Protective Effect of *Panax ginseng* Against Carbon Tetrachloride (CCl_4) - Induced Oxidative Brain Injury in Rats

Ali KARADENİZ[1*] Abdulkadir YILDIRIM[2] Fikret ÇELEBİ[1]

[1]Department of Physiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Turkey
*e-posta:karadenizali@atauni.edu.tr

Summary: This study was designed to investigate the protective effects of *Panax ginseng* against acute brain damage induced by CCl_4 in rats. A total of 30 healthy female Sprague–Dawley rats were divided in to three groups. Sedentary control group (group C) injected intraperitoneally (i.p.) with physiological saline as placebo for 7 consecutive days. CCl_4 toxication group was injected by i.p. a single dose of CCl_4 (group CCl_4). *Panax ginseng* plus CCl_4 group (200 mg/kg) was feeding through an orogastric tube for 7 consecutive days prior to CCl_4 injection (group CCl_4 + PG). The degree of protection in brain tissue was evaluated by the levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and nitric oxide (NO). *Panax ginseng* showed a significant brain-protective effect by decreasing the level of lipid peroxidation (MDA) and elevated the activities of GSH and SOD ($p<0.05$). Consequently *Panax ginseng* was blocked oxidative brain damage induced by CCl_4 in rats

Key words: *Panax ginseng*, Carbon tetrachloride, Brain damage, Rat

Sıçanlarda Karbon Tetraklorürün (CCl_4) Neden Olduğu Oksidatif Beyin Hasarına Karşı *Panax ginseng'* in Koruyucu Etkisi

Özet: Bu çalışma CCl_4 ile beyin hasarı oluşturulmuş sıçanlarda *Panax ginseng*' in koruyucu etkilerini incelemek için yapılmıştır. Toplam 30 adet dişi Sprague – Dawley sıçan üç eşit gruba ayrıldı. Kontrol grubuna placebo olarak 7 gün boyunca intraperitoneal (i.p.) serum fizyolojik uygulandı (grup C). CCl_4 toksikasyon grubuna ise i.p. tek doz CCl_4 uygulandı (grup CCl_4). *Panax ginseng* + CCl_4 grubuna ise i.p. CCl_4 uygulamasından önce 7 gün boyunca orogastrik tüp aracılığıyla *Panax ginseng* (200 mg/kg) verildi (grup CCl_4 + PG). Beyin dokusundaki korumanın derecesi malondialdehit (MDA), glutathion (GSH), superoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ve nitrik oksit (NO) düzeyleri ölçülerek belirlendi. *Panax ginseng* lipit peroksidasyon (MDA) düzeyini azaltırken, GSH ve SOD ($p<0.05$) düzeylerini ise artırdı. Sonuç olarak *Panax ginseng* sıçanlarda CCl_4'ün neden olduğu oksidatif beyin hasarını engelledi.

Anahtar kelimeler: *Panax ginseng*, Karbon tetraklorür, Beyin hasarı, Sıçan

INTRODUCTION

Reactive oxygen species (ROS) including oxygen free radicals are causative factors in the etiology of degenerative diseases. The enhanced production of free radicals and oxidative stress can be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics (e.g., carbon tetrachloride) (Kim et al., 1990). Carbon tetrachloride (CCl_4) intoxication in animals is an experimental model that mimics oxidative stress in many pathophysiological situations (Mc Gregor and Lang, 1996). CCl_4 intoxication in various studies have demonstrated that CCl_4 causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, brain and blood (Dashti et al., 1989). The toxicity of CCl_4 probably depends on formation of the trichloromethyl radical (CCl_3), which in the presence of oxygen interacts with it to form the more toxic trichloromethylperoxyl radical (CCl_3O_2)(Behar-Cohen et al., 1996). Oxidative stress resulting from increased free radical production after CCl_4 intoxication may play an important role in the degenerative processes in the tissues (Recknagel et al., 1989). Although toxic effects of CCl_4 were shown in the brain, heart or kidney, the major injury after CCl_4 intoxication was investigated in the liver.

