Assiut Vet. Med. J. Vol. 48 No. 95. October 2002

Dept. of Zoology,
Faculty of Science, Assiut University

ROLE OF TANNIC ACID AS AN ANTIOXIDANT IN REDUCTION OF THE OXIDATIVE DAMAGE OF ALUMINIUM CHLORIDE IN THE RAT'S BRAIN

(With 7 Tables and 2 Figures)

By

K.A. HASSAN; H.M. OMAR, S.KH. ABD-ELGHAFFAR* and E.A.A. ABDEL-GABBER

*: Dept. of Pathology and Clinical Pathology, Fac.Vet. Med., Assiut Univ.

دور حامض التانيك كمضاد للأكسدة في تقليل الضرر الناتج عن الضغط الأكسيجينى في مخ الفئران المعرضة لكلوريد الألمونيوم

خديجة عبد الحميد حسن ، حسام الدين محمد عمر ، ساري خليل عبد الغفار عماد أحمد عبد الجابر

صممت هذه التجربة لبيان دور حامض التانيك كمضاد للأكسدة في تقليل الضرر الناتج عن الضغط الأكسيجينى في مخ الفئران المعرضة لكلوريد الألمونيوم لفترة طويلة. تم إستخدام عـدد ٣٢ فـأر قسمت إلى ٤ مجموعات أعطيت المجموعة الأولى كلوريد الألمونيوم (٣٠ مجم/كجم من وزن الجسم) في مياه الشرب ، أعطيت المجموعة الثانية حامض التانيك (٥٠ مجم/كجم من وزن الجسم) ، أعطيت المجموعة الثالثة مخلوط من الجرعتين السابقتين في مياه الشرب ، بينما أستخدمت المجموعة الرابعة كمجموعة ضابطة. إستمرت التجربة لمدة ٨٠ يومـاً و بعدهـا تـم ذبـح الحـيوانات و أخـذ المـخ و الـدم للدراسـة البيوكيمائية والهسـتوباثولوجية. أظهرت النتائج و جود زيادة معنوية في بيروكسيدات الدهون و أكسيد النيـتريك و إنـزيم الكتالايز في المجموعة التي تعرضت لكلوريد الألمونيوم, بينما إنخفض معنوياً كل من إنزيم السوبرأكسيد الديسميوتيز، الجلوتاثيون و فيتامين هـ في المجموعة الـتى أخـذت كلوريد الألمونيوم و حامض التانيك معاً و قد كان الفحص الباثولوجى متطابقاً ومؤيداً لهذه النتائج. وهذا يوضح أن إستخدام حامض التانيك يمكن أن يلعب دوراً وقائي في تقليل التأثيرات الضارة عند التسمم بكلوريد الألمونيوم في الفئران.

SUMMARY

The experiment was designed to investigate the role of tannic acid as an antioxidant in reduction of the oxidative damage of aluminium chloride in the brain of rat. Thirty-two rats were used, divided into four groups (8 rats each). The rats of the first group were given 30 mg/kg body weight aluminium chloride in drinking water, while those of the second one were given tannic acid 50 mg/kg body weight in drinking water. The rats of the third group were given a combination of aluminium chloride and tannic acid in the same doses. The fourth one served as control. After 80 days, the rats were sacrificed. Blood and brain samples were taken for biochemical and histopathological studies. The results revealed that there was a significant increase in lipid peroxides and nitric oxide levels and the activity of catalase in the aluminium chloride treated rats. However, superoxide dismutase activity, glutathione and vitamin E content were significantly decreased in aluminium/tannic acid treated group. The histopathological investigation documented these results. In conclusion, it is clear that tannic acid could play a prophylactic role in reducing the oxidative damage in the brain tissue of aluminium chloride exposed rats.

Key words: *Tannic acid, antioxidant, aluminium chloride, brain, rat.*

INTRODUCTION

Oxidative stress is a disturbance in the proxidant-antioxidant balance leading to cellular damage. Antioxidants play an important role in the metabolism of reactive oxygen species and hence responsible for protecting cell against oxidative stress (Halliwell and Cutteridge, 1985).

