ISSN 1991- 8690 الترقيم الدولي ٨٦٩٠ ـ ١٩٩١
website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq

Tannic acid(TA) protects against cadmium acetate induced toxicity in female rats (Role of tannic acid AS antioxidant)

Khalid G. Al-Fartosi

Department of Biology - College of Education - University of Thi-Qar

Summary

Cadmium is a highly toxic element that can be ingested or inhaled from a variety of industrial and dietary sources. Tannic acid (TA) is a plant polyphenol found in many dietary plant materials such as grain sorghum, coffee, tea and cocoa. The purpose of this study was to investigate the role of tannic acid against cadmium acetate induced toxicity in female rats.Three groups of adult female rats were treated orally with normal saline (0.09 Nacl) as a control group, 200 ppm cadmium acetate (first treated group), and 200 ppm + 200 ppm tannic acid (second treated group) for 12 weeks. The results indicated that cadmium acetate caused a significant decreased in RBCs count, Hb concentration, and PCV of female rats compared with control group, while that tannic acid enhanced these parameters. Also, the results showed that cadmium acetate induced a significant alternative in total and differential count of WBC, while these changes were attenuated by the tannic acid administration. Total protein and albumin were decreased significantly in female rats treated with cadmium, while there was non significant differences between these parameters in control female rats compared with Cd + TA treated rats. AST, ALT, ALP, Cholesterol, and TG were increased significantly in cadmium treated rats, but these values were decreased according to (TA) administration.

Key words: Cadmium, Tannic acid, Female rats.

khalidalfartosi@yahoo.com

Introduction :

Cadmium (Cd) is an important environmental pollutant heavy matal with high toxicity to animals and plants. It is released into the environment by traffic, metal-working industries, mining, as a by-product of mineral fertilizers, and from other sources (Nriagu and Pacyna, 1988). Heavy metal toxicity is also an important issue in reclamation of industrial sites. It has been reported that administration of cadmium via different routes causes increased lipidperoxidation in membranes of erythrocytes and tissues such as the liver, kidney, brain and testes where malondialdehyde (MDA) is used as an indicator of oxidative damage(Gutteridge, 1995). The high dietary levels of cadmium results in suppressed feed intake, reduction in bone mineralization and anemia (McDowell, 1992). The biochemical alteration occur prior to morphological changes in the organs and changes in certain enzyme levels in the extracellular fluids may reflect the extent of cadmium induced damage in target organs(Khandelwal *et al*., 1991).

Animals are generally tolerance to low doses of Cd exposure, but respond considerably to high lethal doses. Mortality in animals due to Cd toxicity does not occur due to cardiotoxicity or nephrotoxicity, but rather by liver injury, because the liver accumulates substantial amount of cadmium after both acute and chronic exposures (Klaassen & Liu , 1998). The soluble salts of cadmium accumulate in the organism and affect toxicity not only in liver and kidney , but also the brain, lungs, testicles, central nervous system and other tissues (Pinot et al., 2000).

Tannic acid (TA) has numerous chemical, food and pharmacological application. TA, a polyphenolic protein-denaturing agent was shown to reduce oxygen to superoxide anion and has neither carcinogenicity potential in F344 rats nor modifying effects on spontaneous tumor development (Ogasawara *et al*., 1990; Bhat and Ha, 1994; Onodera *et al*., 1994).

Tannic acid (TA) is naturally plant phenol present in fruits and vegetables (Block *et al*., 1992). In addition of its use as an additive in medicinal products for human, it has been used for treatment of burns, diarrhea and chemical antidotes in poisoning and as a local astringent (Hirono, 1987). It has been shown to be anti-oxidant and it is a potent antagonist of the mutagenicity of polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP) (Daniel and Stoner, 1991,Huang *et al*., 1985). According to the extensive experimental data it is now known that PAHs must be metabolically activated by peroxy radical dependent pathway and the electrophilic bay-region diol-epoxides act as the ultimate carcinogenic metabolites of PAH (Kapitulnik *et al*., 1979).

The aim of this study is to assess the effect of long-term uptake of cadmium acetate on selected hematological and biochemical parameters and the role of tannic acid against cadmium acetate induced toxicity in female rats.

Materials and Methods :

The experiments were carried out on 30 female rats, (8-9) weeks old weighting 200-250 g. They were housed in groups in stain less-steel cages (6 rats/cage) in a room maintained at 22±3 on a 12-h alternating light-dark cycle. The animals were divided into 3 groups of 6 individuals each.

