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THE EFFECT OF ALUMINUM POLLUTION ON THE MALE REPRODUCTIVE SYSTEM IN RATS: ROLE OF OXYRADICALS

(With 3 Tables and 3 Figures)

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تأثير التلوث بالألومنيوم على الجهاز التناسلي في ذكور الفئران : دور الأوكسجين الشارد

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يعد الألومنيوم من العناصر النادرة في الجسم ولكن ليس لها دور أو وظيفة بيولوجية . وفي هذه الدراسة اعطى كلوريد الألومنيوم المذاب في الماء المقطر (٢ ملي / ملـــي جـــم وزن الجسم) عن طريق الفم إلى ذكور الفئران غير البالغة (عمر ٤ أسابيع) لمدة شهرين ولمـــدة ثلاث شهور. ولقد أدى ذلك إلى تأخر في نمو الخصية ووظيفتها وفي مجموعة أخرى أعطى كلوريد الألومنيوم بنفس الجرعة السابقة يوميا ولكن لذكور فئران بالغة لمـدة شـهر ولمـدة شهرين وقد أدى أيضا إلى تثبيط وظائف الخصية هستولوجيا عن طريق اختلال في مراحـل تكوين الحيوانات المنوية وكذلك في عدد وحيوية خلايا ليدج المنتجة لهرمون التستـــستيرون. وبفحص الحيوانات المنوية المأخوذة من ذيل البربخ وجـــد أن نســبه الحيوانـــات المنويـــة المتحركة ونسبه الحيوانات المنوية الحية قد نقص بدرجة معنوية وكذلـــك وجـد أن نســبه التشوهات الكلية ونسبه وجود نقط البروتوبلازم على الحيوانات المنوية قد زاد فـــي جميـــع الفئران التي أخذت الألومنيوم في فترة ما قبل أو بعد البلوغ. أيضا أدى إعطاء الألومنيـــوم إلى نقص معنوي في مستوى هرمون التستستيرون ون في كل من السيرم ومطحون نســـيج الخصية في جميع الفئران التي أخذت الألومنيوم وكذلك وجد أن مستوى الـــهرمون المنشـــط لخلايا ليدج قد نقص معنويا في الفئران التي بدء إعطاء الألومنيوم لها قبل مرحلـــة البلـــوغ ولمدة ثلاث اشهر. وبدراسة مستوى نشاط أنزيم SOD وكمية الزنك في مطحون الخصيـــة وجد أن الألومنيوم يؤدى إلى نقصهما معنويا في كل الفئران التي أخذت الألومنيـــوم. أمـــا مستوى فوق أكسدة الدهون (MDA) في مطحون الخصية فقد ارتفع ارتفاعـــا معنويـــا فـــي الفئران التي أخذت الألومنيوم لمدة شهرين فقط قبل سن البلوغ وفي مرحلة ما بعد البلوغ، وقد وجد من الدراسة أن هناك علاقة تراجع معنوية بين حركة الحيوانات المنوية ومســـتوى فوق أكسدة الدهون بالخصية في الفئران التي اخذت الألومنيوم قبل البلوغ اما الفئران التي بدأ

فيها إعطاء الألومنيوم بعد البلوغ فكانت هذه العلامة معنوية مع كل من نشاط أنزيم SOD، مستوى فوق أكسدة الدهون ومستوى هرمون التستستيرون، كذلك وجدت علاقة تراجعية معنوية بين نسبة الحيوانات المنوية الحية ونشاط أنزيم SOD في الفئران التي تأخذ الألومنيوم قبل البلوغ ، وكذلك وجد علاقة تراجعية معنوية بين نسبه التشوهات الكلية للحيوانات المنوية ، نقط البرتوبلازم ، مستوى نشاط أنزيم SOD ومستوى الزنك في الفئران التي بدء إعطاء الألومنيوم قبل البلوغ في حين ان هذه العلاقة تكون مع نسبه وجود نقط البرتوبلازم في حالة إعطاء الألومنيوم بعد البلوغ، ومن هذه الدراسة نستنتج أن الألومنيوم يؤدى إلى عدم توازن بين مستوى الأكسدة والشوارد الحرة وبين مضادات الأكسدة في نسيج الخصية وأن هذه الاختلال يكون نتيجه ارتفاع الشوارد الحرة بالخصية والذي يؤدي إلى اعتلال في وظيفة الخصية من حيث الاضطراب في إنتاج الحيوانات المنوية وإنتاج هرمون التستستيرون وكذلك ارتفاع نسبة التشوهات في الحيوانات المنوية.

