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# A Comparative Study on the Effects of Glutathione and Green Tea Extract (Camellia sinensis L.) on Thioacetamide-induced Hepatotoxicity in Male Adult Wistar Rats

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***Abstract***

***Background*:** Flavonoids play significant role in the treatment of many diseases. Green tea (*Camellia Sinensis* L.) is a common beverage all over the world with antioxidant and detoxification effects related to the presence of flavonoids and catchins. This study aimed to investigate the protective effect of green tea on thioacetamide-induced hepatotoxicity.

***Materials and Methods*:** In this experimental study, 64 male Wistar rats were allocated to eight groups. The control group received a normal diet alone, sham group received normal saline, hepatotoxic group received thioacetamide (50 mg/kg thioacetamide for three days), other groups received a thioacetamide for three days and the alcoholic extract of bgreen tea, at minimum (50 mg/kg), moderate (100 mg/kg), and maximum (200 mg/kg) doses, glutathione (250 mg/kg), green tea (200 mg/kg) with glutathione (250 mg/kg) for 21 days (i.p.). After that, blood samples were drawn and the levels of serum alanine aminotransferase, aspartate aminotransferase, Alkaline phosphatase, total protein and albumin, as liver injury indices, were measured.

***Results*:** The decrease of aminotransferase and alkaline phosphatase activity in the receptors of different dosages of green tea and glutathione was significant compared with the group treated by thioacetamide. Also, a significant increase was observed in total protein and albumin of serum in green tea receptors compared with thioacetamide group.

***Conclusion*:** The study results show the protective effect of green tea on thioacetamide-induced hepatotoxicity which is likely caused by the antioxidant effect of polyphenol compounds controlling thioacetamide activity which in turn controls the cytochrome P450 activity and neutralization of free radicals.



## Introduction

Liver is one of the critical organs of the body. Due to its key role in metabolism and detoxification processes it continuously deals with different substances and compounds resulted from metabolism paths which may have destructive impacts on it [1]. The use of medicinal plants for disease treatment purposes has been in vogue since a long time ago so that by half century ago, plants were one of the main sources of preparing remedies for diseases [2]. Tea is a plant likes within single based angiosperms category, dicotyledonous class and partial row of Teacae family and Camellia kind with the scientific name of *Camellia sinensis*. Oxidative enzymes are available in more than 20% of different kinds of tea. Green tea has no oxidative enzyme which is considered as an advantage. Moreover, green tea consists of polyphenol ingredients including catchins, epigallocatechingallate, epicatechingallate, epigallocatechin and epicatechin. Green tea's catechins show strong antioxidant effects [3-5]. It is stronger than famous antioxidants like vitamins C and E. [5-7].

**Figure 1.** Green tea plant

Glutathione (gammaglutaminsistenilgylicin) is the most abundant non-protein thiol compound with low molecular mass. The most important physiological roles of glutathione are: 1- antioxidant role, 2- detoxification role, 3- adjusting growth and reproduction and 4- safety [8, 9].

The studies demonstrated the protective effect of green tea against the formation of toxic metabolite of thioacetamide in liver by decreasing the activity of P450 cytochrome in one hand and on in the other hand it promotes liver detoxification capability by increasing glutathione concentration [1]. However, there is no study to compare the effects of green tea with glutathione on the hepatotoxic effect of thioacetamide. Thus, the aim of this research was to compare the effect of green tea extract on thioacetamide-induced hepatotoxicity with that of glutathione.

## Materials and Methods

This was an experimental study and all animals were collected from Tehran Pars Institute and transferred to the animal house of Arsanjan Azad University. The required glutathione and thioacetamide were provided from Sigma (UK) and Merck (Germany) companies, respectively.

This study was carried out by observing all ethical codes related to dealing with laboratory animals prepared by the Ministry of Health and Medical Education. The collected animals were kept in the following conditions: tempreture: 25±2ºC, 12 h of light and 12 h of dark. Before study, their weight was measured in order to make sure that all of them are within a specific weight range. The average weight of rats was 190±5 g and their age was 2.5-3 months. A total of 64 rats were divided randomly into 8 groups with 8 members.

Control group: during experiment they received no solvent or medicine.

Sham group: intraperitoneal (i.p.) injection of normal saline (5 mL/day) for 21 days.

Hepatotoxic group (thioacetamide): intraperitoneal (i.p.) injection injection of 50 mg/kg thioacetamide at last three days [9].

Experiment group 1: i.p. injection of 50 mg/kg green tea alcoholic extract for 21 days + 50 mg/kg thioacetamide at last three days [10].

