J. Ginseng Res.
Vol. 24, No. 2, 94-98(2000)

Protective Effect of *Panax ginseng* Against Tetracycline Toxicity in Rats

Somaia A. Nada# and Mahmoud S. Arbid

Pharmacology Department, National Research Center, P.O. Box : 12311, El-Tahrir St., Dokki, Cairo, Egypt

(Received December 9, 1999)

Abstract : Tetracycline hydrochloride (TC) caused 100%, 80% and 20% mortality rates among rats injected with 40 mg, 30 mg and 20 mg/100g. b.w. respectively; while the mortality rates were decreased to 50%, 20% and 10% when *Panax ginseng* (2 mg/100g. b.w.) injected with TC during 72 hrs. post-injection. Subacute-toxicity study demonstrated that TC caused severe hepato-nephrotoxicity (demonstrated by biochemical analysis of serum including: transferases , alkaline phosphatase, total protein , glucose, cholesterol urea and creatinine) in rats injected i.p. with 10 mg and 5 mg/100g. b.w. for 7 days of daily injection . These signes of toxicity were greatly diminished by *P. ginseng* addition to TC doses.

Key words : *Panax ginseng*, tetracycline hydrochloride, rats, toxicity, hepatotoxicity, nephrotoxicity.

INTRODUCTION

Tetracyclines (TC) are valuable antibiotics effective against a wide variety of gram positive and gram negative pathogenes,[1] rikettsia, myocoplasma, spirocketa and actinomyces organisms.[2,3] It has a good antiprotozoal activity.[4] Nowadays, the use of TC has been discouraged because of the toxic effect of TC and their decomposition products formed during storage or for its abuse.

TC caused adverse effect on gastrointestinal tract in the form of: anorexia, epigastric distress, nausea, vomiting, diarrhea, bulky stool, stomatitis, dysphagia, enterocolitis, pancreatitis, inflammatory lesions in the anogenital region. Moreover, TC induced hepato-nephrotoxicity and hypersensitivity reactions, these conditions usually resolve soon after discontinuation of TC,[5] inhibit bone development;[6] decrease iron absorption.[7] TC induced hepatic injury through two ways: (i) TC could to inhibit the mitochondreal oxidative phosphorylation;[8] (ii) it inhibit protein synthesis which ended by fatty changes in hepatocytes.[9]

P. ginseng is a herbal root that has been used in folk medicines. It is most often used as a general tonic. Recently, more of its effects have been attributed to its antioxidant action[10] against the primary and secondary free radicals.[11] *P. ginseng* is one of the most popular natural tonics and has been shown to possess various biological activities such as protein anabolic effect, antitumor activities, and an inhibitory effect of tumor angiogenesis and metastasis.[12] *P. ginseng* was reported to repair the damaged tissues of the kidney,[13] liver[14] and brain.[15] It was found that *P. ginseng* minimized the toxic effect of ochratoxin A (a mycotoxin) on liver and kidney tissues.[16] The aim of this study is to investigate the effect of *P.ginseng* on TC-induced toxicity in rats and suth, to renew TC preparations to become less toxic for long administration.

To whom correspondence should be addressed.
(Tel) 202-3669963; (Fax) 202-3370931
(E-mail) sanada@menanet.net

MATERIALS AND METHODS

1. Materials

(1) Chemicals

Tetracycline hydrocholride 250 mg capsules (El-Nasr Pharma. Chem. Co., Egypt) in a concentration of 60 mg/m*l* in water. *P. ginseng* was obtained from EPICO, 10th Ramadan City, Egypt. Powder of *P. ginseng* was soked with water (1:25, w/w) in a glass flask for 3 hours, then the container was put on boiling water for 40 minutes. After filtration, the solid residue was subjected to the same process once again, then two filtrates were mixed and freez dried. The dried extract equaled about to 20% (w/w) of the raw drug. The extract was diluted to 2 mg/m*l*.

Diagnostic Kits were obtained from Biomerieux, Laboratory Reagents and Products, Marcy L'Etoile, France. For determination of serum glucose, cholesterol, total protein (T.P.), transaminasis (ALT & AST), alkaline phosphatase

(A.P.), urea and creatinine.

