



Hepatoprotective effect of the root extract of green tea against malathion-induced oxidative stress in rats

Negar Mehri¹, Hamed Felehgari¹, Amir Larki Harchegani², Hamid Behrooj¹, Nejat Kheiripour¹, Hassan Ghasemi³, Mahmoud Mirhoseini⁴, Akram Ranjbar^{2*}

¹Student Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

²Department of Toxicology and Pharmacology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

³Abadan school of Medical Sciences, Abadan, Iran

⁴Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

ARTICLE INFO

Article Type:

Original Article

Article History:

Received: 25 January 2016

Accepted: 23 April 2016

Keywords:

Green tea

Malathion

ALT

AST

Oxidative toxic stress

Rat

ABSTRACT

Introduction: Organophosphorus (OPs) pesticides such as malathion intoxication has been shown to generate oxidative stress due to the production of free radicals and alteration of the antioxidant defense system. The aim of this study was to evaluate the effects of extracts from green tea (GT) hydroalcoholic extract on liver function.

Methods: Male Wistar rats were separated into 4 groups of 8 rats each. Group I (control), group II was given GT (10 mg/kg/day). Animals of groups III received only malathion, group IV was given GT+ malathion. Animals received malathion 150 mg/kg by gavage and GT 30 mg/kg for 1 week through intraperitoneal injection. Twenty-four hours after treatment, blood samples were collected. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations as well as biomarkers of oxidative stress such as lipid peroxidation (LPO), total antioxidant capacity (TAC) and total thiol groups (TTG) were measured.

Results: A decrease in ALT and AST levels in GT group were observed compared with the ones in control group. Also, the results showed that malathion could increase liver toxicity in rats through reduction of ALT and AST.

Conclusion: Amelioration of malathion toxicity through reduction of inflammation may suggest a prolonged therapeutic option against pesticides-induced hepatotoxicity.

Implication for health policy/practice/research/medical education:

Green tea (GT) could ameliorate malathion toxicity in rats through reduction of inflammation. Hence, it may provide a cushion for prolonged therapeutic option against pesticides-induced hepatotoxicity with no serious side effects.

Please cite this paper as: Mehri N, Felehgari H, Larki Harchegani A, Behrooj H, Kheiripour N, Ghasemi H, et al. Hepatoprotective effect of the root extract of green tea against malathion-induced oxidative stress in rats. J HerbMed Pharmacol. 2016;5(3):116-119.

Introduction

Pesticides fall into several chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms (1). Organophosphorus (OPs) insecticides are the largest and the most diverse group of insecticides (2). The broad application of OPs insecticides in public health and agricultural programs are accompanied by potentially hazardous impacts on humans, animals, plants and environment (water, air, soil and food) and severe acute and chronic poisoning (3,5). In fact, mechanisms of the OPs toxicity have been proposed, including inhibition of acetylcholinesterase, leading to accumulation of acetylcholine and subsequent activation of cholinergic, muscarinic and nicotinic recep-

tors (6). In addition, OPs insecticides exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues with simultaneous generation of reactive oxygen species (ROS) (6,7). ROS have been implicated in hepato- and neuro-toxicities induced by several OPs which are associated with lipid peroxidation (LPO) and phospholipids degradation (8,9). Oxidative stress occurs when the generation of ROS in the body exceeds the ability of the body to neutralize and eliminate them (7). The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of overall balance between the degree of oxidative stress and the antioxidant capacity (10). Malathion [O,O-dimethyl-S-(1,2-dicarcethoxyethyl) phosphorodithioate is an OP pesticide