Experiment group 2: i.p. injection of 100 mg/kg green tea alcoholic extract for 21 days + 50 mg/kg thioacetamide at last three days [10].

Experiment group 3: i.p. injection of 200 mg/kg green tea alcoholic extract for 21 days + 50 mg/kg thioacetamide at last three days [10].

Experiment group 4: i.p. injection of 250 mg/kg glutathione for 21 days + 50 mg/kg thioacetamide at last three days [11].

Experiment group 5: i.p. injection of 200 mg/kg green tea along with 250 mg/kg glutathione for 21 days + 50 mg/kg thioacetamide at last three days [11]. To prepare green tea extract, at first green tea leaves were powdered and then were macerated by ethanol 80% for 24 h and after screening the obtained solution, extraction process was performed through vacuumed concentration method.

**Biochemical investigations:** Forty eight h after the last injection, the rats were anesthetized with ether and their bloods were collected from their hearts. The samples were kept for 30 min in laboratory temperature and then serum was separated by centrifugation at 3000 rounds per minute during 10 min. To evaluate and compare the liver performance of different groups, serum aminotransferases including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured by enzyme method using commercial kits. The mentioned factors are from clinical indices for diagnosing liver diseases. To measure liver enzymes via RIA method, Pars Azmoon kit as well as RIA 1000 device, manufactured by USA, was used.

**Statistical Methods:** The data was presented as mean±SEM. The activity level of liver enzymes of different groups was analyzed with ANOVA. All analysis were performed by SPSS-11.5 and $p<0.05$ was considered as the significance level.

## Results

The injection of 50 mg/kg thioacetamide in 3 successive days increased the significance level of ALT, AST and ALP enzyme activities compared with control group. However, the decrease of total protein and albumin was significant compared with control group.

The injection of alcoholic extract of green tea at different dosages decreased significantly the activity level of ALT, AST and alkaline phosphatase liver enzymes compared with thioacetamide group. Also, total protein ($p=0.003$) and albumin showed a significant increase ($p<0.001$) in the groups compared with thioacetamide group. A significant decrease was shown in the activity level of ALT ($p=0.001$), AST ($p=0.002$) and ALP ($p=0.00$) liver enzymes in the receptors of glutathione compared with thioacetamide group and total protein and albumin showed a significant increase level in the groups compared with thioacetamide group (Table 1).

The decrease of ALT and AST enzymes decrease as well as total protein and albumin increase in the experimental 1 and 2 groups was not significant while the decrease of ALP was significant. Also, the injection of green tea extract at dosages of 50 and 100 mg/kg had more effects on the significant increase of albumin level and total protein of blood, compared with the injection of glutathione. Simultaneous injection of green tea extract and glutathione decreased significantly the activity level of ALT, AST and ALP enzymes compared with thioacetamide group. A significant increase ($p\leq0.05$) was observed in total protein and albumin in the group compared with thioacetamide group. The injection of green tea extract at dosages of 100 and 200 mg/kg decreases liver enzymes levels more than the injection of glutathione (Table 1).

**Table 1.** The effects of glutathione and green tea extract (Camellia sinensis L.) in different doses on hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), after thioacetamide-induced hepatotoxicity

| Groups | Total Protein(mg/dl) | Albumin(mg/dl) | ALP(IU/l) | AST(IU/l) | ALT(IU/l) |
|---|---|---|---|---|---|
| Control | 7.68±0.09 | 4.1±0.08 | 535.6±4.2 | 183.38±3.22 | 63.9±1.3 |
| Sham | 7.8±0.1# | 4.3±0.2 | 573.3±8.9 | 206.8±2.3 | 68.7±1.7 |
| Hepatotoxic (Thioacetamide) | 7.3±0.1* | 3.05±0.06 | 787.1±10.2 | 409.3±4.4 | 107.3±3.7 |
| Experimental (50 mg/kg) | 7.9±0.13 | 3.95±0.06 | 735.8±7.7 | 262.2±9.7 | 76±3.08 |
| Experimental (100mg/kg) | 8.06±0.1 | 4±0.05 | 571.5±7.1 | 259.7±7.2 | 75.2±1.5 |
| Experimental (200mg/kg) | 7.3±0.1 | 3.7±0.05 | 569.7±19.3 | 210.1±28 | 69.9±0.83 |
| Experimental (250mg/kg) | 7.1±0.5 | 3.7±0.08 | 755.2±9.04 | 249.4±7.7 | 67±1.4 |
| Experimental (200 mg/kg+ Glutation) | 7.05±0.12 | 3.55±0.1 | 613±15.3 | 212.2±6.7 | 64.1±0.8 |

* Significant difference with the Sham group ($p \leq 0.05$)
# Significant difference with the thioacetamide group ($p \leq 0.05$)

Also, the injection of 200 mg/kg green tea extract has a more appropriate effect on decreasing liver enzymes level compared with the injection of glutathione. This means that the extract exhibits better protective effects on liver cells against thioacetamide injuries.