(2) Animals

Sprague Dawly rats weighing 100±5g. b.w. were obtained from Animal House Lab., National Research Center, Cairo, Egypt. The animals were allowed free access to food and water.

2. Experimental design

(1) Experiment I (acute toxicity study)

Eighty rats were divided into 8 equal groups (10 rats each). Rats injected i.p. with a single doses of TC (20 mg, 30 mg, 40 mg/kg b.w.) for group 1, 2 and 3; while groups 4, 5 and 6 were injected with *P. ginseng* (2 mg/Kg b.w.) mixed with TC different doses and alone for group 7 and control group administered 1 m*l* normal saline i.p. All the injected animals were kept under observation during 72 hours recording any singes of toxicity and the mortality rate.

2. Experiment II (sub-acute toxicity study)

Sixty rats (both sex) were divided into 6 equal groups (10 rats each); they were injected i.p. daily for 7 successive days as follows: group (1) was the control group injected with normal sterile saline solution (1 m*l*) i.p.; group (2) injected with *P. ginseng* (2 mg/100 g. b.w.); groups (3 & 4) were given TC 5 mg and 10 mg/100 g. b.w. respectively; groups (5 and 6) were administered a mixed injection of *P. ginseng* (2 mg/100g. b.w.) and TC (5 mg and 10 mg/100g. b.w.) i.p.respectively. Blood samples were collected from retro-orbital venous plexus at the end of experimental time for the following biochemical assays: serum T.P.,[17] cholesterol,[18] glucose,[19]' AST & ALT activities,[20] AP,[21] urea[22] and creatinine.[23]

All data were analyzed statistically using ANOVA and LSD (least significant differences) according to Snedecor and Cochran (1967).[24] The level of significance chosen in all cases were $p<0.05$.

RESULTS

1. Acute toxicity study

Fig. 1. showed that three doses of TC injections (20 mg, 30 mg, 40 mg/100g. b.w.) caused an increase in the mortality rate as a dose-dependent manner (30%, 50%, 80%, respectively). When TC-treatments were combined with *P.ginseng* (2 mg/100g. b.wt.) the mortality rate decreased up to 10%, 20% and 50%.

2. Subacute toxicity study

Rats which were given *P. ginseng* alone, significantly

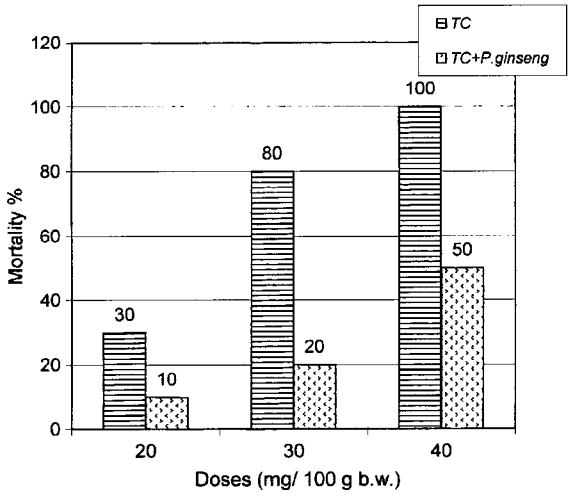

Fig. 1. Effect of P. ginseng on mortality percent induced by different doses of tetracycline HCl in rats after 72 hr.of i.p. injection.