Ginseng has been used medicinally in the Far East for hundred years and is currently one of the most widely taken herbal products throughout the world. *Panax ginseng* (PG) is well-known to have immunostimulatory, anti-inflammatory effects, antihepatoxicity effects, and a protective action against mammalian tumor cell lines. It is well known for its antioxidant property due to its ability to scavenge free radicals and to neutralize ferryl ion-induced peroxidation (Bastianetto et al., 2000; Shin et al, 2000). These properties of the ginseng are thought to provide many beneficial effects against organ damages. Therefore, the purpose of this study was to investigate the protective effect of ginseng against acute brain damage induced by CCl_4 in rats.

MATERIALS and METHODS

Chemicals

Panax ginseng was purchased from local herbal store in Ankara, Turkey. All other chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA) or Merck (Darmstadt, Germany).

Animals and Housing

Thirty adult male Sprague – Dawley rats (n = 10 x 3) weighing about 250 g were used. The animals were fed with standard rat pellets and tap water *ad libitum*. The rats were housed in individual cages (360 x 200 x 190 mm^3), each containing 2 or 3 animals for 15 days before the start of the experiment. All animals were housed in stainless steel cages under standard laboratory conditions (light period 07.00 a.m. to 8.00 p.m., 21 ± 2 °C, relative humidity 55 %), and received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health.

Experimental design

Rats were divided into three groups [Control group (C), CCl_4 toxication group (CCl_4), and CCl_4 toxication + *Panax ginseng*-treated group (CCl_4 + PG] each containing 10 animals. The first group served as a control group and was injected intraperitoneally (i.p.) with physiological saline for 7 consecutive days. The second group was given a single i.p. dose of CCl_4 10 ml/kg. Third group received *Panax ginseng* (200 mg/kg) orally through an orogastric tube for 7 consecutive days prior to CCl_4 injection. The dose and duration for *Panax ginseng* treatment were chosen according to previous study on *Panax ginseng* (Kim et al., 1999).

Sample collection and biochemical assays

Brain cell damage was biochemically assessed twenty-four hours after CCl_4 administration. Rats were anesthetized with 50 mg/kg body weight ketamine and 5 mg/kg body weight xylazine intramuscularly injected. The brain samples were removed and stored -80 ºC for biochemical examination.

All tissues were maintained at +4 ºC throughout preparation. A portion of each brain tissues (1:9, w/v) for all assays were homogenized in 0.9 % NaCl solution with an OMNI TH International homogenizer (Warrenton, VA, USA). Tissue homogenates were centrifuged for 15 min at 15.000 g, and then the clear upper supernatants were removed for analyses.

GPx activity in tissues was measured by the method of Paglia and Valentine (1967) and expressed as U/g protein. Cu,Zn-SOD activity in tissues was detected by the method of Sun et al. (1988) and expressed as U/g protein. Glutathion (GSH) levels in tissues were assessed according to the method of Tietze (1969) and Anderson (1985) and expressed as (µmol/g protein). Tissue NO levels were measured using the Griess reagent by method Moshage et al. (1995) expressed as µmol/g protein. MDA levels in tissues were determined spectrophotometrically according to the method described by Ohkawa et al. (1979) and expressed as nmol/g protein.

Statistical Analysis

Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's posttest. A p value of < 0.05 was considered to be significant. All data were expressed as mean ± S.E.

RESULTS

The results are shown in Table 1. As seen from the table, MDA, GPx and NO levels were found to increase, on the contrary SOD and GSH activities were found to decrease in the brain tissues from rat treated CCl_4 compared with control ($p<0.05$). PG supplementation concurrently applied with CCl_4 caused significant increases in GSH and SOD activities and decreases in MDA, GPx and NO levels compared with CCl_4 group ($p < 0.05$).

Table 1. The levels of lipid peroxidation and antioxidant enzyme activities in brain tissue of rats with *Panax ginseng* against CCl_4-induced oxidative damage (n=10 for each groups).