Group 1 (control group): the experimental animals were treated orally with 0.9% NaCl.
Group 2 (First treated group / Cd): The experimental animals received orally a 200 ppm cadmium acetate.
Group 3 (Second treated group / Cd+TA): The experimental animals received orally 200 ppm cadmium acetate and 200 ppm tannic acid.

After 12 weeks of the experiment, the control and experimental animals were antherized and 5 ml of blood samples were collected from heart and then 2 ml were put in EDTA tubes for measurement of the blood parameters, while 3 ml were put in a tubes without EDTA for measurement of the biochemical parameters. The blood parameters included red blood cell counts (R.B.C.) , hemoglobin (Hb.) , hematocrite (P.C.V) value , erythrocytes sedimentation rate (E.S.R) ,Total white blood cells count (W.B.C.) and Differential W.B.C.) which were measured according to (Baker and Silverton, 1976; Lewis *et al*., 2001) . The biochemical parameters included Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Cholesterol , Albumin , Sugar , Urea , Alkaline phosphate and Triglycerladehyde (TG) were determined with Bio- Merieux apparatus and reagents .

Statistical analysis:

Statistical analysis of the results was performed by SPSS test, data are presented as Mean ± Stander Error .

Results:

The results of the effect of cadmium acetate on blood parameters of female rats are presented in table (1). These results showed the significant decreased ($p<0.05$) in blood parameters (RBCs,Hb.,PCV and total WBC) of female rats treated with cadmium acetate compared with control group. Also the results indicated that the tannic acid enhanced these parameters.

Table (1): The role of tannic acid against cadmium toxicity on some blood parameters of female rats.

Treatments	Blood parameters(Mean +S.E.)			
	R.B.C.	Hb.	PCV	Total WBC
Control group	7.53±0.37 a	13.43±0.21 a	38.14±0.33 a	4.80±0.13 a
First treated group	5.42±0.41 c	10.12±0.19 c	33.21±0.24 c	2.92±0.12 c
Second treated group	6.73±0.39 b	12.55±0.18 b	36.74±0.19 b	3.99±0.09 b

The different letters refers to a significant differences at $p<0.05$.

Table (2) showed a significant alternation of differential count of WBC of female rats treated with cadmium acetate (the first treated group) compared with control group. These changes were attenuated by the tannic acid administration.

Table (2): The role of tannic acid against cadmium toxicity on differential count of leukocytes of female rats.

Treatments	Blood parameters(Mean +S.E.)			
	Neutrophils	Acidophils	Lymphocytes	Monocytes
Control group	30.76 b ±0.36	1.87 b ±0.03	62.38 a ±0.53	3.45 a ±0.09
First treated group	35.38 a ±0.37	2.24 a ±0.07	59.29 b ±0.95	3.17 b ±0.07
Second treated group	31.48 b ±0.84	2.16 a ±0.08	59.93 b ±0.58	3.19 b ±0.07

The different letters refers to a significant differences at $p<0.05$.

The present study demonstrated that treatment of female rats with cadmium caused a significant decreased ($p<0.05$) in total protein and albumin, while there was non significant differences in globulin level compared with control group, as well as the study indicated non significant changes in these parameters in female rats treated with cadmium acetate and tannic acid compared with control group (table 3).

Table(3): The role of tannic acid against cadmium effect on serum Proteins of female rats.

Treatments	Level of protein(Mean±S.E.)		
	Total protein	Albumin	Globulin
Control group	7.14±0.05 a	4.64±0.12 a	2.49±0.15 a
First treated group	5.59±0.23 b	3.58±0.08 b	2.54±0.12 a
Second treated group	6.79±0.06 a	4.48±0.11 a	2.18±0.10 a

The different letters refers to a significant differences at $p<0.05$.

AST and ALT activity were increased significantly in the cadmium treated rats compared with control group, while the tannic acid caused improving these parameters (table 4). ALP of cadmium treated group and cadmium plus tannic acid were increased significantly ($p<0.05$) compared with control group, but it is appear that TA improving the level of this enzyme.

Table(4): The role of tannic acid against cadmium effect on serum Enzymes of female rats.

Treatments	Level of enzyme(Mean±S.E.)		
	AST	ALT	ALP
Control group	10.45±0.18 b	9.27±0.16 c	28.42±0.73 c
First treated group	14.81±0.15 a	11.88±0.26 a	38.16±0.95 a
Second treated group	11.10±0.14 b	10.34±0.19 b	32.81±1.16 b

The different letters refers to a significant differences at $p<0.05$.