Table 1. Effect of tetracycline hydrocholoride (TC) (5 mg & 10 mg/100 g. b.w.m i.p.) and/ or P. ginseng (2 mg/100 g. b.w., i.p.) daily doses for 7 days of teratment on some liver and kidney function tests in rats. (means ± S.E., n=10)

Parameters Groups	T.P. (g/d*l*)	Cholesterol (mg/d*l*)	Glucose (mg/d*l*)	Ap (K.A./d*l*)	AST (IU/L)	ALT (IU/L)	Urea (mg/d*l*)	Creatinine (mg/d*l*)
Control	A 7.04±0.14	A 73.25±2.14	A 80.41±3.88	A 14.25±0.84	A 108.25±1.60	A 55.04±4.81	A 39.49±2.44	A 0.73±0.04
P.g. (2 mg/100g)	B 8.30±0.11	C 65.20±0.25	B 69.60±0.42	A 13.32±0.36	B 97.20±2.27	A 48.60±2.40	B 32.10±1.49	B 0.56±0.03
TC 10 mg/100 g.b.w	C 5.75±0.18	C 87.23±1.45	C 114.43±2.43	B 80.50±1.55	C 167.22±3.80	B 98.23±4.63	C 50.47±3.64	C 1.12±0.08
TC 5 mg/100 g.b.w	AC 6.30±0.12	A 75.14±2.43	CD 109.84±4.33	C 33.70±3.92	D 118.73±3.83	C 68.44±3.83	AC 46.56±2.84	D 1.03±0.05
TC (10 mg/100 g.b.w) & *P.g.* (2 mg/100 g.b.w)	A 6.60±0.12	B 61.81±2.08	D 101.50±3.54	C 30.70±0.72	E 133.78±2.43	D 85.45±5.10	AC 44.32±1.53	E 0.93±0.03
TC (5 mg/100 g.b.w) & *P.g.* (2 mg/100 g.b.w)	A 6.80±0.22	AC 70.72±2.44	A 85.42±4.81	A 18.40±1.62	A 104.50±3.32	AC 63.57±2.43	AC 43.55±2.48	E 0.87±0.04

ANOVA within the column means superscribed with different letters are significantly different ($P<0.05$).

increased total protein level and decreased cholesterol value comparing with the control group. At the same time, no detectable changes were observed in the levels of glucose, AP, AST, ALT, urea, and creatinine by *P. ginseng* injection (Table 1).

Hypoproteinaemia was observed in the rats injected with two dose levels of TC (10 mg & 5 mg); the levels of total protein were elevated to the normal values by the addition of *P. ginseng* to TC. The lower dose of TC (5 mg/100g. b.wt.) and the combined treatment P.g. plus TC (5 mg) did not change cholesterol values. Meanwhile, the higher dose of TC (10 mg/100g. b.wt.) caused significant hypocholesterolaemia ($P<0.05$) and it became less than control values when *P. ginseng* combined with TC-dose (Fig. 2C).

Rats injected with TC doses (10 mg and 5 mg/100g. b.w.) showed significant hyperglycaemic effect. The addition of *P. ginseng* to TC-doses changed the elevated glucose concentration from 42.31% (TC 10 mg) and 36.6% (TC 5 mg) to 26.2% (*P. ginseng* + TC 10 mg), 6.23% (*P.ginseng* + TC 5 mg) . But the levels were still higher than control group (Table 1, Fig. 2B).

