J HerbMed Pharmacol. 2016; 5(3): 99-102.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Effect of green tea on inflammation and oxidative stress in cisplatin-induced experimental liver function

Nilofar Amidi[1], Shirin Moradkhani[2], Mahsa Sedaghat[2], Nejat Khiripour[1,3], Amir Larki-Harchegani[4], Nahid Zadkhosh[4], Mahmoud Mirhoseini[5], Akram Ranjbar[4*]

[1]Student Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Pharmacognosy and pharmaceutical biotechnology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Biochemistry, Hamadan University of Medical Sciences, Hamadan, Iran
[4]Department of Toxicology and Pharmacology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[5]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 3 January 2016
Accepted: 14 May 2016

Keywords:
Cisplatin
Green tea
Inflammation
Liver function
Oxidative toxic stress

ABSTRACT

Introduction: Cisplatin is one of the most potent chemotherapeutic antitumor drugs. Also, oxidative stress has been established to be involved in cisplatin-induced toxicity. Therefore, the present study was undertaken to examine the antioxidant and anti-inflammation potential of green tea hydroalcoholic extract (GTE) against the liver function of cisplatin in male rats.
Methods: Adult male Wistar rats (180–250 g) were divided into 4 groups (n = 5) treated as follows: (1) control group (saline solution, 1 ml kg–1 body weight, i.p.), cisplatin group (7 mg kg–1 body weight, i.p.). Animals of Groups III received only green tea extract (30 mg/kg/day, by gavage). Group IV was given green tea extract+ cisplatin once daily for 24 hours. Liver function was evidenced in the cisplatin group by the increased serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The mechanism of cisplatin induced liver function was considered as being decreased the total antioxidant power (TAP). Systemic inflammation was assessed by tumor necrosis factor-alpha (TNF-α) levels.
Results: A decrease in TAP level in cisplatin group was observed compared with control group. GTE administration decreased TNF-α and increased TAP compared to cisplatin group, but showed no significant differences between groups.
Conclusion: The results suggested that green tea could ameliorate cisplatin liver function in rats through reduction of oxidative toxic stress and inflammation.

Implication for health policy/practice/research/medical education:
Green tea polyphenols are potent antioxidants with anti-inflammatory property, and may have a protective role in hepatoprotective of cisplatin toxicity through reduction of inflammation and oxidative stress. Hence it may be beneficial in patients using cisplatin.
Please cite this paper as: Amidi N, Moradkhani S, Sedaghat M, Khiripour N, Larki-Harchegani A, Zadkhosh N, et al. Effect of green tea on inflammation and oxidative stress in cisplatin-induced experimental liver function. J HerbMed Pharmacol. 2016;5(3):99-102.

Introduction

Cisplatin, one of the most effective and powerful anti-cancer drugs, is used in the treatment of a wide variety of both pediatric and adult malignancies (1). However, the chemotherapeutic use of cisplatin is limited by serious side-effects such as nephrotoxicity and ototoxicity, sometimes requiring a reduction in dose or discontinuation of treatment (2). It has been previously shown that cisplatin-induced-nephrotoxicity is closely associated with an increase in oxidative damages (3). Cisplatin stimulates renal production of oxidative stress markers and generates reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical (4). Although the exact mechanism of cisplatin-induced toxicity is not well known, specially in liver, some studies have now documented the importance of oxidative stress in cisplatin-induced hepatotoxicity (5). Many preclinical trials have been done to evaluate the protective effects of some antioxidants on antagonizing the side effects related with cisplatin (6). Green tea *(Camellia sinensis* L.) is very potent naturally occurring antioxidant (7). In addition the free radical scavenging and antioxidant activity of Green tea exhibit vasodilatory, immune-

***Corresponding author:** Akram Ranjbar, Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. Tel/Fax: +98 8138380031; Email: akranjbar2015@gmail.com

stimulating, anti-carcinogenic, anti-allergic, anti-inflammatory, anti-bacterial, cardio-protective and antiviral activities (8). Furthermore, the researchers suggested that green tea may be useful in ameliorating the cytotoxic effects of chemotherapeutic agents (9). The green tea polyphenols have been shown to possess potent antioxidant activity that is several folds higher than that of vitamins C and E (10). Therefore, in the present study the effect of total green tea extract (GTE) was investigated on inflammation and oxidative stress in cisplatin-induced experimental liver function.

Materials and methods

Plant materials

The leaves of *Camellia sinensis* L. (Theaceae) was purchased from the market in September 2013. A voucher specimen was deposited at the Herbarium unit of the Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran (No: 230).