Oral aluminium bioavailability from water appears to be about 0.3%. Aluminuim may be distributed to the brain from nasal cavity, but the significance of this exposure route is unknown. Brain uptake of aluminium may be mediated by aluminium transferrin and aluminium citrate complexes (McNamara and Yokel, 2001)

Long-term treatment with aluminium was reported to induce oxidative injury in rat brain (Katyal *et al.*, 1996). The black tea which is the main drunk in Egypt, contained a complex mixture of polyphenols to which aluminium was partly bound (Baxter *et al.*, 1989). Polyphenolic tannic acid is used as antioxidant in various food and beverages (Khan

and Hadi, 1998). Although the neurotoxic actions of aluminium have been well documented, its contribution to neurodegenerative disease such as Alzheimer's disease remains controversial (Canales *et al.*, 2001 and McNamara and Yokel, 2001). In the present study, evaluation of the role of tannic acid as an antioxidant against the oxidative damage induced by long-term aluminium exposure on the brain of male rats was done.

MATERIAL and METHODS

Chemicals:

Thiobarbaturic acid (TBA), sodium dodecyl sulfate, butanol, 1,1,3,3-tetra-methoxypropane (TMP), pyridine, phosphoric acid, triton x, 1-chloro 2,4 dinitrobenzene (DTNB), 5,5 dithiobis, 2 nitrobenzoic acid, glutathione (GSH), superoxide dismutase enzyme (SOD), and epinephrine were purchased from Sigma Chemicals Co.,Sant Louis USA. All other chemicals were the highest grade available.

Animal and the design of experiment:

Thirty-two normal healthy male Sprague-Dawely rats with body weight average 100 gm. were purchased from Helwan breading farm, Ministry of Health, Egypt. Animals were maintained in the animal house of Faculty of Medicine, Assiut University at 25 ^{0}C and normal day and night cycle for 80 day. A standard bellet diet *ad labitum* were provided. Rats were then categorized randomly into four groups each one 8 rats.. Rats of the first group received orally aluminium chloride (30 mg/Kg body weight/day) dissolved in drinking water. Rats of the second group received orally tannic acid (50 mg/Kg body weight/day) dissolved in drinking water. However, the third group received orally the previous dose of aluminium chloride and tannic acid in combination. Animals of the fourth group drank normal tapwater water

Collection of samples:

All groups of animals were maintained at the experiment condition for 80 day. All rats were sacrificed, and blood was drawn into a tube containing heparin. Brains were excised immediately and washed in phosphate buffer (pH 7.4). Blood samples were centrifuged at 4000 rpm for 10 min to separate plasma and erythrocytes. Erythrocytes lysate were made by washing in saline solution three times and then hemolyzed by the addition of bidistilled water. 10 % homogenate (w/v) of brain was made in 0.1 M phosphate buffer (pH 7.4) by using glass

homogenizer. The cytosols were made by centrifugation of homogenates 250000 rpm for 30 min at 4°C. All samples of plasma, erythrocytes lysate, and the homogenates and cytosols of brain were stored at – 20 °C for the subsequent biochemical determination.

Biochemical determination:

Lipid peroxidation products as Thiobarbitunic reactive substances (TBARS) were measured according to the method of Ohkawa *et al.* (1979). Nitric oxide was measured as nitrite concentration colorimetrically by the method of Ding *et al.* (1988). Glutathione was determined by using the method of Beutler *et al.* (1963). Vitamin E (-tocopherol) was determined by using Emmerie-Engel reaction based on the reduction of ferric to ferrous ions which form a red complex with α- α -dipyridyl (Roe, 1961).

Superoxide dismutase (SOD) activity was determined according to its ability to inhibit the autooxidation of epinephrine in alkaline medium according to the method of Misra and Fridovich (1972). Catalase activity was measured basing on its ability to decompose hydrogen peroxide (H_2O_2) according to Lück (1963).

Total protein in plasma , tissues homogenates and cytosoles were determined by the method of Lowry *et al.* (1951). Hemoglobin content in the erythrocytes lysate was determined by commercial kit (Sclavo cat.No.81222).