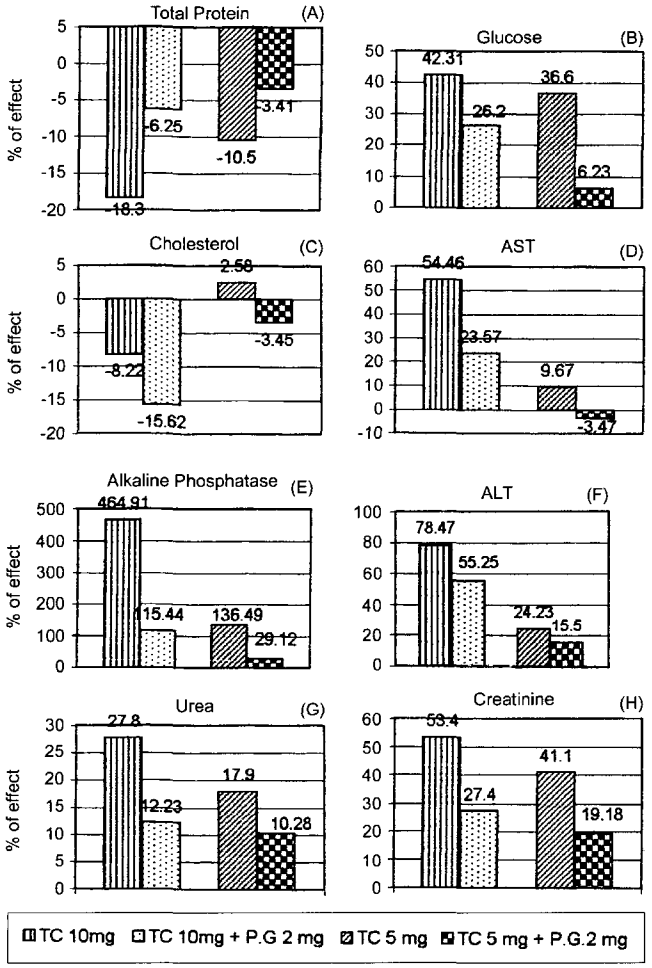

Fig. 2. The effect of P.ginseng (2 mg/100g. b.wt.) on tetracycline HCl treated rats (10 mg & 5 mg/100g. b.wt.).

AP and AST activities were increased by TC-doses (10 mg and 5 mg/100g. b.w.), these values were decreased to in the groups injected with the combined treatments of *P. ginseng* plus TC-doses. AP and AST values were normalized after P.g. was mixed with TC (5 mg), whereas TC (10 mg) plus *P. ginseng*-treated rats had lower activities in the values of AP and AST compared with TC (10 mg)-group (Table 1, Fig. 2D & 2E).

ALT activity was altered than control value in both groups administered TC (10 mg and 5 mg/100g. b.w.). ALT activity showed non-significant differences between TC alone and *P. ginseng* + TC (Table 1, Fig 2F).

Significant inrease in urea concentration as the resulte of TC(10 mg)-injection. Meanwhile, urea level did alter by other treatments comparing to the control (Table 1, Fig. 2G).

TC-doses caused a significant increase in creatinine level . Addition of *P.g.* to TC-doses decreased creatinine level to became within the normal lemit (Table 1, Fig. 2H).

DISCUSSION

The mortality rate was decreased by the combined treatment of *P. ginseng* and TC-different doses; TC-injected rats produced severe toxicity especially in the groups received 30 and 40 mg/100g. b.w. Generally these changes were dose related. The signes of toxicity were suggestive of hepato-nephrotoxicies as confermed by the serum biochemical analysis. Similar results were obtained by previous study on ochratoxins and P.g.[16] Suggested mechanisms of TC-induced toxicity are as follows: (i) TC caused inhibition of mitochondrial oxidative phosphorylation[8]; (ii) TC inhibit protein synthesis via the initial step in the pathogenesis of TC-induced fatty changes in hepatocytes.[9,25]

The marked decrease in T.P and the increase in urea level in TC-treated groups have been observed by previous studies, and were attributed to incorporation of amino acids into protein synthesis and accumulation of amino acids and accumulation of protein precursors metabolites in the liver.[26,27] The present results show that suppression of protein synthesis is not the only cause of urea elevation. Impairment of renal function in TC-

treated rats could be a contributary cause.[28] TC acts on the renal function of rats by a mechanism independent of antiduretic hormone.[29]

Hyperglycemia was resulted from binding activity of TC to Ca^{+2} and inorganic phosphate[30] and impairment of protein synthesis.[31] These effects are independet of the action of insulin on glucose entry the cell.[32]

The decrease of the serum cholesterol level in TC-treated rats (10 mg/100g. b.w.) may be attributed from lowering of cholesterol biosynthesis by the antibiotic.[33] Simillar findings were reported by Bocker *et al.*,[34] in mice treated with TC.