Plant extraction

Dried and finely powdered aerial parts (1000 g) were extracted with methanol 80% (3×5 L) at room temperature, three times each time 3 days. After removal of the solvent in vacuum at 50°C, the residue (300 g, 30%, w/w) was stored at 4°C in sealed vials until usage.

Chemicals and drugs

Dithiononitrobenzoic acid (DTNB), Tris base, 2,4,6-tripyridyl-s-triazine (TPTZ), from Merck Chemical Co. (Tehran), TNF-α kit from Abcam and green tea were prepared and used in this study.

Animals and experimental design

Male albino rats of Wistar strain weighing approximately 250–300 g, obtained from the Pasteur Institute of Iran, were used throughout this study. They were maintained at an ambient temperature of 25 ± 2°C and 12/12 hours of light–dark cycle.

The experimental animals were divided into four groups, each group contained 5 animals: (1) control group (saline solution, 1 ml 100 g−1 body weight, by gavage), cisplatin group (7 mg kg−1 body weight, i.p.). Animals of Group III received only green tea (30 mg/kg/day, by gavage). Group IV was given green tea+ cisplatin once daily for 24 hours. The animals were killed 24 hours after the treatment (11).

Liver function evaluation

Alanin aminotransferase (ALT) and Aspartate aminotransferase (AST) were assayed by Pars Azemon kit.

TNF-α assay

Serum TNF-α was measured by Enzyme-linked immunoadsorbent assay (ELISA) using an enzyme-linked immunoassay kit (rat TNF-α ELISA kit, ab46070, Abcam, USA) according to the manufacturer's protocol. The TNF-α content was expressed as pg/mL.

Estimation of oxidative stress parameters

Assay of total antioxidant power

It was measured by ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (12).

Assay of total thiol groups

To evaluate the plasma total thiol molecules, DTNB was used as a reagent. DTNB reacts with thiol molecules and creates a yellow complex which has good absorbance at 412 nm in spectrophotometer (13).

Statistical analysis

Results were expressed as the mean ± standard error (SE) for all animals in each group. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by post hoc Tukey test. Results were considered significantly different if $P<0.05$.

Results

Effect of green tea extract on the oxidative stress parameters and blood TNF-α level of rats

Table 1 shows the mean ± SE of variables related to either oxidative stress in animals tested. A significant increase ($P=0.04$) in total antioxidant power (TAP) was observed in green tea vs cisplatin group. The values for green tea and cisplatin groups were 5.04 ± 0.11 and 2.9 ± 0.26 μmol/mL, respectively. TNF-α of green tea group decreased significantly compared with cisplatin group ($P=0.045$) (16.67± 2.5 vs 23.03 ± 2.05 pg/mL). Also, green tea reduced ALT compared to cisplatin group ($P=0.032$) (58.22 ± 4.77 vs 97.76 ± 4.47 U/mL). AST increased in cisplatin group compared to green tea group ($P=0.035$) (124 ± 15 vs 42.08 ± 7.9 U/ml). No significant difference was observed in TTG between groups.

Discussion

The present study established that administration of GTE improves the level of parameters of oxidative stress and inflammatory such as TAP and TNF-α. Also, GTE improved liver function throughout reduction of ALT and AST compared to cisplatin group. In this study, administration of GTE decreased the plasma oxidative status. A significant decline in antioxidant enzymes activities and increase in free radicals in experimental models as well as in subjects were typical during the regimens of commonly used chemotherapy, which is particularly related to cisplatin treatment. In this regard, a significant increase in kidney lipid peroxidation and decrease in the activities of antioxidant enzymes have previously been reported (14). The investigators have also reported an increase in lipid peroxidation and decrease in the activities of antioxidant enzymes upon similar cisplatin treatment of rats (15). Similarly, previous studies, reported significant increase in hepatic lipid peroxidation and decrease in antioxidant enzymes in rats treated with cisplatin (16). It has been suggested that oxidative stress is an important mechanism

Table 1. Effect of green tea extract on the oxidative stress parameters and blood TNFa level rats

Groups	TNFα (pg/ml) Mean ±SE	TAP (umol/mL) Mean ± SE	TTG (nmol/mL) Mean ± SE	ALT (U/mL) Mean ± SE	AST (U/mL) Mean ± SE
Control	21.0 ± 2.2	3.2 ± 0.32	0.16 ± 0.01	73.4 ± 1.5	72.25 ± 12.6
Cisplatin	23.03 ± 2.05	2.9 ± 0.26	0.15 ± 0.02	97.76 ± 4.47	124 ± 15
Green tea	16.67 ± 2.5	5.04 ± 0.11**	0.18 ± 0.02	58.22 ± 4.47**	42.8 ± 7.9**
Cisplatin + green tea	18.6 ± 2.5	3.1 ± 0.28	0.16 ± 0.02	78 ± 2.06	52.6 ± 16