*Corresponding author: Akram Ranjbar, Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, 6517838678. Iran. Tel/Fax: +98 8138380031; Email: akranjbar2015@gmail.com

that is widely used to control pests (11). It is extensively used to control or eliminate disease-inducing arthropods targeted by public health programs. It is also used to reduce animal ectoparasites, human head and body lice, and household insects, and to protect grains in storage (12). Malathion is known to inhibit acetylcholinesterase activity in target tissues and has been linked to the dysfunction of several organ systems, including the liver, the pancreas and the reproductive system (13). In addition, malathion as one of the OPs affects mitochondrial membrane transport in rat liver, and it disturbs cytochrome P450 system in human liver (13). The liver is the largest glandular organ in the body and performs many vital functions to keep the body pure of toxins and harmful substances. It is a vital organ that supports nearly every organ in the body. Without a healthy liver, a person cannot survive. High levels of liver enzymes ALT and AST are predictive of disease and all-cause mortality and can reflect liver injury, fatty liver and/or oxidative stress. Difference in ALT and AST levels is heritable (13).

Green tea (GT) is a favorite beverage and its extracts are popular components of dietary supplements. GT, prepared from the leaves of *Camellia sinensis* L., is a beverage that is popular worldwide and possesses many pharmacological effects, such as anti-mutagenic, anti-proliferative, anti-carcinogenic properties. It is a potent neuro-protective remedy in models of degenerative disorders (14). Therefore, the present study was undertaken to evaluate the ameliorative property of GT on hepatotoxicity during malathion-induced oxidative stress in male rat.

Materials and methods

Plant materials

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

Plant extraction

Dried and finely powdered of aerial parts (1000 g) were extracted with methanol 80% (3×5 L) at room temperature for 4 weeks. 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

Dithionitrobenzoic acid (DTNB), Tris base, 1,1,3,3'-tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, 2,4,6-tripyridyl-s-triazine (TPTZ), from Merck Chemical Co. (Tehran) and GT were 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 $25 \pm 2^\circ\text{C}$ and 12/12 hours

of light–dark cycle. The experiments were conducted according to the ethical norms approved by Ethics Committee of Hamadan University of Medical Sciences (No: 930222666). The experimental animals were separated into four groups; each group contained 8 animals: (i) control rats; (ii) GT treated control rats; (iii) malathion rats; (iv) malathion treated GT rats. Animals received malathion 150 mg/kg by gavage and GT 30 mg/kg for 1 week through intraperitoneal injection (14).

Biochemical indicators of liver function & malathion toxicity

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and cholinesterase activity (ChE) were assayed by Pars Azemon kit, Tehran, Iran.

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 (15).

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 (16).

Assay of lipid peroxidation

The LPO product in tissues was determined by thiobarbituric acid (TBA) reagent expressed as the extent of malondialdehyde (MDA) productions during an acid heating reaction. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA+MDA adducts in samples (17).

Statistical analysis

Results were expressed as the mean \pm 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 was $P < 0.05$.

Results

Effects of green tea extract on the oxidative stress parameters and liver enzymes levels in male rats

Table 1 shows the mean \pm SE of variables related to either oxidative stress in animals test. Malathion caused a significant increase in LPO when compared to GT group ($P = 0.04$). Co-administration of malathion with GT significantly reduced malathion induced LPO ($P < 0.045$). Administration of malathion decreased total antioxidant power (TAP) in comparison to GT group ($P = 0.048$). Treatment with GT increased TTG as compared to malathion group ($P = 0.046$). Malathion significantly decreased ChE activity as compared to control group ($P = 0.039$).

Table 1. Effect of GT extract on the oxidative stress parameters and liver function rats

Groups	LPO (nm/ml)	TAP (umol/ml)	TTG (nmol/ml)	ALT (U/ml)	AST (U/ml)	ChE activity (U/ml)
	Mean ±SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ±SE	Mean ± SE
Control	2.3 ± 0.8	1.56 ± 0.24	0.18 ± 0.03	40.8 ± 5.2	79.7 ± 2.9	0.89 ± 0.23
Malathion	6.2 ± 1.8	1.4 ± 0.35	0.12 ± 0.02	47.1 ± 4.1	130 ± 21	0.70 ± 0.25 ^a
GT	1.1 ± 0.2 ^b	3.05 ± 0.55 ^b	0.38 ± 0.15 ^b	39.2 ± 4.3	77.1 ± 13 ^b	1.8 ± 0.4 ^b
Malathion+ GT	2.9 ± 1.3	2.4 ± 0.43	0.23 ± 0.06	43.5 ± 10	97.5 ± 16	1.23 ± 0.2

Abbreviations: GT, green tea; LPO, lipid peroxidation; TAP, total antioxidant power; TTG, total thiol groups; ALT, Alanine aminotransferase; AST, aminotransferase; ChE, cholinesterase activity.