Histopathological investigation:

Paraffin sections of brain tissues (5-7 μ) were prepared and stained with H& E for the histopathological examinations (Bancroft and Stevens, 1982).

Statistical analysis:

All data were statistically analyzed by analysis of Variance (Anova-Tukey test).

RESULTS

Table (1) showed the lipid peroxides as thiobarbituric reactive substances in plasma and erythrocytes lysate. Plasma lipid peroxides level was significantly increased in aluminium treated group compared to control one however, it significantly decreased in the aluminium/tannic acid treated group compared to that of control and

aluminium treated groups. The level of lipid peroxides in erythrocytes lysate showed a significant increase in aluminium treated rats compared to the control rats. The level of lipid peroxides in the brain tissues of all experimental groups showed no significant changes

The data in Table (2) indicated that nitric oxide as nitrite concentration in plasma and brain tissue was significantly increased in aluminium treated group versus control group. Aluminium/tannic acid treated group showed a significant increase in its plasma level of nitric oxide compared to control group. However, nitric oxide levels in the brain tissue showed no significant change in the aluminium/tannic acid treated rats versus control rats.

Table (3) showed a significant inhibition in the activity of superoxide dismutase in the plasma of aluminium treated rats compared to that of control group however, it increased in the aluminium/tannic acid treated rats versus aluminium treated rats. In erythrocytes lysate, the activity of superoxide dismutase significantly increased only in the aluminium/tannic acid treated group compared to that of aluminium treated group. Also, there is a significant inhibition in the activity of superoxide dismutase in the brain tissues of all the treated groups compared to the control group.

The activity of catalase in plasma, erythrocytes lysate, and the brain tissues was represented in Table (4). It showed that catalase activity in plasma was significantly increased in aluminium treated group compared to the control group and significantly inhibited in aluminium/tannic treated rats compared to aluminium treated rats. Catalase activity in erythrocytes lysate showed no significant changes in the four experimental groups. In the brain tissue the catalase activity significantly inhibited in aluminium treated group compared to the control group, but it significantly increased in aluminium/tannic treated group versus aluminium treated group.

Table (5) showed the activity of glutathione S-transferase plasma, erythrocytes lysate, and the brain tissue. There is no significanct changes in the plasma between the four experimental groups. In erythrocytes lysate and the brain tissue the activity of glutathione S-transferase was significantly increased in aluminium/tannic acid treated group compared to that of aluminium treated group..

Glutathione level in plasma, erythrocytes lysate, and brain tissues was represented in Table (6). Glutathione level in plasma of aluminium treated group significantly increased compared to control and

aluminium/tannic acid treated groups. Glutathione content in erythrocytes lysate significantly decreased in aluminium treated group and aluminium/tannic treated group compared to the control group. The content of glutathione in the brain tissues was relatively increased in aluminium/tannic acid treated rats versus aluminium treated rats.

Table (7) showed that vitamin E content in plasma was significantly decreased in aluminium treated group versus controls while plasma vitamin E of aluminium/tannic acid treated group was significantly increased compared to aluminium treated group. Vitamin E content in erythrocytes lysate did not show any significant differences between the four experimental groups. Vitamin E content in the brain tissues of aluminium treated rats was significantly decreased compared to control rats.

Histopathological results:

The brain of aluminium chloride treated rats showed large areas of demylination in the white matter, in which small to large vacuoles were numerously seen in the vicinity of the white matter (Fig. 1a). The grey matter of the cerebrum showed multiple focal areas of gliosis and malacia (Fig 1b). Perivascular cuffing of mononuclear cells were also frequently seen in the cerebral cortex (Fig.1c).

In aluminium chloride /tannic acid treated rats there were only fine minute vacuoles of demylination in the cerebral cortex (Fig.2a), while the gliosis, malacia and perivascular cuffing subsided (Fig.2b). The brain of rats in the tannic acid treated group did not showed any remarkable pathological changes.