SUMMARY

Aluminum (Al) is one of the trace elements that has no any biological function in the body. In this study, oral administration of Al chloride (2mg/kg B.W) to 4 weeks immature male rats for two and three months produced regression in testicular development and functions. In another experiment adult rats ingested orally the same dose of Al chloride for one and two months, it produced suppression in testicular functions with interruption in stages of spermatogenesis and reduced in the number of leydig cells. The epididymal spermatozoa showed a significant suppression in motility and alive percentage with significant elevation in total abnormalities and protoplasmic droplets percentage in spermatozoa in both immature and mature treated rats. Al significantly produced a reduction in testosterone of serum and testicular tissue homogenates, in both immature and adult treated rats. While serum LH levels were reduced significantly in immature treated rats for three months. The testicular superoxid dismutase (SOD) activities and zinc contents were reduced significantly in all rats (immature and adults). The levels of malondialdehyde (MDA), as an indicator for lipid peroxidation, were elevated significantly in testicular tissue homogenates in immature treated rats for two and three months, while it was elevated significantly in testicular tissues in adult rats treated for two months only. The motility of spermatozoa regressed significantly with MDA levels in immature treated rats and with testosterone levels in adult treated rats. The alive percentage of spermatozoa was regressed significantly with testicular SOD activities in immature treated rats. The total abnormalities and protoplasmic droplets regressed significantly with

testicular SOD activities, zinc content in immature treated rats while protoplasmic droplets percentage regressed significantly with SOD activities and zinc content and MDA levels of testicular tissues in adult treated rats. The present study indicates that Al significantly induced inbalance between antioxidant/prooxidant system in testicular tissue and this effects may be due to elevation of free radicals. There may be affected the viability of leydig cells as well as disruption of spermatogenesis. Moreover, elevation in sperm abnormalities due to testicular and/or epididymal origin.

Key words: *Aluminum, male rat reproduction, antioxidant, lipid peroxidation, spermatozoa, leydig cells.*

INTRODUCTION

Aluminum (Al) is abundant in the earth's crust, but is present in very small amounts in living organisms because of its insolubility of Al hydroxide complexes at neutral pH (Ganrot, 1986, Mcdonald and Martin, 1988). Al is not unessential element for mammals and microorganisms but causes impairment of energy metabolism (Yashino et al., 1998 and 1999) relation to some neurological and skeletal disorders (Perl and Brody, 1980 and Perl et al., 1982). Naturally, tea, some spices and herbs contain high Al concentration however, its concentration increased dramatically in food kept in containers which were manufactured from Al. It is primarily absorbed in the gastrointestinal tract and these is a relationship between amount which ingested and amount absorbed (Skalsky and Carchman, 1983).

The effect of Al on body accompanied with increases in the rate of lipid peroxidation (Parkinson et al., 1981). However, the effects on male reproductive system was received a low attention from investigators in comparison to its effects on nervous system. The relation between free radicals and reproduction was close related. The unusually high content of phospholipid bound polyunsaturated fatty acids in the sperm plasma membrane drew attention to their potential physiological significance (Kim and Parthasarathy, 1998). They reported also, that there high polyunsaturated fatty acids of sperm membranes makes them vulnerable to peroxidative changes by oxygen radicals. Al was one of the potent substances that increased free radicals in the body and produce its damaging effects through its prooxidant action (Katyal et al., 1997).

In this study we will investigate the effects of Al on male reproductive system and on the development of gonads when Al administration started at immature stage. Moreover, the degree of lipid peroxides and superoxide dismutase (SOD) as well as serum and testicular tissue homogenate testosterone and serum LH were measured. Also, histological examination were done for semineferous tubules and leydig cells.

MATERIALS and METHODS

Animals:

Fourty eight male albino rats (Sprague Dawely), twenty four immature and another twenty four adult, were used in this study. The animals were purchase from animals house, Faculty of Medicine, Assiut University.

Chemicals:

Al chloride, thiobarbituric acid, adrenaline and sodium citrate were purchased from sigma chemical Co.

Study Protocol:

Two experiment sets were done as the following:

Experiment I: Immature (4 weeks) male rats were divided into three groups, 8 each. The first kept as control and ingested the vehicle of Al chloride. The second and third groups were ingested daily with 2mg/kg B.W Al chloride for two and three months respectively.

Experiment II: Adult (2.5 months) male rats were divided into three groups (8 each). The first kept as control and rats were ingested daily with the vehicle of Al chloride. The second and third groups tread daily with 2mg/kg B.W Al chloride orally for one and two months respectively.

Blood Samples:

At the end of each experiment, blood samples were collected from each rat in clean sterile centrifuge tubes without anticoagulant by puncture of the retro-orbital sinus. Serum was separated after centrifugation for 5000 rpm for 7 min. and kept in aliquets at -20°C.