	Groups		
Parameters	C	CCl_4	CCl_4 + PG
MDA (nmol/ g protein)	0.65 ± 0.05^{a}	0.81 ± 0.12^{b}	0.70 ± 0.05^{c}
GPx (U/g protein)	2.30 ± 0.10^{a}	2.92 ± 0.25^{b}	2.40 ± 0.15^{c}
GSH (µmol/ g protein)	1.65 ± 0.12^{a}	1.25 ± 0.20^{b}	1.50 ± 0.10^{c}
SOD (U/ g protein)	110 ± 10.50^{a}	89.50 ± 9.50^{b}	102.3 ± 8.40^{c}
NO (µmol/ g protein)	0.40 ± 0.05^{a}	0.58 ± 0.09^{b}	0.42 ± 0.08^{c}

$^{a-b}$ $p < 0.05$ compared to control group ($p<0.05$).
^{c} $p < 0.05$ compared to CCl_4 group ($p<0.05$).

DISCUSSION

CCl_4 when administrated is distributed and deposited to organs such as the liver, brain, kidney, and heart (Recknagel et al., 1989). In this experimental study was investigated the protective effect of PG on CCl_4-induced brain toxicity. The level of brain MDA in CCl_4 treated group was significantly higher than the control group. The increase in MDA level in the brain suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant mechanisms to prevent the production of excessive free

radicals. Similar results were previously reported in kidney (Ogeturk et al., 2005) and liver (Yang et al., 2008) tissues. Melin et al. (2000) stated that CCl_4 metabolised by cytochrome p-450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids. Neuronal cell death from oxidative stress has been implicated in ischemia, traumatic brain injury, Alzheimer's disease and Parkinson's disease (Maier and Chan 2002). However pretreatment of PG significantly prevents CCl_4-induced lipid peroxidation in brain tissue. This was probably due to less damage by oxygen free radicals with PG.

GSH is involved in several defense processes against oxidative damage. It protects cells against free radicals, peroxides and other toxic compounds (Sies, 1999). Indeed, glutathione depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury (Limon-Pacheco et al., 2007). In our study the brain GSH level in CCl_4 treated group was significantly decreased compared with control group. However same parameter for CCl_4 with ginseng treated group was increased compared with CCl_4 groups. It shows that this situation is sourced by antioxidant feature of ginseng. Recent studies have provided considerable support for the *in vitro* and *in vivo* protective effects of ginseng on oxidative stress. For example, Recknagel (1983) stated that GSH plays a key role in the detoxification of the reactive toxic metabolites of CCl_4 in liver necrosis. However, Harputluoglu et al. (2006) stated that *Panax ginseng* improved hepatic damage in thioacetamide-induced fulminate hepatic insufficient. The antioxidant action of this *Panax ginseng* extract is due to its components, the flavonol glycosides, which are known for scavenging superoxide and then preventing anion and hydroxyl radical lipid peroxidation in the membranes (Attele et al., 1999).

GPx is partially located within the cellular membrane. It is generally belived that the protective effect of GSH against the oxidative breakdown lipids is mediated through GPx by reduction of endogenously formed hydrogen peroxides of unsatured fatty acids to hydroxyl derivates (Sing and Pathak, 1990). SOD is a scavenger of peroxide anion radicals, which could inhibit the initiation of lipid peroxidation by free radicals (Escobar et al., 1996). In the brain, SOD activity decreased after CCl_4 application, but GPx activity significantly increased statistically at the end of the study. The high concentration of polyunsaturated fatty acids and aerobic metabolic activity of the brain increase the susceptibility of this organ to peroxidative damage induced by reactive oxygen species after CCl_4 ingestion (Kitajka et al., 2002). It has been reported that in the brain the content of cytochrome p-450 and arachidonic acid is lower than other organs, demonstrating that the antioxidant defense system has limited capacity in the brain (Nakata et al., 1985). These results suggest that GPx is more important than SOD for detoxification of reactive oxygen species in brain tissue. Furthermore, GPx can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell membrane (Sun et al., 1988). These findings suggest that brain have different coping mechanisms to deal with oxidative stress than other organs such as liver, kidney and etc.. However, neuroprotective properties of ginsenosides have also been demonstrated *in vitro* (Rudakewich et al., 2001) and *in vivo* (Koc et al., 1995). Oyama et al. (1996) stated that EGb-761 was shown to increase the viability of neuronal cells under hydrogen peroxide-induced oxidative stress. On the other hand, Ni et al. (1996) stated that the EGb-761 were shown to protect against apoptosis, increase cell viability, inhibit lipid peroxidation and scavenge hydroxyl radicals in cultured rat cerebellar cells.