DISCUSSION

The question-whether aluminium is toxic or not had never been answered satisfactory, however aluminium overload was frequently associated with neurological disorders such as alzheimer's disease (Doll, 1993). The mechanisms of aluminium neurotoxicity are presently unclear but evidence has emerged suggesting that aluminium accumulation in the brain can alter neuronal signal transduction pathways associated with glutamate receptors.

Results of the present study indicated that there was a significant increase in the plasma levels of lipid peroxides in rats treated with aluminium compared to control group. However, there was a significant decrease in plasma lipid peroxides levels of aluminium /tannic acid

treated group compared to that of aluminium treated group, which might be related to the action of tannic acid as antioxidant. The level of lipid peroxides in eryhrocytes lysates significantly increased in aluminium treated group compared to that of control group. It is reported that aluminium increased lipofuscin and lipid peroxides products, due to its accumulation in erythrocytes (Sridhar *et al.*, 1995). It was also, shown that chronic aluminium exposure to rats caused no significant changes in lipid peroxides levels in the brain tissues. The same results in rabbit brain was obtained by Bertholf *et al.* (1987). Also, Katyal *et al.* (1996) reported that oral administration of aluminium to rats for 6 weeks produced oxidative injury but failed to induce lipid peroxidation. However, Verstraeten *et al.* (1997) mentioned that aluminium ion could stimulate in vitro and in vivo lipid oxidation by promoting phase separation and membrane rigidification thus accelerating lipid peroxidation.

Nitric oxide levels in the plasma of aluminium /tannic acid treated group was significantly increased compared to control group, which may due to the effect of tannic acid as modulator of nitric oxide production by endothelial cells (Fitzpatrick *et al.*, 1993). At the same time chronic aluminium exposure to rats produced a highly significant elevation of nitric oxide levels in the brain tissue compared to that of control group. It is known that aluminium impaired neuronal glutamate nitric oxide c-GMP pathway (Hermenegildo *et al.*, 1999) and cerebral nitric oxide synthestase (Bondy *et al.*, 1998). Nitric oxide levels in brain tissue of aluminium/tannic acid treated group did not show any significant differences compared to that of control group, which might reflect the protective effect of tannic acid on the toxicity of aluminium on the brain tissues. Polyphenols such as quercetin and tannic oxide modulate the production of nitric oxide by vascular endothelial cells (Fitzpatrick et al., 1993). Moreover, addition of chelating agents as ethylene diamine tetraacetic acid inhibited lipid peroxidation induced by aluminium, but the addition of superoxide dismutase did not show such effects in mice brain homogenate (Toda and Yase, 1998).

In the plasma, catalase activity showed a significant increase in aluminium treated group, while superoxide dismutase activity significantly increased in aluminium/tannic acid treated group compared to that of aluminium treated group. In the brain tissues superoxide dismutase and catalase activities were significantly inihibited in the aluminium treated groups. The results of the present study indicated that

glutathione content in lysate and brain tissues was significantly decreased in aluminium treated group compared to the control. This reduction of the brain glutathione could be due do the competitive binding of aluminium to the active enzyme site (Katyal *et al.*, 1996). Bondy *et al.* (1998) reported that treatment of rats with aluminium over a 3 week period increased the glutathione content in brain tissue. The depletion of glutathione in erythrocytes following aluminium exposure, which accompanied by a reduction in the activity of ATPase and acetylcholinesterase was reported by Desigan et al. (1995). In the present study tannic acid failed to modulate the reduction in erythrocyte lysate glutathione due to the reduction of NADPH production; the substrate of glutathione peroxidase as a result of aluminium exposure (Desigan *et. al.*,1995). In addition, glutathione S-transferase activity was significantly increased in the brain tissue of aluminium/tannic acid treated group compared to that of aluminium treated group. Plasma levels of glutathione showed a significant elevation in aluminium treated group compared to that of control group, which might be due to the attachement of aluminium to the thiol (-SH) group, thus preventing it from functioning in certain chemical reactions. Aluminium had been reported by ohtawa *et al.* (1983) to inhibit superoxide dismutase activity in the brain tissues of rats treated orally with aluminium hydroxide. Also, Julka and Gill (1996) found that the activities of superoxide dismutase, catalase and glutathione peroxidase were seen to decrease in the brain tissues following chronic aluminium treatment for 4 weeks.