Sperm evaluation:

Testes and corresponding epididymis were collected from sacrified rats directly after blood collection. Within few minutes, testis and its epididymis were carefully separated from surrounding tissue and weighed. Each epididymis was carefully separated from testis and weighed the testis only. The caudal epidiymis was squeezed on a clean dry warm (37°C) slide then few drops of warm sodium citrate buffer

solution (2.9%) were added as well as gently mixed and covered with a cover-slip to be examined microscopically for the detection and estimation of sperm motility. Thin smears were made from the squeezed fluid and stained with alkaline methyl violet stain for detection of sperm primary and secondary abnormalities. The alive and unripe (with protoplasmic droplets) % were determined by using eosin-nigrosin and Indian ink stains respectively. Testes specimens were rinsed with 6.3 mM EDTA phosphate buffer and then absorbed excess buffer, specimens were immediately frozen and kept at -80°C. Other specimens were kept in Bouin's fluid for histological examination.

Biological measurements:

At the time of lipid peroxidation assay, testicular samples were thawed and homogenized in ice-cold phosphate buffer (pH 7.4) yielding a 1/10 homogenate, these were centrifugated at 3000 rpm for 30 min. The supernatant were collected and assay immediately with the serum samples, lipid peroxidation by measuring MDA by thiobarbituric acid and provide a convenient index of lipid peroxidation according to (Thayer, 1985). The rest of supernatant and serum were kept at -20°C for another assays. SOD activities were measured in both supernatants of testicular homogenate and serum using epinephrine according to (Misra and Fridovich, 1973). Protein in testicular supernatant were determined by the Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Hormonal assay:

Testosterone levels in testicular homogenate and serum in each rat were determined using ELISA kit (Biosource, Co.). The minimal detection limit in the assay was 0.05 ng/ml and the intra-and interassay coefficients of variation were 8.5% and 7.3% respectively. Serum LH levels were measured by rat RIA kit, the minimal detection limit in the assay was 0.02ng/ml and the intra-and interassay coefficients of variation were 6% and 8% respectively.

Histological examination:

Immediately after sacrified animals, testes were removed and fixed in Bouin's fluid, dehydrated in ascending series of ethyle alcohol, cleared in xylol and embedded in paraffin wax. Paraffin sections were cut at 7μ thickness and stained with haematoxylin and eosin according to Drury and Wallington (1980).

Statistical analysis:

Data were expressed as the mean ± SEM for all parameters in both serum and tissue homogenates, then analyzed by using Analysis of

Variance (ANOVA) with Bonferroni's post-test for multiple comparisons with confidence intervals at 90% as appropriate. Linear analysis regression were also done using prism 3 Graphapad computer programme.

RESULTS

The obtained results are presented in Tables 1-3 and Figures 1-3.

I. Effects of Al on gonads and epididymal spermatozoa (Tables 1&2):

i- Immature treated rats:

Al produced significant ($P<0.001$) decrease in testicular and epididymal weights after two months of treatment while after three months of treatment, testicular and epididymal weight decreased significantly at levels 0.05 and 0.001 respectively. Epididymal spermatozoa showed a significant ($P<0.001$) decrease in motility and alive % after two or three months of treatment. The total abnormalities and protoplasmic droplet % were significantly ($P<0.001$) increased in these rats treated for both two and three months.

ii- Adult treated rats:

The epididymal weights were reduced significantly ($P<0.001$) in rats treated for one months. The motility and alive % of the epididymal spermatozoa showed significant decrease ($P<0.05$) after one month of treatment, while they decrease significantly ($P<0.001$ and $P<0.01$) after two months of treatment respectively. The total abnormalities and protoplasmic droplets were significantly ($P<0.001$) increased after one and two months of treatment.

II- Biological effects of Al treatment:

i- Immature treated rats (Fig. 1):

Serum testosterone levels were reduced significantly ($P<0.05$ and $P<0.001$) in two and three months treated rats respectively. While it was reduced significantly ($P<0.001$) in all rats in testicular tissue homogenates. Serum LH levels only decreased significantly ($P<0.01$) in rats treated for three months. Testicular tissue homogenates showed a significantly ($P<0.01$ and $P<0.001$) decrease in SOD activities and zinc concentration in rats treated for two and three months respectively. The levels of MDA as an indicator for lipid peroxidation were increased significantly ($P<0.05$) in all rats.

ii- Adult treated rats (Fig. 2):

Al treatment for one month produce a significant ($P<0.05$) decrease in both serum and testicular tissue homogenates of testosterone,

while it was (P<0.001) in serum and (P<0.01) testicular homogenates after three months of treatment. SOD activities in testicular tissue homogenates decreased significantly (P<0.001) in one and two months of treatment. The levels of MDA increased significantly (P<0.05) after two months of treatment in tissue homogenates. Zinc content in tissue homogenates was decreased significantly (P<0.05 and P<0.01) in treated rats for one and two months respectively.

iii- Linear analysis of regression (Table 3):

It was found a significant regression in motility of epididymal spermatozoa and testicular homogenates of MDA levels (P<0.05) and testosterone levels (P<0.001) in immature treated rats. This relation of linear regression were significantly (P<0.05) with testicular homogenate for SOD activities, MDA levels and testosterone level in adult treated rats. Alive % of epididymal spermatozoa were significantly (P<0.05) regressed with testicular homogenates of SOD activities in immature treated rats. The total abnormalities % in spermatozoa were regressed significantly (P<0.01, P<0.05, P<0.01) with SOD activities, zinc content and testosterone in tissue homogenates respectively in immature treated rats. The protoplasmic droplets % in spermatozoa was regressed significantly (P<0.05) with SOD activities and zinc contents in tissue homogenates in immature treated rats, while in adult treated rats it significantly (P<0.01) regressed with SOD activities, zinc contents and MDA levels.