Cholesterol and triglyceraldehyde were increased significantly ($p<0.05$) in cadmium treated rats, while the tannic acid caused a significant decreased in cholesterol and triglyceraldehyde of female rats compared with these treated with cadmium acetate only (table 5).

Table(5): The role of tannic acid against cadmium effect on some Parameters of lipid profile of female rats.

Treatments	Lipid profiles(Mean±S.E.)	
	Cholesterol	Triglyceraldehyde
Control group	68.81±1.61 c	81.92±1.89 c
First treated group	91.60±1.49 a	92.98±1.99 a
Second treated group	76.80±1.21 b	85.00±1.65 b

There different letters refers to a significant differences at $p<0.05$.

Discussion :

The results of present study indicated the effect of cadmium on hematological and biochemical parameters of female rats. Female rats administrated cadmium acetate orally showed decreased values of erythrocyte counts, hemoglobin, hematocrit and total WBC. , as well as alternations of differential WBC values . This findings is consistent with previous studies of anemia in fish, rats and rabbits exposed to cadmium, lead, nickel, copper and zinc (Dhanapakiam and Ramasamy, 2001; Bersenyi *et al*., 2003). It has been reported that chronic treatment with cadmium inhibits the bone marrow or induced oxidative damage in erythrocytes of rats, causing destruction of cell membrane and increasing lipid peroxidation , as well as alternation of the oxidative enzyme system, energy metabolism and the appearance of anemia (Kostic *et al*., 1993; Ognjanovic *et al*., 2000; Dallak, 2009). Demir and Oner (1995) showed that Cadmium induced alternations in the phospholipids and protein content of the red blood cell membrane which are accepted normally as evidence of disturbed membrane fluidity which associated in this case with unaltered membrane fragility. Also, cadmium ions significantly affect regulatory genes for erythropoietin, which may be the cause of inhibiting its expression (Chun *et al*., 2000).

The treatment of female rats with cadmium acetate caused the decrease of total protein and albumin, while AST, ALT, ALP, cholesterol and TG were increased. These characteristics features of cadmium-induced liver toxicity were similar to those previously reported by other investigations (Rikans and Yamano, 2000; Tzirogiannis *et al*., 2003). Cadmium hepatotoxicity is probably affected in two ways, on the one hand by the occurrence of inflammatory state, on the other hand by direct toxic action of cadmium on liver cells (Al-Hashem *et al*., 2009). Cadmium is one of the most dangerous occupational and environmental toxins. It accumulates in the human organism mainly in liver and kidney, where it cause functional changes and then interstitial fibrosis (Nguyen and Chien, 1989 ; Theocharis *et al*., 1994). Rikans *et al.* (2000) observed kupffer's cell activation and liver cell infiltration with proinflammatoty cytokines after cadmium intoxication , whereas Shaikh *et al.* (1999) observed oxidative stress intensification of liver peroxidation of fats and liver glutathione exhaustion.

The present study indicated that tannic acid (TA) protects against cadmium induced hematoxicity and hepatotoxicity , this role may be according to reduce the oxidative damage by preventing reduction in glutathione and decreasing level of malonaialdehyde in liver of Cd treated rats (Kulbhushan *et al*., 2008). Omar *et al.* (2003) showed that treatment of rats with aluminum chloride for 80 days caused moderate toxicity on liver, kidney's and spleen as shown by elevation of free radicals (lipid peroxidation and nitric oxide) and reduction of antioxidants (superoxide dismutase, catalase, gluathione transferase, and glutathione and vitamin E) as well as histopathological changes, in addition to the improvement was noticed in the rats treated with tannic acid in addition to aluminium chloride. This study proved that tannic acid has a role as an antioxidant in protection of

rats from the aluminum toxicity. Mitjavila *et al.* (1977) showed the effect of tannic acid on the functional state of rat intestinal epithelium show an inhibition of oxygen consumption and succinic dehydrogenase activity in the homogenate and an exresion of sialic acid and glucosamine. Tannic acid post treatments enhance the antioxidant and antitumor-promoding effects of TA pretreatments. TAs inhibit the second rather than the first stage of tumor promotion. Plant TAs, therefore, may be valuable against tumor propagation but their efficacy may vary considerably depending on their origin(Gali *et al*., 1993).

References :

Al- Hashem, F.; Dallak, M.; Basher N.; *et al.* (2009). Camel's milk protects against cadmium chloride induced toxicity in white albino rats. Am. J. Pharm. Toxicol., 4(3): 107-117.

Baker, F. T. and Sliverton , R. E. (1976). Introduction to medical laboratory technology , 5th ed . London , ISBN ., 408:519-532.