Vitamin E, being the major lipid-soluble chain breaking antioxidant in mammalian tissues. In the present study, vitamin E content significantly decreased in the brain tissue of aluminium treated group compared to control and tannic acid treated group .Abdel-Fattah *et al.* (1998) found that long term administration of vitamin E prevent aluminium-stimulated oxidative injury in the brain.

The histopatological results revealed that chronic aluminium exposure in rats caused demylination of the nerve fiber in the cerberal cortex as well as occasionally seen focal areas of malacia and gliosis. This may be attributed to the oxidative stress induced by aluminium toxicity as increase generation of nitric oxide and depletion of glutathione, superoxide dismutase and catalase activities as well as vitamin E. Campbell *et al.* (1999) reported that the cerebral target following an exposure to aluminium may be glial reaction rather than neuronal degenartion. Aluminium was reported to be a neurotoxic agent

used experimentally to produce neurofibrillary degeneration in brain tissues was similar but not identical to that produced in the case of Alzheimer's disease (Kowall *et al.*, 1989). Earlier investigations proposed that lipid peroxidation might contribute to the oxidation of membrane lipids, but only after exhaustion of antioxidant defenses. Aluminium in the present study failed to induced lipid peroxides, but was shown to increase nitric oxide levels in brain tissues The use of tannic acid as prophylaxis in aluminium intoxicated rats caused marked reduction in nitric oxide generation and improves the activities of antioxidant enzymes, which subsequently reduced the histopathological lesion in the brain tissues.

REFERENCES

Abdel -Fattah, A.A.; Al-Yousef, H.M.; Al-Bekairi, A.M. and Al-Sawaf, H.A. (1998): Vitamin E protects the brain against oxidative injury stimulated by excessive aluminium intake. Biochem. Mol.Bial., 46: 1175-1180.

Baxter, M.J.; Burell, J.A.; Crews, C. and Massey, R..C. (1989): Aluminium in Infant formula and tea leaching during cooking in aluminium in food and the environment. Edited by Massy R.C. and Taylor PP. 77-87 Royal Society of Chemistry Cambridge.

Bertholf, R.L.; Nicholson J.R.; Wills, M.R. and Savory, J. (1987): Measurement of lipid peroxidation products in rabbit brain (response to aluminium exposure). Aut. Clin. Lab. Sci., 17: 418-423.

Beutler E; duron O; and Kelly, B.M. (1963): Improved method for the determination of blood glutathione. J. Lab. Clin. Meth., 61:882-888.

Bonccroft, J.D. and Stevens, A. (1982): Theory and practice of histological Technique Sed. Ed. Charchil Living Stone. Edinbergh, London.Melbourne and N.Y.

Bondy, S.C.; Lui, D. and Guo-Ross, S.X. (1998): Aluminium treatment induces nitric oxide synthesis in rat brain. Neurochem. Int., 33: 51-54.

Campbell A., Prasad K.N. and Bondy S.C (1999): Aluminium-induced oxidative events in cell lines: glioma are more respective than neuroblastoma. Free Radical Biology and Medicine, Vol. 26: 1166-1171.

Canales J.J, Corbalam R., Montoliu C., Llansola M., Monfort P., Erceg S., Hernander-Viadel M., Felipo V. (2001): Aluminium impairs the glutamate-nitric oxide cGMP pathway in cultured neurons and in rat brain in vivo: molecular mechanisms and implications for neuropathology. J. Inorg. Biochem, 87:63-69.

Desigan, B.; Katyal, R.and Ohaja , S. (1995): Hematological changes in erythrocytes after chronic oral administration of aluminium. Med. Sci. Res., 23: 665-666.

Ding A.H.; Nathan C.F.; and Stuchr D.J. (1988): Release of reactive Nitrogen intermediate and reactive oxygen intermediate from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. J. Immunol., 141: 2407-2412.

Doll, R. (1993): Alzheimer's disease and environmental aluminium. Age and Aging, 22 : 138-153.