III. Histological findings (Fig. 3):

The semniferous tubules of control rats are lined with spermatogenetic cells which is highly modified stratified cuboidal epithelium. The semniferous epithelium contains two distinct categories of cells; Sertoli cells and the germ or spermatogenetic cells in various stages of development. In between the seminiferous tubules, a layer of loose connective tissue highly vascular containing a group of polyhedral cells named the interstitial cells of leydig (A).

Treatment of immature rats with Al for 2 months showed alternation in the spermatogenetic and interstitial cells. The spermatogenetic cells appear small in size with scanty and small deeply stain cytoplasm. The spermatogenetic cells were separated from each other by irregular wide spaces. Few sperms were observed inside the lumen of the seminiferous tubules. The interstitial cells of leydig showed homogenous deeply stained cytoplasm with rounded nuclei (B). Treated immature rats with Al for 3 months showed vacuoles of various shape

and size between spermatogenetic cells with few sperms in lumen of seminiferous tubules. The leydig cells smaller in size with more deeply stained nuclei and cytoplasm (C).

Treatment of Al to adult rats for one month lead to marked histological changes in the seminiferous tubules. Decrease in the diameter of the seminiferous tubules accompanied with decrease in the interstitial connective tissue. The spermatogenetic cells loss in normal arrangement with deeply stained nuclei and scanty cytoplasm. Vacuoles were observed between the spermatogenetic cells (D).

Adult rats treated for 2 months showed similar observation of those treated for one month but with marked changes. No sperms can be detected in the seminiferous tubules and numerous detached cells were observed in lumen of seminiferous tubules (E).

DISCUSSION

Al is one of the trace elements where it is present in very small amounts in living organisms (Ganrot, 1986 and Mcdonald and Martin, 1988). The present study investigated the effects of Al chloride administrated orally on male rat reproductive system. It was observed that, Al produced marked interruption in the spermatogenesis and reduced testicular and epididymal weights. Epididymal sperm examination showed a marked decrease in motility and alive percentages as well as elevated the total abnormalities and protoplasmic droplets in spermatozoa. These results were inagreement with Hovatta et al. (1998). They reported that reduction in semen qualities was observed with elevation of Al concentration in seminal plasma and spermatozoa. Moreover, the long-term ingestion of Al in rats affected sexual behaviour and fertility (Bataineh et al., 1998). They reported also, a regression, in fertile capacity of testes after Al ingestion included suppression in spermatogenesis and dropped in seminal vesicles weight.

Al produced reduction in both serum and testicular tissue homogenates testosterone levels. From the histological point of view, the number of leydig cells decreased with degenerating changes, as well as failure in spermatogenesis. Moreover, these observation were more prominent in rats start to receive Al in the immature stage. Llobet et al. (1995) reported that Al produced changes in reproductive system of male mice. They found a reduction in both testicular and epididymal weights as well as significant decreases in testicular spermatid and epididymal spermatozoa counts. They added that necrosis of

spermatocytes/spermatides were observed, whereas the tubular diameters were unaffected.

To investigate the role of Al in inbalance between antioxidant/prooxidant, MDA levels and SOD activities were measured in testicular tissue homogenates. The MDA levels were elevated in tissue homogenates as well as reduction in SOD activities in immature and adult treated rats. Oteiza et al. (1993) and Abd-El-Fattah et al. (1998) reported that one of the most important effects of Al in the body is elevation of free radicals. Moreover, they reported that vitamin E as antioxidant, could protect the body from oxidative stress induced by Al.

Sikka et al. (1995) investigated that spermatozoa possess an inherent but limited capacity to generate free radicals to aid in the fertilization process, but increase free oxygen which suppress the functions spermatozoa and reduced their survival.

There is a relation between testosterone levels and antioxidant system in spermatozoa and epididymal fluid. In this study, the reduction in testosterone levels may be potentiate the elevation of free radicals. Purohit et al. (2000) suggested that glutathione (antioxidant) content in the spermatozoa and epididymal fluid were increased significantly after testosterone treatment. Further, they reported that testosterone hormone reduced superoxide anion generated by caudal epididymal spermatozoa. The reduction in testosterone levels may be reduce sperm counts by two mechanisms, the first through its direct effects on spermatogenesis and the second may be through elevation of superoxid anion and suppression of SOD activities. Moreover, the elevation of protoplasmic droplets after Al ingestion in rats may be due to elevation of free radicals in epididymal fluid as a result of Al. The fertilizing capacity of sperm also decreased as a results of elevation of free radicals in epididymal fluid where, zona-binding index were decreased.

