chavda *et al*., 2010). Our histopathological observations, consistent with biochemical findings, showed that liver injury with ethanol chronic exposure including necrosis and degeneration, disruptor of radiated hepatocyte, fatty infiltration, hyperplasia of kupffer cells and infiltration of mononuclear cells and severe fatty change and inflammation (Fig. 1b, e). These histopathological changes were attenuated by GTE treatment in which the presence of portal inflammation and infiltration of mononuclear cells were significantly reduced (Fig. 1c, f).

Secretion of cytoplasmic liver enzyme GGT into blood has also been attributed to altered liver metabolism and damage from increased consumption of alcohol. GGT index has been reported high in alcoholic liver disease (Kirpich *et al*., 2008) and it is claimed that GGT measurement is an extremely sensitive marker of ethanol-induced hepatic damage. In our study GTE treatment remarkably protected hepatocytes by reducing the GGT raise induced by ethanol (Table 1).

Ethanol induced liver injury may be produced via several ways (Plaa and Carbonneau, 2001). Acetaldehyde and lipid peroxidation-derived adducts are generated in the early phase of alcohol-induced liver disease (Niemela *et al*., 1998). Chronic ethanol consumption can alter metabolic activity of hepatocytes by inducing CYP2E1 which causes an increase in acetaldehyde production (Bruckner and Warren, 2001). This event may result in morphologically changes in hepatocytes including cellular hypertrophy, micro and macro vesicular structure, fatty changes, liver necrosis and hemorrhages (Jaeschke *et al*., 2002). Formation of MDA, the index of lipid peroxidation, was significantly increased with ethanol (Table 2). Based on histopathological observations in this study and attenuation of MDA levels in GTE treated group; it may be concluded that GTE can potentially prevent zonal hepatocellular alterations probably through decreasing acetaldehyde production. This finding is in accordance with the findings of another research (Gujrati *et al*., 2007) showing that aqueous extract of *Tylophora indica* (Asclepiadaceae) prevents ethanol induced hepatotoxicty in rats.

Epigallocatechin-3-gallate (EGCG), a polyphenol derived from green tea, has been shown to alleviate oxidative stress biomarkers including MDA, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) through modulating the hepatic nuclear transcription factors (nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2)) in heat-stressed quails (Sahin *et al*., 2010).

Tea flavonoids, depending on seasonal conditions, growing place and infusion time are different quantitatively and qualitatively (Labbe *et al*., 2006). They are water-soluble antioxidants, t may contribute to the protective effects of GTE against ethanol-induce oxidative damage in liver. Glutathione is a naturally producing antioxidant in mammalian cells that plays an important role in preventing oxidative stress and cellular damage caused by various toxins (Dickinson and Forman, 2002). Determination of total GSH, concomitant to its reduced and oxidized fractions, is a key factor to show the amount of antioxidant reserve in the organism (Lu *et al*., 1999; Odukoya *et al*., 2007). Thus, the levels of GSH are very crucial in liver injury caused by toxic substances such as ethanol. One study has demonstrated that green tea administration to

ethanol-treated rats of different ages normalized the activity of biomarkers of liver damage (ALT and AST) and the level of glutathione (GSH and GSSG) (Augustyniak *et al*., 2005). In the present study, GTE treatment restored the cellular antioxidant capacity and reduced generation of lipid peroxidation index which is probably due to its natural antioxidants and scavenging properties (Feng *et al*., 2001) and prevented intracellular leakage of AST, ALT, ALP and GGT that might happen via membrane stability (Kew, 2000). The biochemical findings were supported by histopathological examination.

CONCLUSION

Decrease in the activity of antioxidant enzymes such as GSH following ethanol exposure may be due to the damaging effects of free radicals on these enzymes. Furthermore, natural antioxidants and scavenging agents in GTE might be effective as plant hepatoprotectors and therefore may have some major therapeutic applications. Taken collectively, from the results of this study it is suggested that GTE has a hepatoprotective activity against ethanol liver injury in rat. Thus, the present study provides a scientific rationale for the application and commercial uses of GTE in management of ethanol-induced liver diseases.

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