Activities of ALT, AST and AP were elevated in all TC-injected rats. Such findings demonstrate the cytotoxic effect of TC on hepatocytes. It has been reported that the liver of female rats injected with TC (10~30 mg/100g. b.w.) developed fatty metamorphosis.[35,36] Several recent studies show the utilization of *P. ginseng* for controlling the side effects of several toxicants as insecticide (2,3,7,8-tetrachlorodiabenz-p-dioxine),[37] thioacetamide,[38] aflatoxin B_1[39] and glutamate induced neuron damage.[40]

The combined administration of *P. ginseng* plus TC improved liver and kidney function including prevention of the increase in AST, ALT, AP, glucose, cholesterol, urea and creatinine values as well as decrease in total protein concentration. These data confirmed that *P. ginseng* has ability to repair the damaged liver, kidney, heart and brain tissues.[13,15] *P. ginseng* exerts its effect through the activation of hepatic epoxide hydrolase and glutathione S-transferase[41] which are involved in detoxification of electophilic metabolites of carcinogenic agent such as benzo [a] pyrene, aflatoxin B_1 and ochratoxin A.[16,39,42]

Further pharmaceutical studies are necessary on the modification of TC conjugation with *P. ginseng*

REFERENCES

1. Steigbigel, N. H., Reed,C. W. and Finland, M : *Am. J. Med. Sci.* **255**, 179 (1968).
2. Finland, M: *Med. Clins. North. Am.* **36**, 1239 (1952).
3. Weinstein , L. : Antibiotics, III the tetracyclines. Quoted from Goodman, L. S. and Gilman, A. (1970): The Pharmacological Basis of Therapeutics. 4th ed., The Mcmillan Company Limited, London.
4. Huber, W. G. : Tetracyclines. Quoted from Dones,L.M., Booth , N. H. and McDonald, L. E. (1977):Veterinary Pharmacology and Therapeutics. 4th ed. Iowa State University Press, Ames., Iowa. U.S.A.
5. Krogh, C. M. E. ed. (1995) : "Compendium of Pharmaceuticals and Specialties". Pub. Canadian Pharmaceutical Association, Ottawa, Ontario, Canada.
6. Simmons, D. J., Tteitelbaum, S. L., and Rosenberg, G. D. : *Metabol. Bone Dis. Relat. Res.* **3**, 51 (1981).
7. Djaldetti, M., Fishman, P., Notti, I. and Bessler, H. : *Biomedicine*, **35**, 150 (1981).
8. DuBuy, H. G. and Showacare, J. L.: *Science* **133**, 196 (1961).
9. Zia, H. and Price, J. C. : *J. Pharm. Sci.* **65**, 226 (1976).
10. Sohn, H. O., Lim, H. B., Lee, Y. G., Lee, D. W. and Kim, Y. I. : *Arch. Toxicol.* **67**, 667 (1993).
11. Okada, S.: *Pathol. Interntl.* **46**, 311 (1996).
12. Sato, K., Mochizuki, M., Saiki, I., Yoo, Y. C., Samukawa, K. and Azuma I : *Biol. Pharmacol. Bull.* **17**, 635 (1994).
13. Hattori, T., Nagamatsu, T., Ito, M. and Suzuki, Y. : *Jap. J. Pharmacol.*, **50**, 477 (1989).
14. Yamamoto, M., Miki, S., Deuchi, H., Uemiya , M., Uemura , T. and Kumagai, A. : Combined effect of red ginseng with XIAO-CHAI-HU-TANG in patients with chronic hepatitis. Proceeding of the 6th International Ginseng Symposium, Seoul, Korea, pp. 57-60 (1993).
15. Okamura, N., Kobayashi, K., Akaike, A., and Yang, A. : *Biol. Pharmacol. Bull.* **17**, 270 (1994).
16. Nada, S. A. Arbid, M. S. and Ramadan, H. A. I. : The possible protective role of Korean ginseng on ochratoxicosis : with special references on chromosomal aberrations in rats. Proceeding of the International Symposium on Ginseng "Advances in Ginseng Research", Seoul, Korea, Sept. 22-25 (1998).
17. Gornall, A. G., Bardawill, C. J., and David, M. M.: *J. Biol. Chem.* **177**, 75 (1949).
18. Charles C. A. and Richmond W. : *Clin. Chem.* **20** , 470 (1974).
19. Trinder, P. : *Ann. Clin. Biochem.* **6**, 24 (1969).
20. Reitman, S. and Frankel, S. : *Am. J. Clin. Path.* **28**, 56 (1959).
21. Kind, P. R. N. and King, E. J. : *J. Clin. Pathol.* **7**, 322 (1954).
22. Henry, J. B., Todd, M., Sanford, A. and Davidsohn H.: Clinical Diagnosis and Measurement by Laboratory Methods, 16th ed., W.B. Saunder and Co., Philadelphia PA. pp. 260 (1974).
23. Husdan H. and Rapoport A. : *Clin. Chemist.*, **14** , 222 (1968).
24. Snedecor G. W. and Cochran W. G. : Statistical Methods. 6th. ed. The Iowa State Univ. Press, Ames, Iowa, USA (1967).
25. Yeh, S. D. and Shils, M. S. : *Porc. Soc. Exp. Biol. Med.* **121**, 729 (1966).
26. Morgan, T. and Ribush, N. : *Med. J. Aust.* **1**, 55 (1972).
27. Ganguly, R., Pennock, D. G. and Kluge, R. M.: *Allerg. Immunol. (Lepi 1)* **30**, 104 (1984).
28. Korkeila, S. : *Lancet* **1**, 974 (1971).
29. Bocker, R., Estler, C. J., Maywald, M. and Weber, D. : *Arzneim. Forsch.* **31**, 2118 (1981).
30. Kelly, R. G. and Buyske, D. A. : *J. Clin. Pathol.* **7**, 322 (1960).
31. Dijkhuis, H. J. P., and Van Meurs, A. J. : *J.A.M.A.*, **233**, 441 (1973).
32. Murray, R. K., Granner, D. K., Mayes, P. A. and Radwell, V. W. ed. : "Harper's Biochemistry" 23rd ed. Appleton and Lange, Norwalk, Connecticut/San Mateo, California. pp. 562 (1993)
33. Mukherjee, D., Ghosh, H. N., Mukherjee, S. : *J. Antibiot.* **24**,

