Original Article

Hepatoprotective property of organic green tea in change liver cell line

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ABSTRACT

Introduction: To assess the hepatoprotective property of organic green tea in Chang liver cell line. Green tea is found to have anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic properties and also helps in preventing cardiac disorders, gives protection against solar UV rays, maintains body weight and prevents Intestinal Dysbiosis and infection. Materials and methods: Green tea extract was evaluated for hepatoprotective property against CCl4 induced toxicity. This is done by MTT assay and by evaluating the ALT and AST levels in vitro study. Results: This study proves that green tea extract can be used as a potent hepatoprotective drug against CCl_4 induced hepatotoxicity. Results of MTT assay show that the cell viability is >80% even at higher concentration of 120µg/ml of green tea and hence considered to be nontoxic. Also from the results of in vitro study, it has been proved that pre-treatment with green tea exact prevented Chang liver cells from CCl_4 induced hepatotoxicity. This can be appreciated by the reduced levels of ALT and AST in green tea extract treated Chang cell lines in contrast with untreated Chang cell lines upon CCl_4 induced toxicity. Conclusion: This study proves that green tea extract can be used as a potent hepatoprotective drug against CCl4 induced hepatotoxicity.

Keywords: Green tea, hepatoprotective property of green tea, CCL_4, liver toxicity, hepatoprotective drugs.

Introduction

Camellia sinensis (green tea) is a shrub of the Theaceae family, grown in semi- tropical environment [1]. Green tea is a famous beverage in East Asia and is used as an herbal remedy in Europe and North America [2]. Green tea is found to have anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic properties and also helps in preventing cardiac disorders, gives protection against solar UV rays, maintains body weight and prevents Intestinal Dysbiosis and infection [3-5]. Herbal medicines derived from plant extracts are being increasingly utilised to treat a wide variety of clinical disease [6]. More attention is being given to the protective effects of natural antioxidants compared to drug induced toxicities especially whenever free radical generation is involved [7].

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Crespy and Williamson [8] reported that green tea extract (GTE) displays antioxidants and free radicals' scavenger properties. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress, especially in case of cancer [9, 10]. Fresh tea leaves are rich in flavonoids monomers known as catechins such as epicatechins which are 13.6g/100g in green tea and 4.2g/100g dry weight in black tea [11, 12]. In animal studies done by Defeudis et al. [13], Banskota et al. [14], Ostrowska et al. [15], it has been proved that green tea may protect liver and brain cells against oxidative stress induced by ethanol intoxication.

Carbon tetrachloride (CCl_4) is a major inducer of hepatic damage frequently used while evaluating hepatoprotective activity. There are a few reports on their use and in vitro characteristics [16-18]. CCl4 undergoes metabolic activation in a cytochrome P-450-dependent step to produce free radicals, which can initiate lipid peroxidation. The toxicity induced by CCl_4 in vivo and in cultured hepatocytes involves stimulation of lipid peroxidation, which is detected as an increase in malondialdehyde (MDA) formation [19].

The aim of this study is to assess the hepatoprotective property of organic green tea against liver damage induced by CCl_4 in Chang liver cell line.

Materials and Methods

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Plant material

Green tea extract used in the study were obtained from Green Chem Herbal extracts and formulations, Bengaluru, India.

Chemicals used

Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS) and Dulbecco's modified Eagle medium (DMEM) were obtained from HiMedia Lab Pvt. Ltd, Mumbai. 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Carbon tetrachloride (CCL_4) and Trypsin were purchased from Sigma Aldrich Co, St Louis, USA. Accurex kits for serum aspartate aminotransferase (AST), alanine aminotransaminase (ALT) were purchased from Radiant diagnostics, Mumbai. All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Cell culture

Chang liver cells (obtained from National Center for Cell Sciences, Pune, India) were seeded ($1x10^5$ cells/T_{25} Flask) and cultured in DMEM containing 10% FBS and penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. Cells were maintained in continuous passage by trypsinization of sub confluent cultures using trypsin EDTA solution (0.2% trypsin, 0.02% EDTA in PBS). The stock culture was grown in $25cm^2$ culture flasks and all experiments were carried out in 96 microtitre plates.

MTT assay

Chang liver cells ($5x10^3$cells/well) were maintained in 96 well culture plate for 24h in presence of 100µl of organic green tea extract at the concentrations of 10, 20, 40, 60, 80, 100 or 120 µg/ml. At the end of incubation period, the extract containing media in the wells were discarded and 50µl of MTT prepared in DMEM was added in each well. The plates were gently shaken and incubated for 4h at 37°C in 5% CO_2 atmosphere. After 4h, the supernatant was removed. Later on, 150µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan followed by 30min incubation at room temperature with constant shaking. Absorbance (OD) was read at 540 nm using microplate reader (Multimode reader, Perkin elmer, USA). The dose response curve was generated using % viable cells and different concentrations of green tea extract.

CCl4 induced toxicity in Chang cell line

The Chang livers cells were trypsinized and the cell count was adjusted to $1x10^5$ cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24h of incubation, the cells were treated with indicated concentration of green tea extract with/without 100µl of toxicant (medium containing 40mM CCl_4) for time interval of 4h. ALT and AST assays were carried out to determine the hepatoprotective effect of green tea extract. The assays were done based on the manufacturer's protocol.

Statistical analysis

The results are expressed as mean ± standard error mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test using Prism software (v. 6.0; GraphPad, San Diego, CA, USA). Differences between means were considered significant at $P < 0.05$.