## Discussion

Our results showed that green tea extract at doses of 50, 100 and 200 mg/kg significantly reduced the elevated activities of serum aminotransferases (ALT and AST), alkaline phosphatase and increased the production of proteins including albumin compared to the thioacetamide-treated group. In addition, daily injection of 200 mg/kg green tea had more efficacy compared to that of glutathione. Thioacetamide is used as an appropriate model in the studies of anti poisoning as well as protective effects of medicines and different compounds on liver [11].

Thioacetamide is a strong liver poison. When it enters the body it is metabolized by the enzymes of cytochrome P450 detoxification system [12, 13]. The metabolism of thioacetamide produces S thioacetamide oxide producing oxidative stress in liver cells. Studies show that thioacetamide results in liver cell necrosis and apoptosis [14, 15].

The study of Sai et al. showed the controlling effect of green tea on nitropropane toxicity of liver [16]. In another study carried out by Sugiyama et al., it was revealed that green tea extract mitigates beta-di galactosamine toxicity of liver [17] which agrees with the results of this study. The protective mechanism of green tea is likely generated due to its antioxidant effect [18, 19]. Moreover, the injection of green tea extract increases the concentration of glutathione [20]. Muto et al. and Yang and Raner showed in their studies the controlling effect of green tea in the statement of P450 cytochrome [21, 22].

The polyphenols of green tea increase the anti-apoptotic factor generated due to Bcl-2 in thioacetamide toxicity of liver in rats [23].

Xu et al. showed that the injection of green tea controls the biochemical parameters as well as histopathological changes due to micromicin [24]. In this research, the increase of the activity of aminotransferases and alkaline phosphatase due to treatment by thioacetamide implies the liver cells injuries due to thioacetamide because the enzymes are available inside cells and in the event of damaging cells they enter to serum. Polyphenol compounds are from the most important antioxidants. The compounds especially flavonoids have a protective effect on liver against liver poisoning and free radical injuries. The polyphenol compounds inside cells could act as electron donor and show both antioxidant and anti-prooxidant effects by two enzyme and no enzyme methods. Khorsandi et al. showed that the oral consumption of green tea extract affects severe poisoning of liver due to acetaminophen, improves liver necrosis and decreases serum transaminases [25].

In his study on the tea-cardamom aquatic essence, Baghy-Nia et al. showed that the essences have antioxidant effects and could decrease oxidative stress by decreasing free radicals [26]. Glutathione is the most efficient cellular tool for detoxification of medicines, pesticide substances and other xenobiotic substances and neutralize them before reaction with cellular elements like nucleic acids and proteins [27, 28]. Yuan et al. showed in his study that by its oxidation and reduction states, glutathione plays a key role in protecting liver against liver necrosis, liver cells apoptosis, hepatitis, kidney ischemia and stress oxidative of liver cells [11]. Dulundu investigated the effect of grape essence on the stress oxidative and liver cells necrosis and reported that the decrease of glutathione level in liver cells is the main reason of liver cells fibrosis and stress oxidative [29].

Green tea decreases cellular death due to thioacetamide probably by decreasing or controlling apoptosis which in turn decreases serum transaminase and increases serum protein and albumin and it shows a better performance in these situations than glutathione.

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### Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of Interest

The authors declare no conflict of interest.