It is known that NO is very important for antioxidant defense system in organism. Although NO is stable in absence of oxygen, but it isn't stable in the existence of oxygen and free radicals (Karadeniz and Cemek, 2006). In our study, the level of brain NO in CCl_4 treated group was significantly higher than the control group. However NO level for CCl_4 with ginseng treated group was decreased compared with only CCl_4 treated group. This result assign that ginseng is blockage for the occurrence of NO and therefore it has supporting effect on antioxidant system. Similar antioxidant properties have also been reported in studies using ischemia re-perfusion injury rat brain (Zhang and Liu, 1994). More recently, EGb-761 has been shown to markedly decrease neuronal nitrix oxide syntase (NOS) immunoreactivity following heat stress in rats and to protect and rescue hippocampal cells from NO-induced toxicity (Bastianetto et al., 2000). Furthermore Behar-Cohen et al. (1996) informed that EGb-761 reduce peroxynitrite-induced cell death in cultured retinal epithelial cells.

In conclusion, our data indicated that CCl_4-induced brain toxicity might be related to oxidative damage. Co-administration of PG decreased the harmful effects of CCl_4-induced brain toxicity probably by inhibiting free radical mediated process.

REFERENCES

Anderson, ME., 1985. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol. 113, 548–555.

Attele, AS., Wu, JA., Yuan, CS., 1999. Ginseng pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol. 58, 1685-1693.

Bastianetto, S., Zheng, WH., Quirion R., 2000. The Ginkgo biloba extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity: involvement of its flavonoid constituents and protein kinase C. J Neurochem. 74, 2268–2277.

Behar-Cohen, FF., Heydolph, S., Faure, V., Droy-Lefaix, MT., Courtois, Y., Goureau, O., 1996. Peroxynitrite cytotoxicity on bovine retinal pigmented epithelial cells in culture. Biochem. Biophys. Res. Commun. 226, 842–849.

Dashti, H., Jeppsson, B., Hagerstrand, I., Hultberg, B., Srinivas, U., Abdulla, M., Bengmark, S., 1989. Thioacetamide- and carbon tetrachloride-induced liver cirrhosis. Eur. Surg. Res. 21, 83-91.

Escobar, JA., Rubio, M.A., Lissi, EA., 1996. SOD and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic. Biol. Med. 20, 285–290.

Harputluoglu, MM., Demirel, U., Ciralik, H., Temel, I., Firat, S., Ara, C., Aladag, M., Karincaoglu, M., Hilmioglu, F., 2006. Protective effects of Gingko biloba on thioacetamide-induced fulminant hepatic failure in rats. Hum Exp Toxicol, 25, 705-713.

Karadeniz, A., Cemek, M., 2006. Protective Effect of Spirulina platensis Against Lead Toxication in Rats. J. Anim. Vet. Adv. 5, 1113-1116.

Kim, HJ., Odend'hal, S., Bruckner, JV., 1990. Effect of oral dosing vehicles on the acute hepatotoxicity of carbon tetrachloride in rats. Toxicol. Appl. Pharmacol. 102, 34-49.

Kim, W., Hwang, S., Lee, H., Song, H., Kim, S., 1999. Panax ginseng protects the testis against 2,3,7, 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. BJU Int. 83, 842-849.

Kitajka, K., Puskás, LG., Zvara, A., Hackler, LJr., Barceló-Coblijn, G., Yeo, YK., Farkas, T., 2002. The role of n-3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by dietary n-3 fatty acids. Proc Natl Acad Sci USA, 99, 2619-2624.