Fitzpatrick, D.F.; Hirschfield, S.L. and Caffey, R.G. (1993): Endothelium-dependant vasorelaxing activity of wine . Am. J. Physiol.,265: 774-778.

Halliwell B.; and Gutteridge J.M.C. (1985): Free radicals in biology and medicine. Clarendon Press Inc.Oxford.

Hermenegildo, C.; Saez,R. ; Minoia, C. ; Manzo. L. and Felipo, V. (1999): Chronic aluminium esposure impairs the glutamate-nitric oxide-cyclic GMP pathway in the rat in vivo. Neurochem. Int., 34:2465-253.

Julka , D. and Gill, K.D.(1996): Effect of aluminium on regional brain antioxidant defense status in Wister rats. Res. Exp. Med. (Berl), 196: 187-194.

Katyal,R. ., Desigan,B. and Ojaha, S. (1996):Oral aluminium Administration and oxidative injury. Bio. Trace Elem. Res., 56: 125-130.

Khan N.S.;and Hadi SM .(1998): Structural features of tannic acid important for DNA degradation in the presence of Cu^{+2}. Mutagenesis, 13:271-274.

Kowall, N.W.; Bendlebury, W.W.; Kessier, J.B.; Perl, D.B. and Beal, M.F. (1989): Aluminium induced neurofibrillary degeneration affects a subset of neurons in rabbit cerebral cortex. Neurosci, 29: 329-337.

Lowry, O.H; Rosenbrough N.J; Far A.L; and Randall R.J (1951): Protein measurement with folin phenol reagent. J.Biol. Chem., 193:265-275.

Luck, H. (1963): Catalase. In methods of enzymatic analysis edited by Bergmer HU. Academic Press, New York, PP.885-888.

McNamara, P.J. and Yokel R.A. (2001): Aluminium toxicokinetics: an updated minireview. Pharmacol. Toxicol., 88:159-167.

Misra H.P. and Fridovich I. (1972): The role of superoxide anion in the autooxidation of epinephrine and a simple method for superoxide dismutase determination. J.Biol. Chem., 274:3170-3175.

Ohkawa H.; Ohishi N.; and Yagi K. (1979): Assay for lipid peroxides in animal tissue by thiobarbaturic acid reaction. Anal. Biochem., 95:351-358.

Ohtawa, M.; Seka, M. and Takayama, F. (1983): Effect of aluminium on lipid peroxidation in rats. chem. Pharm . Bull., 15: 1418.

Roe J.H. (1961): Standard methods of clinical chemistry, Vol.3 edited by David Selgson, Acad. Press. New York, p.35.

Sridhar, M.K.; Shoeni, O.I. and Kepeayong, T. E. (1995): Aluminium toxic or beneficial? A study with laying chicken. 3rd congress of toxicol . in developing countries . Egypt., 19-23 Nov., 1995.

Toda, A. and Yase, Y. (1998): Effect of aluminium on iron-stimulation of iron-induced lipid peroxidation oxidative modification of brain homogenate. Bio. Trace Elem. Res., 61: 207 -217.

Verstraeten, S.V.; Gloub, M.S.; Keen, C.L. and Oteiza, P. I.(1997): Myelin is a preferential target of aluminium mediate oxidative damage. Arch. Biochem. Biophys, 344: 289-294.

Table 1: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the lipid peroxides as thiobarbituric reactive substances (TBRAS) in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (nmoles/ml)	o.76 ± 0.16	0.98 ± 0.16**a,c	0.71 ± 0.11	0.60 ± 0.16*a,**b
Erythrocyte lysate (nmoles/mgHb)	0.65 ± 0.09	0.79 ± 0.06*a,**c	0.45 ± 0.09**a	0.66 ± 0.18**c
Brain (nmoles/mg protein)	2.94 ± 0.37	2.86 ± 0.23	2.81 ± 0.16	2.70 ± 0.34