^aSignificantly different from control group at $P < 0.05$. ^bSignificantly different from malathion group at $P < 0.05$.

Also, treatment with malathion increased ALT and AST as compared to GT group ($P = 0.047$).

Discussion

In the present study, the protective effect of GT was carried out against malathion-induced toxicity in the rat liver. Malathion induced remarkable hepatic oxidative damage. According to the results, malathion treatment promotes LPO content in blood of rat treated with malathion. In the present study, rat treated with malathion for 1 week showed an increase in LPO when compared to control group. LPO is known to disturb the integrity of cellular membranes, leading to the leakage of cytoplasmic enzymes (18). The increase of LPO observed in liver following malathion exposure was probably ascribed to the excessive production of ROS, which could be related with hepatocyte enzyme leakage (19). Malathion primarily acts by irreversibly inhibiting AChE at cholinergic junctions of the nervous system which produces hepatotoxicity in rats and induces oxidative stress (20). Overproduced ROS could directly attack the hepatocellular membrane as a result of LPO, followed by a cascading series of cellular events such as the massive release of inflammatory cytokines, which ultimately lead to liver injuries (21). Administration of malathion elevates the formation of lipid peroxides and ROS, leading to the inactivation of enzymatic and non-enzymatic antioxidants in the liver (13). Antioxidants are used to counteract the formed ROS due to malathion toxicity (22). The major nutraceutical compounds in GTs have the most effective antioxidant activities in insecticides toxicity (23). Results of the current study revealed that GT extract reversed the elevation of LPO. Hence, it is possible that the mechanism of hepato-protection 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 radicals which initiate LPO (24). Polyphenols have additional mechanisms in which they reduce oxidation level besides direct role as antioxidants (1). They cause prevention of redox sensitive transcription inflammatory reactions (3), suppression of oxidation stimulants such as induced nitric oxide synthase (iNOS), cyclooxygenase 2 (COX), (2- lipoxygenase (LOX-2) and xanthine oxidase (4), induction of antioxidant enzymes such as glutathione S-transferase and superoxide dismutase (25). In the current study, GT extract reversed the elevation of LPO. Hence, the mechanism of hepato-protection of GT extract might be attributed to polyphenolic compounds (e.g. epicatechins) that

scavenge a wide range of free radicals including the most active hydroxyl radicals, which initiate LPO (26). Then, it may diminish the concentration of lipid free radicals. Also TAP increased in GT groups compared with control group in this study. This could be attributed to the antioxidant capacity of GT that attenuates liver toxicity. The results of the present study showed that malathion treatments caused oxidative damage, biochemical alterations in the liver of male rats. In contrast GT reduces oxidative damage by virtue of its antioxidant properties thus improving the structural integrity of cell membrane and eventually alleviating the oxidative changes as well as the biochemical perturbations.

Conclusion

Based on the present observations, GT may provide a cushion for prolonged therapeutic option against pesticides-induced hepatotoxicity without harmful side effects.

Acknowledgments

This study was supported by a grant (Grant No:930222666) from Vice Chancellor of Research of Hamadan University of Medical Sciences.

Authors' contributions

NM: Design of the study and sampling. HF: Doing laboratory methods. HB: Sampling and laboratory methods. NK: laboratory methods. ALH: Preparation draft the paper and statistical analysis. HG: 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

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: 930222666).