In this study, Al ingestion produced damaging effects on male gonads particularly when administrated to immature rats. Samanta et al. (1999) suggested that an antioxidant defence system plays a crucial role in development and maturation of rat testis. They found that elevation of lipid peroxidation in immature rats as well as reduction in SOD activities retarded the testicular functions at puberty.

Oxidative stress not only affected seminiferous tubules and epididymal spermatozoa but also, it affects the number and viabilitis of leydig cells. In the present study, elevation of MDA as a results of Al ingestion produced reduction in number of leydig cells as well as decrease the levels of testosterone. Moreover, LH decreased but only in

the immature treated rats for long period (three months). As early, Myers and Abney (1988) found a reduction in testosterone synthesis in leydig cells culture as a results to elevation of free radicals. They reported also, that oxidative injury-mediated damage of enzymes responsible for steroidogenesis in leydig cells. Recently, Mueller et al. (1998) found a close relation between testicular MDA levels and reduction in testosterone synthesis.

Due to the close relation between Al and zinc and between zinc content in testicular tissue and fertility, it was found that zinc contents were dropped in testicular tissue homogenates in all rats ingested Al. SOD an important antioxidant enzymes in testicualr tissues and it is zinc-dependent enzyme (Gutteridge and Halliwel, 1994). Sever zinc deficient diet produced asospermia and depletion of testicular steroidogenesis and may develop hypogonadism (Hamdi et al., 1997). Ai et al. (1997) reported that zinc deficiency decreased body weight, testes and muscles of rats with lower contents of testosterone in testes and serum. The reduction of zinc levels in immature rats treated with Al were associated with testicular development. These results were inaggrement with observations by Li et al. (1998). They reported that zinc was essential to testicular development at puberty. Moreover, they added that this effects of zinc was perhaps carried out in different ways. Zinc deficiency also, reduced seminal plasma zinc, which has a positive correlation with sperm counts and sperm viability (Fuse et al., 1999 and Telisman, 2000).

In conclusion, the Al chloride produced a reduction in testicular and epididymal functions, through decrease the testosterone levels and increase the protoplasmic droplets of spermatozoa. These effects were more prominant when Al ingested to immature rats till after puberty. The damaging effects of Al may be due to oxidative stress in testicular tissue as well as epididymal fluids. These results indicate to the importance of Al in foods as well as elevation of free radicals in the body as an important for development of hypogonadism.

REFERENCES

Abd-El-Fattah, A.A., al-Yousef, H.M. al-Bekairi, A.M. and Al-Sawaf, H.A. , (1998): Vitamin E protects the brain against oxidature injury stimulated by excessive aluminum intake. Biochem Mol Biol Int., 46: 1175-1180.

Ai, H., Chen, J. and He, S., (1997): The effects of zinc deficiency and testosterone supplement on testosterone synthesis and skeletal muscle of rats. Wei Sheng Yen Chiu., 26: 211-215.

Bataineh, H., Al-Hamood, M.H. and Elbetieha, A.M., (1998): Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts. Hum. Exp. Toxicol., 17: 570-576.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 77: 248-254.

Drury, R.A.B. and Wallington, E.M., (1980): Histological technique. 5th edition Oxford, University Press, New York, p. 162.

Fuse, H., Kazama,T., Ohta,S. and Fujiuchi, K., (1999): Relationship between zinc concentrations in seminal plasma and various sperm parameters. Int. Urol. Nephrol., 31 (3): 401-408.

Ganrot, P.O. (1986): Metabolism and possible health effects of aluminum. Environ. Health Perspect, 65: 363-441.

Gutteridge,J. and Halliwel, B. (1994): Antioxidants in nutrition, health and disease. Chapter 5, oxidative stress, 90-102, Oxford Univ. Press.

Hamdi, S.A., Nassif, O.I. and Adawi, M.S., (1997): Effect of marginal or sever dietary zinc deficiency on testicular development and functions of the rat. Arch Androl., 38: 243-253.

Hovatta, O., Venalainen, E., Knusimaki, L., Heikkila, J., Hirvi, T,. and Reima, I. (1998): Aluminum, Lead and cadmium concentrations in seminal plasma and spermatozoa and semen quality in finnish men. Hum Reprod., 13: 115-119.

Katyal, R., Desigan, B., Sodhi, C.P. and Oiha, S., (1997): Oral aluminum administration and oxidative injury. Biol. Trace Elem. Res., 57: 125-130.

Kim, J.G. and Parthasarathy,S. (1998): Oxidation and the spermatozoa. Semen Reprod. Endocrinol., 16: 235-239.

Li, J., Xu, P. and He, Z., (1998): Effect of zinc deficiency on apoptosis of spermatogenic cells of rat testis. Chung Hua I Hsueh Tsa Chih., 78: 91-93.