Table 2: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the nitric oxide as nitrite concentration in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (nmoles/ml)	1.44 ± 0.32	1.88 ± 0.36**a,c	1.45 ± 0.26	1.75 ± 0.14*a,c
Erythrocyte lysate (nmoles/mgHb)	0.53 ± 0.09	0.49 ± 0.06	0.48 ± 0.07	0.57 ± 0.10*c
Brain (nmoles/mg protein)	0.53 ± 0.08	0.69 ± 0.13*a	0.58 ± 0.06	0.64 ± 0.17

Table 3: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the superoxide dismutase activity (SOD) in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (U/ml)	3.07 ± 0.66	2.12 ± 0.37**a,c	3.08 ± 0.33	2.95 ± 0.52**b
Erythrocyte lysate (U/mgHb)	5.61 ± 1.00	5.00 ± 1.09	5.79 ± 0.37	6.10 ± 0.73*b
Brain (U/mg protein)	4.73 ± 0.39	3.25 ± 0.85**a	3.36 ± 0.38**a	3.71 ± 0.63*a

Table 4: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the catalase activity in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (nmoles/ml)	3.64 ± 0.95	5.07 ± 1.22**a	4.20 ± 0.80	3.83 ± 0.90*b
Erythrocyte lysate (nmoles/mgHb)	4.82 ± 0.92	4.22 ± 0.65	4.84 ± 0.54	4.69 ± 0.86
Brain (nmoles/mg protein)	0.77 ± 0.15	0.44 ± 0.09**a,c	0.98 ± 0.08	0.64 ± 0.09**b

Table 5: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the glutathions S-transferase activity toward 1-chloro 2,4- dinitrobenzene (CDNB) in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (nmoles/min/ml)	1.50 ± 0.31	1.20 ± 0.27	1.32 ± 0.18	1.51 ± 0.20
Erythrocyte lysate (nmoles/min/mgHb)	1.28 ± 0.23	1.06 ± 0.20	1.16 ± 0.29	1.51 ± 0.23**b, c
Brain (nmoles/min/mg protein)	822 ± 118	753 ± 100	774 ± 148	944 ± 140**b, c

Table 6: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the glutathione level in the plasma, erythrocytes lysate, and the brain tissue of male rats.

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (µg/ml)	1.69 ± 0.35	2.25 ± 0.16**a,*c	1.95 ± 0.21	1.69 ± 0.28**b
Erythrocyte lysate (µg/mgHb)	31.98 ± 10.64	22.61 ± 2.24**a, c	36.33 ± 2.36	21.43 ± 2.40**b,c
Brain (µg/mg protein)	8.34 ± 1.09	7.50 ± 2.05**c	10.77 ± 1.09**a	8.15 ± 2.45**c

Table 7: The effect of Aluminium chloride ($ALCl_3$), Tannic acid (TA), and the combination of $ALCl_3$ and TA on the vitamin E (α-tocopherol) in the plasma, erythrocytes lysate, and the brain tissue of male rats

	Control	$AlCl_3$	TA	$AlCl_3$ plus TA
Plasma (µg/ml)	1.35 ± 0.32	1.07 ± 0.16**a	1.17 ± 0.11	1.30 ± 0.13 *b
Erythrocyte lysate (µg/mgHb)	0.26 ± 0.04	0.24 ± 0.03	0.26 ± 0.05	0.26 ± 0.05
Brain (µg/mg protein)	0.59 ± 0.05	0.32 ± 0.07**a,c	0.87 ± 0.07**a	0.40 ± 0.11**C