Results

MTT Assay

The effect of organic green tea extract on cell viability was assessed by MTT assay. A drug is considered to be toxic if the percentage cell viability is <40%, according to previously published reports, compared to that of control [20]. Based upon this, the current study results revealed that the extract was non – toxic, which exhibited % cell viability >80% even at higher concentration of 120µg/ml and hence were used to evaluate hepatoprotective property against CCl_4 induction.

Table 1: Effect of Organic green tea extract on Chang liver cell viability

Concentration (µg/ml)	% of Viable cells
0.1% DMSO	99.82±1.09
10	98.28±0.71
20	91.22±0.45
40	88.58±0.46
60	87.48±0.40
80	85.67±0.45
100	84.99±0.24
120	82.14±0.58

Values are expressed as Mean ± SEM (n = 3)

In vitro hepatoprotective effects

The hepatoprotective effect of organic green tea extract was assessed by CCl4 induction model. ALT and AST, the hallmark markers of liver function were assessed. The concentration of CCl4 for induction hepatic cell damage was chosen from previously published journal [21]. Chang liver cells were pre – incubated with indicated concentrations of green tea extract for 20h and subsequently exposed to 40mM CCl4 for 4h. Pre – treatment with green tea extracts at different concentrations prevented Chang liver cells from CCl4 induced hepatotoxicity which was exhibited from the levels of ALT and AST. The CCl4 alone induced group showed higher levels of ALT and AST indicative of cell damage, which was counteracted by the green extract.

Table 2: Effect of Organic green extract on CCl4 induced hepatotoxicity in Chang liver cells

Treatment Groups	ALT (U/L)	AST (U/L)
Control	7.86±0.42	13.99±0.30
CCl_4 (40mM)	$31.85±0.67^{***}$	$55.31±0.45^{***}$
Tea ext (10µg/ml) + 40mM CCl_4	$23.90±0.16^{\#}$	51.33±0.27
Tea ext (20µg/ml) + 40mM CCl_4	$21.30±0.23^{\#}$	48.18±0.30
Tea ext (40µg/ml) + 40mM CCl_4	$19.40±0.24^{\#}$	$44.78±0.49^{\#}$
Tea ext (60µg/ml) + 40mM CCl_4	$17.34±0.22^{\#\#}$	$38.24±0.54^{\#\#}$
Tea ext (80µg/ml) + 40mM CCl_4	$16.18±0.14^{\#\#\#}$	$30.51±0.28^{\#\#}$
Tea ext (100µg/ml) + 40mM CCl_4	$14.69±0.27^{\#\#\#}$	$26.31±0.27^{\#\#\#}$
Tea ext (120µg/ml) + 40mM CCl_4	$10.21±0.28^{\#\#\#}$	$20.98±0.42^{\#\#\#}$

Values are expressed as Mean ± SEM (n = 3). $^{***}P<0.001$, $^{**}P<0.01$ & $^{*}P<0.05$ Vs. Control; $^{\#\#\#}P<0.001$, $^{\#\#}P<0.01$. $^{\#}P<0.05$ Vs. CCl_4 induced group

Discussion

In this study we used Chang liver cell lines to evaluate the hepatoprotective property of green tea extract. This study

proves that green tea extract can be used as a potent hepatoprotective drug against CCl_4 induced hepatotoxicity. Results of MTT assay show that the cell viability is >80% even at higher concentration of 120µg/ml of green tea and hence considered to be nontoxic. Also from the results of in vitro study, it has been proved that pre-treatment with green tea exact prevented Chang liver cells from CCl_4 induced hepatotoxicity. This can be appreciated by the reduced levels of ALT and AST in green tea extract treated Chang cell lines in contrast with untreated Chang cell lines upon CCl_4 induced toxicity.

Many herbal products have been found to protect against hepatic injury. In a study done by Zhen et.al. [22], it has been found that administration of green tea polyphenol epigallocatechin-3-gallate was useful in the treatment and prevention of hepatic fibrosis. In a similar study done by Nevis and Vijayammal [23], it has been reported that a partially purified petroleum ether extractable fraction of the whole plant Aerva lanata composed of antioxidant alkaloids capable of ameliorating the CCl_4 induced hepatic injury by virtue of its antioxidant activity. In a study done by Jain et al. [24], it has been found that Momordica dioica Roxb leaves have potent hepatoprotective action against CCl_4 induced hepatic damage in rats.

Different preparations of tea have been used as liver Protestants. In a study done by Gong et al. [25], it has been proposed that oral consumption of tea polyphenols and tea pigments exhibited protective action against NDEA (N-nitrosodiethylamine) injections, followed by intraperitonial CCl_4 injection. He et al. [26], proved that dietary intake of green tea 0.4-1.2 g/kg body weight for two weeks suppressed the lipopolysaccharide-induced liver injury in D-galactoseamine sensitised rats decreasing the plasma ALT and AST levels. Elhalwagy et al. [27], demonstrated on rats, that green tea extract partially ameliorates the toxic effect of phenytrothion pesticides on the liver and kidney tissues and their functions.

Conclusion

The results of MTT assay show that the cell viability is >80% even at higher concentration of 120µg/ml of green tea and hence green tea can be considered as a non-toxic hepatoprotective drug. Also from the results of in vitro study, it has been proved that pre-treatment with green tea exact prevented Chang liver cells from CCl_4 induced hepatotoxicity. Thus green tea extract can be used as a potent hepatoprotective drug against CCl_4 induced hepatotoxicity.

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