Llobet, J.M., Colomina, M.T., Sirvent, J.J., Domingo, J.L. and Corbella, J., (1995): Reproductive toxicology of aluminum in male mice. Fundam Appl Toxicol., 25: 145-151.

Mcdonald, T.L. and Martin, R.B., (1988): Aluminum ion in biological system. Trends Biochem Sci., 13: 15-19.

Misra, H.P. and Fridovich, I. (1973): The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase.J. Biol. Chem., 247: 3170-3173.

Mueller, A., Hermo, L. and Robaire, B. (1998): The effects of aging on the expression of Glutathione s-transferuses in the testis and epididymis of the Brown Norway rat. J. Androl., 19: 450-465.

Myers, R.B. and Abney, T.O., (1988): The effects of reduced O_2 and antioxidants on steroidogenic capacity of cultured rat leydig cells. J. steroid Biochem., 31: 305-309.

Oteiza, P.I. , Fvaga, C.G. and Keen, C.L., (1993): Aluminum has both oxidant and antioxidant effects in mouse brain membranes. Arch Biochem Biophys., 300(1): 517-521.

Parkinson, I.S., Ward, M.K. and Kerr, D.N., (1981): Dialysis encephalophathy, bone disease and anemia: The aluminum intoxication syndrome during regular hemodialysis. J. Clin Pathol., 34: 1285-1294.

Perl, D.P. and Brody, A.R., (1980): Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science, 208: 297-299.

Perl, D.P., Gajdusek, D.C., Garruts, R.M. , Yanagihara, R.T. and Gibbs, C.J., (1982): Interneuronal aluminum accumulation in amyotrophic lateral sclerosis and Parkinson-dementia of Guam. Science, 217: 1053-1055.

Purohit, S.B., Saxena, D., Laloraya, M. and Kumar, G.P. (2000): Altered molecular dynamics and antioxidant status in the spermatozoa in testosterone-induced oligospermia in mouse. Mol. Reprod. Dev., 55: 316-325.

Samania,L., Roy, A. and Chainy, G.B. , (1999): Changes in rats testicular antioxidant defence profile as a function of age and its impairment by hexachlorocylo hexand during critical stages of maturation. Andrologica, 31: 83-90.

Skalsky, H.L. and Carchman, R.A., (1983): Aluminum homeostasis in man. J. Amer. Col. Toxicol., 6: 405-423.

Sikka, S.C. , Rajasekaran, M. and Hellstrom, W.J., (1995): Role of oxidative stress and antioxidants in male infertility. J. Androl., 16: 464-81.

Telisman, S., Cvitkovic, P. , Jurasovic,J., Pizent, A. and Gavella, M., Rocic, B., (2000): Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc and copper in men. Environ. Health Presspect., 108: 45-53.

Thayer, W.S. (1985): Serum lipid peroxides in rats treated chronically with adiramycin. Biochim. Pharmacol., 33 (14): 2259-2263.

Yashino, M., Murakami, K. and Kawano, K. (1998): Interaction of aluminum ion with A/P Mechanism of the aluminum inhibition of glyceral kinase and its reversal by spermine BioMetals, 11: 63-67.

Yashino, M. Ito, M., Haneda, M., Tsubouchi, R. and Murakami, K. (1999): Prooxidant action of aluminum ion-stimulation of iron-mediated lipid peroxidation by aluminum. Biometals, 12: 237-240.

LEGENDS OF FIGURES

Fig. 1: Testosterone levels in serum (A) and in tissue testicular homogenates (B), SOD activities (C), MDA levels (D), and Zinc content (E) in tissue testicular homogenates and serum LH levels (F) in control and aluminum chloride treated immature rates for two (T_{2m}) and three (T_{3m}) months.

Fig. 2: Testosterone levels in serum (A) and in tissue testicular homogenates (B), SOD activities (C), MDA levels (D), and zinc content (E) in tissue testicular homogenates and serum LH levels (F) in control and aluminum chloride treated adult rats for one (T_{1m}) and two (T_{2m}) months.

Fig. 3: T.S of testis of control rats stained with H and E showing the seminiferous tubules with spermatogenic cells and interstitial cells of control (A), immature treated rats for 2 months (B) and 3 months (C), and adult treated rats for one month (D) and two months (E) (X400).

Table 1: Testicular and epididymal weight, epididymal sperm motility, alive, total sperm abnormalities and protoplasmic droplet % in control and treated immature rats with $AlCl_3$ (200 μg) for one and two months.