*Significantly different from control group at $P < 0.05$. **Significantly different from cisplatin group at $P < 0.05$.

of cisplatin-induced toxicity possibly due to depletion of glutathione and thiol groups (6). TNF-α as an inflammation biomarker has been suggested to be involved in the generation of ROS that causes cisplatin toxicity and initiates inflammation (17). TNF-α can generate free radicals in the myocardium through a variety of mechanisms (18). As mentioned earlier, increased oxidative stress can induce mitochondrial dysfunction, or in turn, result in mitochondrial dysfunction or damage (19). TNF-α can activate caspase and therefore trigger apoptosis through a multitude of molecular mechanisms (20). These mechanisms include the death of receptor pathway that involves death domain-mediated protein-protein interactions, as well as the mitochondrial pathway that involves the release of cytochrome C (21). Also, liver function is considered as the main dose limiting side effect of oxidative damage of cisplatin (5,22). Cisplatin is known to accumulate in mitochondria of renal epithelial cells. It is the primary a target for cisplatin-induced oxidative stress resulting in loss of mitochondrial protein-SH, inhibition of calcium uptake and a reduction in the mitochondrial membrane potential, demonstrating that cisplatin induces ROS in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular antioxidants (23,24). An increase in the production of free radicals exacerbates the cisplatin induced oxidative stress process by deteriorating cellular enzymes (25). Many natural antioxidants are important in cisplatin liver dysfunction (18,26). Also, previous studies have shown that green tea is protective in cisplatin nephrotoxicity (27). Antioxidative enzymes are activated by GTE intake (28), and the antioxidative potency of human plasma increases with continual ingestion of green tea (29). Green tea can act as scavenger of free radicals and prevent oxidative damage (30). Mitochondria are not only one of the main cellular sources of ROS, but they also are a key target of ROS (31). Also, mitochondria are subcellular targets of cytokines, especially TNF; reduction of GSH and thiol groups (32). Green tea polyphenols are potent antioxidants with anti-inflammatory property, suggesting that they may have a protective role in hepato-protection of cisplatin toxicity through reduction of inflammation and oxidative stress. Further studies are required to clarify the protective effect of green tea on molecular toxicity of cisplatin.

Acknowledgments
This study was supported by a grant from Vice Chancellor of Research of Hamadan University of Medical Sciences for student research center.

Authors' contributions
NA: Design of the study and laboratory methods. SM: Preparation of green tea extract. MS: Sampling and laboratory methods. NK: Laboratory methods. ALH: Preparation of the paper draft and statistical analysis. NZ: Help to laboratory methods MM: English editing of the paper. AR: Help in design of the study and English editing of the paper.

Conflict of interests
The authors declared no competing interests.

Ethical considerations
The experiments were conducted according to the ethical norms approved by Ethics Committee Guidelines. It was confirmed by Ethical committee in Vice Chancellor of Research of Hamadan University of Medical Sciences (9112154641). Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support
This study was supported by a grant from Vice Chancellor of Research of Hamadan University of Medical Sciences (Grant No: 9112154641).

References
1. Ries F, Klastersky J. Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. Am J Kidney Dis. 1986;8(5):368-379.
2. Lynch ED, Gu R, Pierce C, Kil J. Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. Hear Res. 2005;201(1):81-89.
3. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. Am J Med Sci. 2007;334(2):115-124.
4. Dehne N, Lautermann J, Petrat F, Rauen U, De Groot H. Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. Toxicol Appl Pharmacol. 2001;174(1):27-34.
5. Waseem M, Bhardwaj M, Tabassum H, Raisuddin S, Parvez S. Cisplatin hepatotoxicity mediated by mitochondrial stress. Drug Chem Toxicol. 2015;38(4):452-429.
6. Kim SJ, Ho Hur J, Park C, Kim HJ, Oh GS, Lee JN, et al. Bucillamine prevents cisplatin-induced ototoxicity through induction of glutathione and antioxidant genes. Exp Mole Med. 2015;47(2):e142.
7. Kim HS, Quon MJ, Kim JA. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin

3-gallate. Redox Biol. 2014;2:187-95.
8. Schroeder EK, Kelsey NA, Doyle J, Breed E, Bouchard RJ, Loucks FA, et al. Green tea epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in neurons. Antioxid Redox Signal. 2009;11(3):469-480.
9. Ahn TG, Kim HK, Park SW, Kim SA, Lee BR, Han SJ. Protective effects of green tea polyphenol against cisplatin-induced nephrotoxicity in rats. Obstet Gynecol Sci. 2014;57(6):464-470.
10. Rice-evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res. 1995;22(4):375-383.
11. Kaur T, Pathak C, Pandhi P, Khanduja K. Effects of green tea extract on learning, memory, behavior and acetylcholinesterase activity in young and old male rats. Brain Cogn. 2008;67(1):25-30.
12. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-76.
13. Hu M, Dillard C. Plasma SH and GSH measurement. Methods Enzymol. 1994;233(385):87.
14. Valentovic MA, Ball JG, Brown JM, Terneus MV, McQuade E, Van Meter S, et al. Resveratrol attenuates cisplatin renal cortical cytotoxicity by modifying oxidative stress. Toxicol In Vitro. 2014;28(2):248-257.
15. Janakiraman M. Antioxidant and protective effects of Vitex negundo against Cisplatin induced nephrotoxicity in male albino rats. International Journal of Pharmaceutical & Biological Archive. 2015;5(5):95-102.
16. Moghadam GT, Hosseini-Zijoud SM, Shayesteh TH, Ghasemi H, Ranjbar A. Attenuation of cisplathin-induced toxic oxidative stress by propofol. Anesth Pain Med. 2014;4:4.
17. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest. 2002;110(6):835-842.
18. Valerio A, Cardile A, Cozzi V, et al. TNF-α downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents. J Clin Invest. 2006;116(10):2791-2798.
19. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol. 2007;47:143-83.
20. Babu D, De Backer O, Soenen S, et al. Influence of CORM-A1 and resveratrol on TNF-a-induced oxidative stress and apoptosis in murine MODE-K intestinal epithelial cells. Joint international Neurogastroenterology and Motility Meeting, USA; 2012.
21. Al-Lamki RS, Lu W, Finlay S, Twohig JP, Wang EC, Tolkovsky AM, et al. DR3 signaling protects against cisplatin nephrotoxicity mediated by tumor necrosis factor. Am J Pathol. 2012;180(4):1454-1464.
22. Waseem M, Pandey P, Tomar B, Raisuddin S, Parvez S. Ameliorative action of curcumin in cisplatin-mediated hepatotoxicity: an in vivo study in Wistar rats. Arch Med Res. 2014;45(6):462-468.
23. Sahu BD, Kuncha M, Sindhura GJ, Sistla R. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. Phytomedicine. 2013;20(5):453-60.
24. Zsengeller ZK, Mukhopadhyay P, Kalyanaraman B. Cisplatin nephrotoxicity involves mitochondrial injury with impaired tubular mitochondrial enzyme activity. FASEB J. 2013;27:1086.
25. Yatin SM, Aksenov M, Butterfield DA. The antioxidant vitamin E modulates amyloid β-peptide-induced creatine kinase activity inhibition and increased protein oxidation: implications for the free radical hypothesis of Alzheimer's disease. Neurochem Res. 1999;24(3):427-435.
26. Ademiluyi AO, Oboh G, Agbebi OJ, Boligon AA, Athayde ML. Sorghum [Sorghum bicolor (L.) Moench] leaf sheath dye protects against cisplatin-induced hepatotoxicity and oxidative stress in rats. J Med Food. 2014;17(12):1332-1338.
27. Khan SA, Priyamvada S, Khan W, Khan S, Farooq N, Yusufi AN. Studies on the protective effect of green tea against cisplatin induced nephrotoxicity. Pharm Res. 2009;60(5):382-391.
28. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr. 2003;133(10):3275S-3284S.
29. Kimura M, Umegaki K, Kasuya Y, Sugisawa A, Higuchi M. The relation between single/double or repeated tea catechin ingestions and plasma antioxidant activity in humans. Eur J Clin Nutr. 2002;56(12):1186.
30. Tavakol HS, Akram R, Azam S, Nahid Z. Protective effects of green tea on antioxidative biomarkers in chemical laboratory workers. Toxicol Ind Health. 2015;31(9):862-867.
31. Ranjbar A, Sharifzadeh M, Karimi J, Tavilani H, Baeeri M. Propofol attenuates toxic oxidative stress by CCl4 in liver mitochondria and blood in rat. Iran J Pharm Res. 2014; 253(1):13.
32. Wang ZM, Gao W, Wang H, et al. Green tea polyphenol Epigallocatechin-3-Gallate inhibits TNF-α-induced production of monocyte chemoattractant protein-1 in human Uumbilical vein endothelial cells. Cell Physiol Biochem. 2014;33(5):1349-1358.