Significant difference * P<0.05 Highly significant difference ** <0.01

a compared to control

b compared to aluminium treated group

c compared to tannic acid treated group

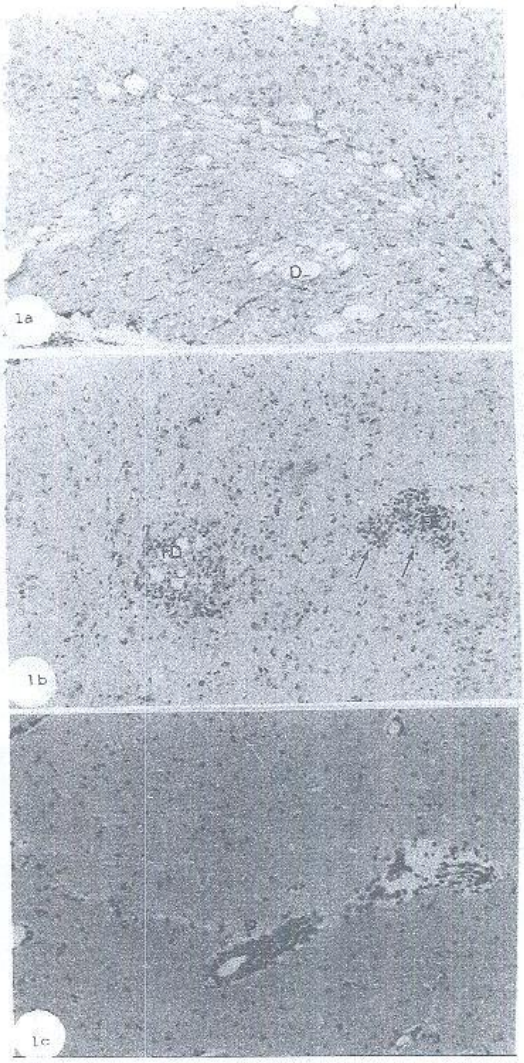

Fig. 1: Brain of aluminium chloride treated rat's showing: a)- Demylination in the cereberal cortex (D) b)- Focal areas of malacia and gliosis (FD)&(arrows). c)- Mononuclear perivascular cuffing (P) (H&E. 10x10)

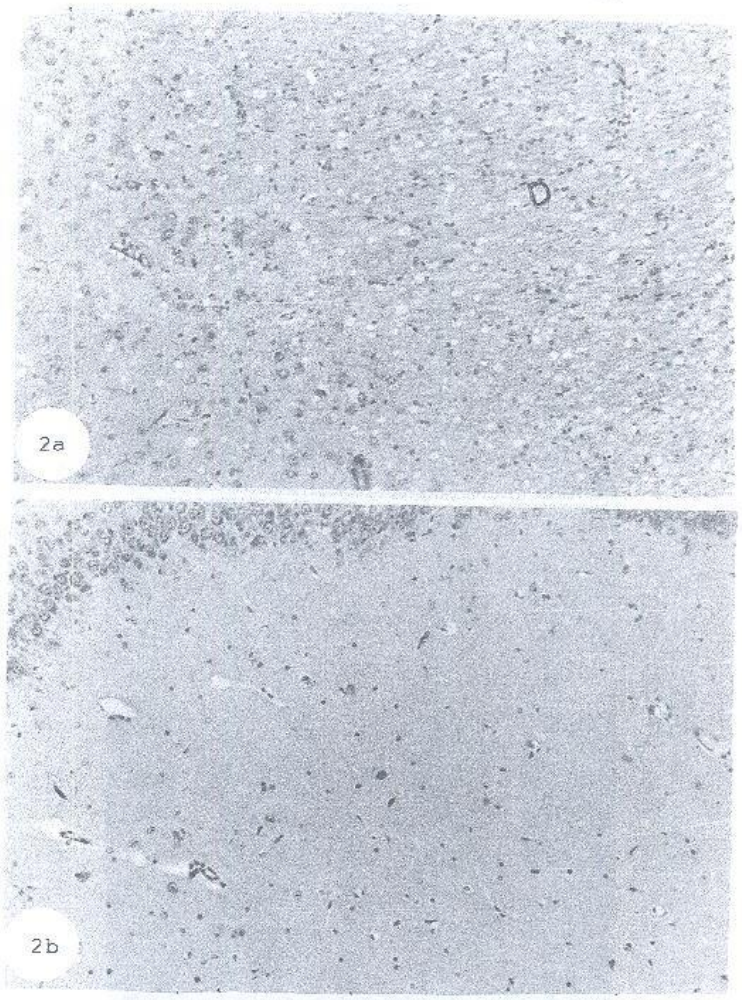

Fig 2: Brain of rats from aluminium chloride/tannic acid treated group showing: a)- Minute vacuoles of demylination (D)
b)- More or less normal gray matter. (H&E 10x10)