	Testicular weight (g.)	Epididymal Weight (g.)	Epid.sperm motility (%)	Epid.sperm alive (%)	Epid.total sperm abnor. (%)	Epid.sperm P. droplets (%)
Control rats (A)	1.03 ± 0.02	0.55 ± 0.06	65 ± 1.70	67 ± 0.71	0.73 ± 0.07	18.77 ± 0.45
Treated rats For 2 months (B)	0.77 ± 0.03	0.25 ± 0.02	29.25 ± 2.2	41.68 ± 0.97	2.82 ± 0.23	35.46 ± 0.45
Treated rats for 3 months (C)	0.92 ± 0.04	0.33 ± 0.01	27.13 ± 1.86	32.58 ± 0.76	3.72 ± 0.12	39.27 ± 0.75
Statistically (A-B-C) P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
(A-B) P<	0.001	0.001	0.001	0.001	0.001	0.001
(A-C) P<	0.05	0.001	0.001	0.001	0.001	0.001
(B-C) P<	0.01	NS	NS	NS	0.01	0.001

NS = non-significant. Epid.= Epididymal.
abnor.= abnormalities. P.= protoplasmic.

Table 2: Testicular and epididymal weight, epididymal sperms motility, alive, total sperm abnormalities and protoplasmic droplets % in control and treated adult male with $AlCl_3$ (200 ug) for one and two months.

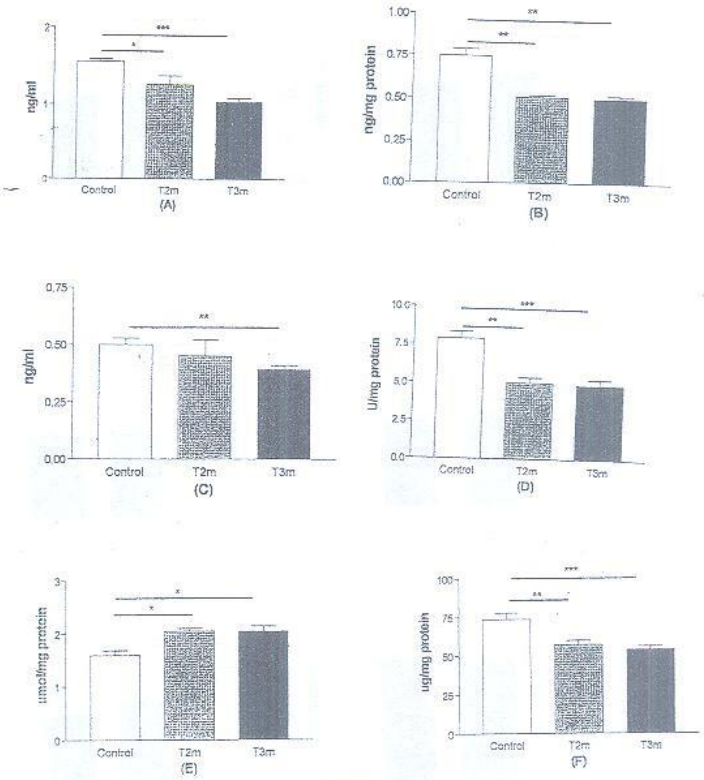
	Testicular Weight (g.)	Epid. weight (g.)	Epid. sperm motility (%)	Epid. sperm alive (%)	Epid. total sperm abnor. (%)	Epid. sperm P. droplets (%)
Control (A)	1.04 ± 0.02	0.58 ± 0.06	65.63 ± 0.71	70 ± 0.71	0.66 ± 0.03	16.77 ± 0.35
Treated rats for one month (B)	0.91 ± 0.05	0.31 ± 0.01	53.75 ± 4.09	64.75 ± 1.44	2.37 ± 0.16	18.99 ± 0.45
Treated rats for two month (C)	1.05 ± 0.06	0.48 ± 0.03	51.75 ± 2.16	58.13 ± 1.59	4.48 ± 0.25	19.79 ± 0.27
Statistically						
(A-B-C) P<	0.0452	0.0003	0.005	0.005	0.0001	0.0001
(A-B) P<	NS	0.001	0.05	0.05	0.001	0.001
(A-C) P<	NS	NS	0.001	0.01	0.001	0.001
(B-C) P<	NS	0.05	0.01	NS	0.001	NS

NS = non-significant. Epid.= Epididymal.
abnor.= abnormalities. P.= protoplasmic

Tables 3. Linear analysis of regression between superoxide dismutase (SOD), Lipid peroxides (MDA) levels, zinc content and testosterone (T) levels in testicular homogenates and epididymal sperm analysis among control and treated immature rats or treated adult rats with Aluminum chloride.