References

1. Tyler-Miller G. Sustaining the Earth: An Integrated Approach. Florence, KY: Thomson/Brooks/Cole; 2004:211–216.
2. Marrs TC. Organophosphate poisoning. *Pharmacol Ther.* 1993;58(1):51-66.

3. Kumari B, Madan V, Kathpal T. Status of insecticide contamination of soil and water in Haryana, India. *Environ Monit Assess.* 2008;136(1-3):239-44.
4. Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. *Environ Toxicol Pharmacol.* 2005;20(1):88-91.
5. Ranjbar A, Pasalar P, Abdollahi M. Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorus pesticide manufacturing workers. *Hum Exp Toxicol.* 2002;21(4):179-82.
6. Galloway T, Handy R. Immunotoxicity of organophosphorous pesticides. *Ecotoxicology.* 2003;12(1-4):345-63.
7. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. Pesticides and oxidative stress: a review. *Medical Science Review.* 2004;10(6): 141-7.
8. Mossa AT, Refaie AA, Ramadan A. Effect of exposure to mixture of four organophosphate insecticides at no observed adverse effect level dose on rat liver: the protective role of vitamin C. *Res J Environ Toxicol.* 2011;5(6):323-9.
9. Oruç EÖ, Usta D. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ Toxicol Pharmacol.* 2007;23(1):48-55.
10. El-Shenawy NS, El-Salmy F, Al-Eisa RA, El-Ahmary B. Amelioratory effect of vitamin E on organophosphorus insecticide diazinon-induced oxidative stress in mice liver. *Pestic Biochem Physiol.* 2010;96(2):101-7.
11. John S, Kale M, Rathore N, Bhatnagar D. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem.* 2001;12(9):500-4.
12. Assini FL, Zanette KD, Brocardo PS, Pandolfo P, Rodrigues ALS, Takahashi RN. Behavioral effects and ChE measures after acute and repeated administration of malathion in rats. *Environ Toxicol Pharmacol.* 2005;20(3):443-9.
13. Akhgari M, Abdollahi M, Kebryaezadeh A, Hosseini R, Sabzevari O. Biochemical evidence for free radicalinduced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum Exp Toxicol.* 2003;22(4):205-11.
14. Choi JY, Park CS, Kim DJ, Cho MH, Jin BK, Pie JE, et al. Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. *Neurotoxicology.* 2002;23(3):367-74.
15. 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):70-6.
16. Hu M, Dillard C. Plasma SH and GSH measurement. *Methods Enzymol.* 1994;233(385):87.
17. Moore K, Roberts LJ. Measurement of lipid peroxidation. *Free Radic Res.* 1998;28(6):659-71.
18. El-Demerdash FM. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. *Food Chem Toxicol.* 2011;49(6):1346-52.
19. Tuzmen N, Candan N, Kaya E, Demiryas N. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. *Cell Biochem Funct.* 2008;26(1):119-24.
20. Selmi S, El-Fazaa S, Gharbi N. Oxidative stress and alteration of biochemical markers in liver and kidney by malathion in rat pups. *Toxicol Ind Health.* 2015;31(9):783-8.
21. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. *Arch Toxicol.* 2009;83(6):519-48.
22. Al-Othman AM, Al-Numair KS, El-Desoky GE. Protection of α -tocopherol and selenium against acute effects of malathion on liver and kidney of rats. *Afr J Pharm Pharm.* 2011;5:1263-71.
23. Elzoghby RR, Ahlam FH, Abdel-Fatah A, Farouk M. Protective role of vitamin C and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. *Am J Pharmacol Toxicol.* 2014;9(3):177.
24. 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):862-7.
25. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. *Am J Clin Nutr.* 2005;81(1):215S-7S.
26. Al-Attar AM, Abu-Zeid IM. Effect of tea (*Camellia sinensis*) and olive (*Olea europaea* L.) leaves extracts on male mice exposed to diazinon. *BioMed Research International.* 2013;2013:461415.