Koc, RK., Akdemir, H., Kurtsoy, A., Pasaoglu, H., Kavuncu, I., Pasaoglu, A., Karakucuk, I., 1995. Lipid peroxidation in experimental spinal cord injury. Comparison of treatment with Ginkgo biloba, TRH and methylprednisolone. Res. Exp. Med. 195, 117-123.

Limon-Pacheco, JH., Hernandez, NA., Fanjul-Moles, ML., Gonsebatt, ME., 2007. Glutathione depletion activates mitogen-activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice. Free Radic. Biol. Med. 43, 1335-1347.

Maier, CM., Chan, PH., 2002. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. Neuroscientist 8, 323-334.

Mc Gregor, D., Lang M., 1996. Carbon tetrachloride: genetic effects and other modes of action. Mutat. Res. 366, 181-195.

Melin, AM., Perromat, A., Deleris, G., 2000. Pharmacologic aplication of Fourier transform IR spectroscopy: in vivo toxicity of carcon tetrachloride on rat liver. Biopolymers 57, 160–168.

Moshage, H., Kok, B., Huizenga, JR., Jansen, PL., 1995. Nitrite and nitrate determinations in plasma: A critical evaluation. Clin. Chem. 41, 892–896.

Nakata, R., Tsukamoto, I., Miyoshi, M., Kojo, S., 1985. Liver regeneration after carbon tetrachloride intoxication in the rat. Biochem. Pharmacol. 34, 586–588.

Ni, Y., Zhao, B., Hou, J., Xin, W., 1996. Preventive effect of Panax ginsengextract on apoptosis in rat cerebellar neuronal cells induced by hydroxyl radicals. Neurosci. Lett. 214, 115–118.

Ogeturk, M., Kus, I., Colakoglu, N., Zararsiz, I., Ilhan, N., Sarsilmaz, M., 2005. Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J. Ethnopharmacol. 97, 273-280.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Oyama, Y., Chikahisa, L., Ueha, T., Kanemaru, K., Noda, K., 1996. Panax ginseng extract protects brain neurons against oxidative stres induced by hydrogen peroxide. Brain Res. 712, 349–352.

Paglia, DE., Valentine, WN., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Recknagel, RO., 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 33, 401-408.

Recknagel, RO., Glende, EA., Dolak, JA., Waller, RL., 1989. Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther. 43, 139–154.

Rudakewich, M., Ba, F., Benishin, CG., 2001. Neurotrophic and neuroprotective actions of ginsenosides Rb(1) and Rg(1). Planta Med. 67, 533-537.

Shin, HR., Kim, JY., Yun, TK., Morgan, G., Vainio, H. 2000. The cancer-preventive potential of Panax ginseng: a review of

human and experimental evidence. Cancer Causes Control 11, 565-576.

Sies, H., 1999. Glutathione and its role in cellular functions. Free Radic. Biol. Med. 27, 916-921.

Sing R., Pathak, DN., 1990. Lipid peroxidation and glutathione peroxidase, and glutathione reductase, superoxide dismutase, catalase and glucose-6-phosphate dehydrogenase activities in $FeCl_3$ induced epileptogenic foci in the rat brain. Epilepsia 31, 15–26.

Sun, Y., Oberley, LW., Li, Y., 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem. 34, 497–500.

Tietze, F., 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione, applications to mammalian blood and other tissues. Anal. Biochem. 27, 502–522.

Yang, YS., Ahn, TH., Lee, JC., Moon, CJ., Kim, SH., Jun, W., Park, SC., Kim, HC., Kim, JC., 2008. Protective effects of Pycnogenol (R) on carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats. Food. Chem. Toxicol. 46, 380-387.

Zhang, YG., Liu, TP. 1994. Protective effects of total saponins of Panax ginseng on ischemia-reperfusion injury in rat brains. Chin. J. Pharmacol. Toxicol. 8, 7-12.