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***References***


1. Neetu D, Ajay KS, Piush S. Review on hepatoprotective plants. Int J Pharm Sci Rev Res. 2011;7(1):14–26.
2. Mehdizade M, Hosseini SA, Ebrahiminia F. [Effect of green tea (Camellia sineisis L) extract on blood glucose and body weight in male induced diabetic rats]Persian. J Gorgan Univ Med Sci. 2009;11(8-12(
3. Lambert JD, Lee MJ, Lu H, Meng X, Hong JJ, Seril DN, et al. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. J Nutr. 2003;133(12):4172–7.
4. Perva Uzunalic A, Skerget M, Knez Z. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. Food Chem. 2006;96(4):597–605.
5. Sasaki H, Matsumoto M, Tanaka T, Maeda M, Nakai M, Hamada S, et al. Antibacterial activity of polyphenol components in oolong tea extract against Streptococcus mutans. Caries Res. 2004;38(1):2–8.
6. Prochazkova D, Bousova I, Wilhelmova N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4):513–23.
7. Nourooz Zadeh J, Eftekhar E. [Physiological importance of glutathione in health and disease] Persian. J Birjand Univ Med Sci. 2007;14(3):9–15.
8. Naderi GA, Bakhtiari S, Almasi A. [Comparison of the Selenium dioxide and green tea extract on serum lipids level in rats] Persian. Iran J Med Aromatic Plants. 2006;5(17):16–20.
9. Madani H, Asgari S, Naderi GA. [Hepatoprotective effects of silybum marianum and calendula officinalis polyphenolic extracts in rat] Persian. Iran Biol J. 2007;19(2):157–63.
10. Ostrowska J, Luczaj W, Kasacka I, Rozanski A, Skrzydlewska E. Green tea protects against ethanol-induced lipid peroxidation in rat organs. Alcohol. 2004;32(1):25–32.
11. Yuan L, Kaplowitz N. Glutathione in liver diseases and hepatotoxicity. Mol Aspects Med. 2009;30(1-2):29–41.
12. Lukivskaya O, Patsenker E, Lis R, Buko VU. Inhibition of inducible nitric oxide synthase activity prevents liver recovery in rat thioacetamide-induced fibrosis reversal. Eur J Clin Invest. 2008;38(5):317–25.
13. Satyabhama S, Padmanaban G. Effect of thioacetamide on cytochrome P-450 synthesis in rat liver. Biochem J. 1984;218(2):371–7.
14. Jeong JS, Han SY, Kim YH, Choi YC. Altered remodeling of nucleolar machineries in cultured hepatocytes treated with thioacetamide. J Korean Med Sci. 2001;16(1):75–82.
15. Kim KH, Bae JH, Cha SW, Han SS, Park KH, Jeong TC. Role of metabolic activation by cytochrome P450 in thioacetamide-induced suppression of antibody response in male BALB/c mice. Toxicol Lett. 2000;114(1-3):225–35.
16. Sai K, Kai S, Umemura T, Tanimura A, Hasegawa R, Inoue T, et al. Protective effects of green tea on hepatotoxicity, oxidative DNA damage and cell proliferation in the rat liver induced by repeated oral administration of 2-nitropropane. Food Chem Toxicol. 1998;36(12):1043–51.
17. Sugiyama K, He P, Wada S, Saeki S. Teas and other beverages suppress D-galactosamine-induced liver injury in rats. J Nutr. 1999;129(7):1361–7.
18. Zi X, Mukhtar H, Agarwal R. Novel cancer chemopreventive effects of a flavonoid antioxidant silymarin: inhibition of mRNA expression of an endogenous tumor promoter TNF alpha. Biochem Biophys Res Commun. 1997;239(1):334–9.
19. Sung H, Nah J, Chun S, Park H, Yang SE, Min WK. In vivo antioxidant effect of green tea. Eur J Clin Nutr. 2000;54(7):527–9.
20. Artali R, Beretta G, Morazzoni P, Bombardelli E, Meneghetti F. Green tea catechins in chemoprevention of cancer: a molecular docking investigation into their interaction with glutathione S-transferase (GST P1-1). J Enzyme Inhib Med Chem. 2009;24(1):287–95.
21. Muto S, Fujita K, Yamazaki Y, Kamataki T. Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450. Mutat Res. 2001;479(1-2):197–206.
22. Yang SP, Raner GM. Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. Toxicol Appl Pharmacol. 2005;202(2):140–50.
23. Oz HS, Chen TS. Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity. Dig Dis Sci. 2008;53(11):2980–8.
24. Xu C, Shu WQ, Qiu ZQ, Chen JA, Zhao Q, Cao J. Protective effects of green tea polyphenols against subacute hepatotoxicity induced by microcystin-LR in mice. Environ Toxicol Pharmacol. 2007;24(2):140–8.
25. Khorsandi LS, Javadnia F, Orazizadeh M, Abdolahi M. [Effect of green tea (Camellia sinensis L.) extract on acetaminophen induced acute hepatotoxicity in mice] Persian. Iran J Med Aromatic Plants. 2010;26(1):22–9.
26. Baghy-Nia N, Fani A, Maleky Rad A, Oryan. [The effect of Cardamom- tea watery extract on oxidative stress] Persian. Arak Univ Med Sci J. 2008;11(4):1–7.
27. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol. 2003;66(8):1499–503.
28. Naderi GA, Asgari S, Taher M. [Antioxidant effect of Pimpinella anisum and Cinnamomum zeylanicum on cell membrane of hepatocytes, LDL and nonenzymatic glycosylation of hemoglobin] Persian. Kermanshah Univ Med Sci J. 2004;7(1):29–36.
29. Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, et al. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. J Gastroenterol Hepatol. 2007;22(6):885–92.


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