263 (1971).
34. Boada,J. N., Padron, F., Feria, M., Alarco, B. and Darias, V. : *Farmaco. Ed. Prat.* **32**, 301 (1977).
35. Gray, J. E.,Weaver, R. N., Skinner, P., Mathews, J. and Day, C. E. : *Toxicol. Appl. Pharmacol.* **30**, 317 (1974).
36. Whalley, P. J., Martin, F. G., Adams, R. H. and Combes, B. : Obstet . *Gynecol.* **28**, 103 (1966).
37. Kim, S., Hwang, S., Kwak, Y., Nam, K. : Protective effect of Korean red ginseng on TCDD-induced toxicity in male guinea pigs. Proceeding of the International Symposium on Ginseng "Advances in Ginseng Research", Seoul, Korea, Sept. 22-25, p.381 (1998).
38. Kim, H. and Kim, K. H. : Hepatoprotective effect of *Panax ginseng* on thioacetamide- intoxicated rat. Proceeding of the International Symposium on Ginseng "Advances in Ginseng Research", Seoul, Korea, Sept. 22-25, p. 377 (1998).
39. Xiaoguang, C., Hongyan, L., Xiahong, L., Zhaodi, F., Yan, L., Lihua T. and Rui, H. : *J. Ethnopharmacol.* **60**, 71 (1998).
40. Kim, Y. C., Kim, R. S., Markelonis, G. J. and Oh, T. H. : Ginsenosides Rb1 and Rg3 attenuate glutamate-induced neurotoxicity in primary culture of rat cortical cells. Proceeding of the International Symposium on Ginseng "Advances in Ginseng Research", Seoul, Korea, Sept. 22-25, pp. 47 53 (1998).
41. Lee, F. C., Park, J. K., Ko, J. H., Lee, J. S., Kim , K. Y. and Kim, E. K. : *Drug Chem. Toxicol.* **10**, 227 (1987).
42. Yun, Y. S., Jo, S. K., Moon, H. S., Kimn, Y. J., Oh, Y. R. and Yun, T. K. : *Cancer Detect. Prev. Sppl.*, **1**, 301 (1987).