Bersenyi, A.; Feket, Z. S. and Berta, E. (2003). Effect of ingested heavy metals (Cd, Pb, Hg) on hematology and serum biochemistry in rabbits. Acta. Vet. Hung., 51: 297-304.

Bhat, R. and Ha, di S.M. (1994). DNA breakage by tannic acid and Cu(II): generation of active oxygen species and biological activity of the reaction. Mutation Research, 313(1): 49-55.

Block, G.; Patterson, D. and Subar, A. (1992). Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, 18:1-29.

Chun, Y.S.; Choi, E.; Kim, G.T.; *et al.* (2000). Cadmium blocks Hypoxia-Indciba Factor(HIF)-1-mediated response to hypoxia by stimulating the proteasome-dependent degradation of HIF-1 alpha. Eur. J. Biochem., 267: 4198-4204.

Dallak, M. (2009). Camel's milk protects against cadmium chloride induced hypocromic microcytic anemia and oxidative stress in red blood cells of white albino rats. Am. J. Pharm. Toxicol., 4(4): 134-141.

Dhanapakiam, P. and Ramasamy, V. K. (2001). Toxic effects of copper and zink mixture on some haematological and biochemical parameters in common crap, *Cyprnus carpio* (Linn). J. Environ. Biol., 22: 105-111.

Daniel, E.M. and Stoner, G.D. (1991). The effect of ellagic acid and 3-cis retinoic acid on N-nitrobenzymethylamine induced esophageal tumorigenesis in rats. Cancer Lett., 56:117-124.

Demir, S. and Oner, G. (1995). The effects of cadmium on the fragility of red blood cell. Journal of Islamic Academy of Sciences, 8(2): 73-78.

Gali, H.U.; Perchellet, E.M.; Gao, X.M.; *et al.* (1993). Antitumor-promoting effects of gallotannins extracted from various sources in mouse skin in vivo. Anticancer Res., 13(4): 915-922.

Gutteridge, J.M. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin.Chem., 41: 1819-1828.

Hirono, I. (1987). Naturally occurring carcinogens of plant origin. Elsevier, Amsterdam, pp: 161-162.

Huang, M.T.; Chang, R.L.; Wood, A.M.; *et al.* (1985). Inhibition of the mutagenecity of the bay region diol-epoxides of polycyclic aromatic hydrocarbons by tannic acid, hydroxylated anthraquinones and hydroxylated cinnamic acid derivatives. Carcinogenesis, 6: 237-242.

Kapitulnik, J.; Wislocki, P.G.; Levin, W.; *et al.* (1979). Tumorigenecity studies with diol-epoxides of benzo(a)pyrene which indicate that (+-)-trans-(+)7a,8a-dihyroxy-9,10-a-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is

an ultimate carcinogen in newborn mice. Cancer Res., 38:354-358.

Khandelwal, S.; Agnihotri N. and Tandon S.K. (1991). Biochemical response to cadmium dose-time effect. Boil. Trace Elem. Res., 29: 157-164.

Klaassen C.D & Liu J. (1998). Induction of metallothionein as an adaptive mechanism affecting the magnitude and progression of toxicological injury. Environ. Health Perspect. , 106(1):297-300.

Kostic, M.M.; Ognjanovic, B.; Dimitrijevic, R.V. *et al*. (1993). Cadmium induced changes of antioxidant and metabolic status in red blood cells of rats: *in vivo* effects. Eur. J. Haematol., 51: 86-92.

Kulbhushan T.; Aupama, T. Ali, I. Y. *et al.* (2008). Tannic acid prevents azidothymidine (AZT) induced hepatotoxicity and genotoxicity along with change in expression of PARG and histone H3 acetylation. Toxicology Letters, 177(2): 90-96.

Lewis, S.; Bain, J. and Bates, I. (2001). Practical Hematology, 9th Chap.3: 19-41.

Mitjavila, S. ; Lacombe, C. ; Carrera, G. and Derache, R. (1977). Tannic acid and oxidized tannic acid on the functional state or rat intestinal epithelium, J. Nutr., 107:2113.

McDowell, L.R. (1992). Minerals in animal and human Nutrition. Academic press, New York,ISBN: 0-12-483369-1, pp: 524.

Nguyen, Q.H. and Chien, P.K. (1989). Cadmium uptake kinetics in human erythrocytes. Biol. Trace Elem. Res., 22:119.

Nriagu J.O. and Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils with trace metals. Nature **333:** 134–139.