Dependent Parameters	Independent Parameters	Immature treated rats		Adult treated rats	
		R2	P	R2	P
Sperm motility %	SOD	0.95	0.13	0.99	0.03[a]
	MDA	0.99	0.04[a]	0.99	0.04[a]
	Zinc	0.97	0.10	0.96	0.12
	T.	1.0	0.001c	0.99	0.03[a]
Alive sperm %	SOD	0.99	0.05[a]	0.84	0.30
	MDA	0.87	0.23	0.85	0.25
	Zinc	0.98	0.09	0.93	0.17
	T	0.90	0.19	0.77	0.32
Total sperm abnormalities %	SOD	0.99	0.01[b]	0.84	0.26
	MDA	0.90	0.2	0.86	0.25
	Zinc	0.99	0.05[b]	0.94	0.16
	T	0.94	0.16	0.78	0.31
Protoplasmic droplets %	SOD	0.99	0.05[a]	0.99	0.05[a]
	MDA	0.96	0.12	0.99	0.04[a]
	Zinc	0.99	0.02[a]	0.99	0.04
	T	0.98	0.08	0.97	0.11

a: Significant at P<0.05. b: Significant at P<0.01.
c: Significant at P<0.001.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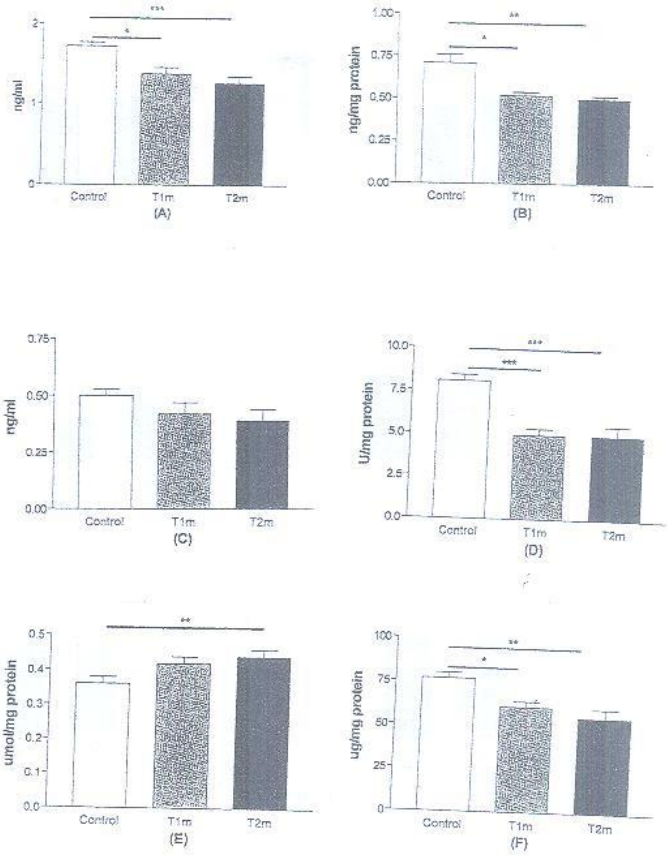
Control
T2m
T3m
(A)
ng/mg protein
(B)
ng/ml
(C)
U/mg protein
(D)
umol/mg protein
(E)
ug/mg protein
(F)

(Fig 1)


ng/ml
Control
T1m
T2m
(A)
ng/mg protein
(B)
ng/ml
(C)
U/mg protein
(D)
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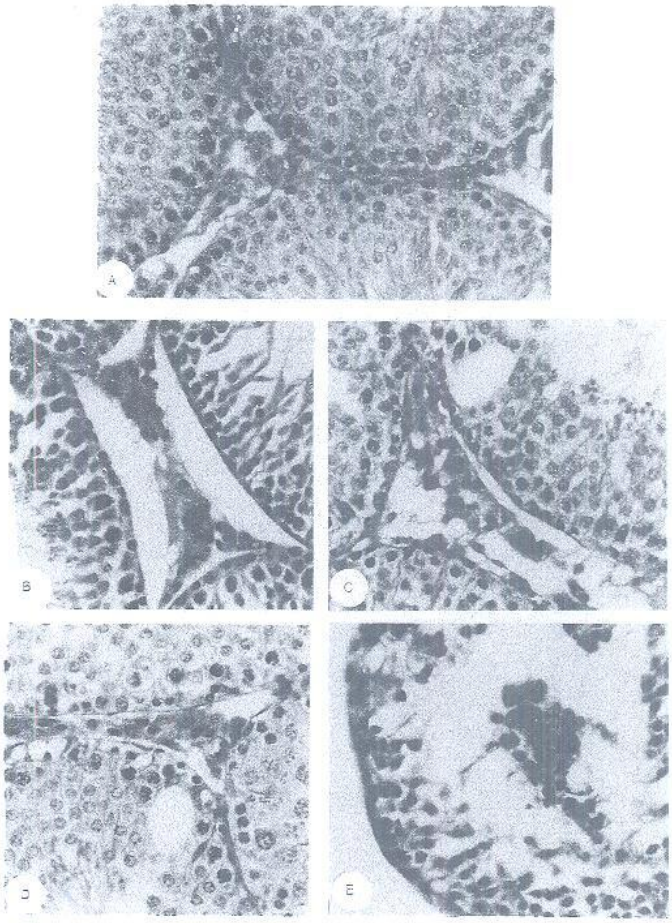
(E)
ug/mg protein
(F)

(Fig 2)



(Fig. 3)