Ogasawara, H.; Todate, A. and Onodera, H. (1990). Subchronic oral toxicity study of tannic acid in F344 rats. Eisei Shikenjo Hokuku, 108:84-89.

Ognjanovic, B.I.; Pavlovic, S.Z.; Ikic Tajn, R.V.A.; *et al.* (2000). The activities and blood hematological values of rats exposed to cadmium. Kragujevac J, Sci., 22:93-99.

Omar , H.M. , Khadiga , A. , Hassan , A.S. and Ahmed , E.A. (2003). Aluminum toxicity in rats : the role of tannic acid as antioxidant. Ass. Univ. Bull. Environ. Res. , 6(2) (Abstract).

Onodera, H.; Kitaura, K.; Mitsumori, K. and Yoshida, J. (1994). Study on the carcinogenicity of tannic acid in F344 rats. Food Chemical Toxicology, 32(12): 1101-1106.

Pinot, F.; Kreps, S.E.; Bachelet, M.; *et al.* (2000). Cadmium in the environment: Sources, mechanisms of biotoxicity and biomarkers. Rev. Environ. Health, 15: 229-323.

Rikans, L.E. and Yamano, T. (2000). Mechanisms of cadmium-mediated acute hepatotoxicity. J. Biochem., 14:110.

Shaikh, Z.A.; Vu, T.T. and Zaman, K. (1999). Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol. Appl. Pharmacol., 154(3): 256.

Theocharis, S.E.; Margeli, A.P.; Vassiliki, A. and Varonos, D. (1994). Thimidyne kinase activity in liver and serum of rats after cadmium administration. Toxicol. Lett.: 71:1.

Tzirogiannis, K. N.; Panoutsopoulos, G.I.; Demonakou, M.D. *et al.* (2003). Time-course of cadmium induced acute hepatotoxicity in the rat liver: The role of apoptosis. Arch. Toxicol., 77: 694-701.

حامض التانك مضاد لسمية خلات الكادميوم في اناث الجرذان المختبرية (دور حامض التانك كمضاد للاكسدة)

الـ فرطو سي كـاطع خالـ د

قسم علوم الحياة - كلية التربية - جامعة ذي قار

الخلاصة :

يعتبر الكادميوم من العناصر عالية السمية التي تنتج من مصادر صناعية وغذائية مختلفة ، كما يمثل حامض التانك متعدد الفينول النباتي الذي يوجد في مواد غذائية نباتية مختلفة مثل حبات الذرة والقهوة والشاي والكاكاو. صممت الدراسة الحالية لبحث دور حامض التانك كمضاد لسمية خلات الكادميوم في اناث الفئران المختبرية. قسمت اناث الفئران المختبرية إلى ثلاث مجاميع الأولى مجموعة السيطرة وعوملت بالمحلول الفسيولوجي ، المجموعة الثانية عوملت بخلات الكادميوم تركيز ٢٠٠ جزء بالمليون ، اما المجموعة الثالثة فقد عوملت بخلات الكادميوم ٢٠٠ جزء بالمليون + ٢٠٠ جزء بالمليون من حامض التانك. عوملت الحيوانات في المجاميع الثلاث فمويا لمدة ١٢ اسبوع. أظهرت نتائج الدراسة الحالية ان خلات الكادميوم سببت انخفاضا معنوية لعدد كريات الدم الحمر،تركيز الهيموكلوبين وحجم الخلايا المضغوط في اناث الجرذان المختبرية مقارنة مع مجموعة السيطرة ، فيما لوحظ ان المعاملة بحامض التانك سببت زيادة في تلك المعايير. النتائج اظهرت ايضا ان الكادميوم سبب تغيرا في قيم العدد الكلي والتفريقي لخلايا الدم البيض ، وقد انخفضت تلك التغايرات بفعل المعاملة بحامض التانك .كما أن تركيز البروتين الكلي والالبومين انخفض معنويا بفعل التعرض لخلات الكادميوم ، فيما لم تكن هنالك فروقا معنويه في هذه المعايير بين أنات جرذان مجموعة السيطرة وإناث الجرذان المعاملة بخلات الكادميوم وحامض التانك. أن فعالية الإنزيمات الناقلة للامين وانزيم الفوسفاتيز القاعدي والكولسترول والكليسيريدات الثلاثية ارتفعت معنويا في الجرذان المعاملة بالكادميوم ، اما المعاملة بحامض التانك سببت انخفاض تلك القيم .

الكلمات المفتاحية : الكادميوم ، حامض التانك ، اناث الجرذان المختبرية .