Proceeding, 2nd International Conference on Radiation Sciences and Applications

28/3 – 1/4/2010 – Marsa Alam, Egypt

The Role of Antioxidants in Biochemical Disorders Induced By Arsenic in Adult male Rats.

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ABSTRACT

The present investigation included biochemical, radiometric, molecular studies and histopathological examination to evaluate the protective role of Antox tablets toward Arsenic toxicity in adult male albino rats (Rattus rattus).

Arsenic were given as sodium arsenate to different groups in drinking water at a dose of 100 mg/L, for 3 and 6 weeks led to severe tissue damage as revealed by an elevation of serum total protein and alteration of serum protein fractions . Using radioimmunoassay it was found that serum total testosterone level was significantly decreased. The decreased level of total testosterone paralleled the observed testicular damage.

Treatment of male rats with antioxidant (Antox) along with arsenic led to an improvement in both the biochemical and histological alterations induced by arsenic. Thus the protective role of Antox is attributed to its antioxidant and free radicals scavenging properties of its components (selenium, vitamin A acetate, ascorbic acid and vitamin E).

Key words: Antioxidants, Antox, Toxicity, Heavy Metals, Arsenic, Radioimmunoassay, Total Testosterone, Protein Electrophoresis, Testes Histopathology, Rats.

INTRODUCTION

Heavy metals include arsenic, gold, iron, lead, manganese, mercury and zinc. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. (1)

Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs.

In addition the symptoms of acute toxicity are usually sever, rapid in onset, and associated with a know exposure or ingestion (2), cramping nausea, vomiting; pain, sweating, headache, difficulty breathing, impaired cognitive, motor, and language skills; mania; and convulsions.

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adult. (3)

Heavy metals are also common in industrial applications such as in the manufacture of pesticides, batteries, alloys, electroplated metal parts, textile dyes, steel, and so forth. (4)

Children are particularly sensitive to lead (absorbing as much as 50% of the ingested dose).

An antioxidants is a substance that when present at low concentrations compared to that of an oxidizable of that substrate.

Antioxidants can act by scavenging biologically reactive oxygen species by preventing their formation or by repairing the damage that they do.

Numbers of vitamins have been found to reduce toxic manifestation of heavy metals. (5 and 6)

Vitamins C,E,A, selenium and zinc are important antioxidants that aid our overall health by increasing our protection from oxidative stress (7)

The plasma proteins produced by the hepatocytes are synthesized on polyribosomes bound to the rough endoplasmic reticulum, from which they are discharged into plasma (8).

Plasma contains a mixture of proteins differing in their origin and function.

The total protein of the plasma is about 6.5 to8.5 g/dl.

The proteins of the plasma are actually a very includes simple proteins and mixed, or conjugated proteins such as glycoproteins and various types of lipoproteins.

There are many different kinds of proteins in the body with many different functions, for examples, enzymes, some hormones, hemoglobin (oxygen transport), LDL (cholesterol transport), fibrinogen (blood clotting), collagen (structure of bone and cartilage), and immunoglobulin antibodies(9).

Increase in total serum protein can be seen in dehydration or as the result of an increase in immunoglobulins (10)

By electrophoresis technique serum protein are grossly separated into albumin and globulin, i.e., total protein = albumin + globulin.

However, globulins are roughly divided into alpha-1, alpha-2, beta and gamma globulins.

These can be separated and quantitied in the laboratory by electrophoresis.

Testosterone serves as a circulating precursor or prohormone for the formation of two types of active metabolites, which in turns mediate many androgen action.

In plasma, testosterone is largely bound to proteins, mainly albumin and sex hormone-binding globulin (SHBG, also called testosterone binding globulin).

In the blood of normal men, about 2% of testosterone is free (unbound), 44% is bound to SHDG, and 54% is bound to albumin and other proteins (11).

Arsenic is the most common cause of acute heavy metal poisoning in adults and is number 1 on the ASTDR's "Top 20 List." Arsenic is released into the environment by the smelting process of copper, zinc, and lead, as well as by the manufacturing of chemicals and glasses. Arsine gas is a common byproduct produced by the manufacturing of pesticides that contain arsenic. Arsenic may be also be found in water supplies worldwide, leading to exposure of shellfish, cod, and haddock. Other

sources are paints, rat poisoning, fungicides, and wood preservatives. Target organs are the blood, kidneys, and central nervous, digestive, and skin systems (3 and 12).

Exposure to arsenic occurs mostly in the workplace, near hazardous waste sites, or in areas with high natural levels. Symptoms of acute arsenic poisoning are sore throat from breathing, red skin at contact point, or severe abdominal pain, vomiting, and diarrhea, often within 1 hour after ingestion. Other symptoms are anorexia, fever, mucosal irritation, and arrhythmia. Cardiovascular changes are often subtle in the early stages but can progress to cardiovascular collapse.

Chronic or lower levels of exposure can lead to progressive peripheral and central nervous changes, such as sensory changes, numbness and tingling, and muscle tenderness. A symptom typically described is a burning sensation ("needles and pins") in hands and feet. Neuropathy (inflammation and wasting of the nerves) is usually gradual and occurs over several years. There may also be excessive darkening of the skin (hyperpigmentation) in areas that are not exposed to sunlight, excessive formation of skin on the palms and soles (hyperkeratosis), or white bands of arsenic deposits across the bed of the fingernails (usually 4-6 weeks after exposure). Birth defects, liver injury, and malignancy are possible.

The aim of this work was to study the effect of Arsenic as sodium arsenate given in drinking water to adult male albino rats and to evaluate the protective role of Antox-a drug containing vitamin C, vitamin E, vitamin A and selenium and their potential effect on heavy metals-induced biochemical toxicity in male albino rats.

MATERIALS AND METHODS

Male adult albino rats (Rattus rattus), obtained from the National research center, weighing 130-150 g were used for the present experiment and acclimated for 1 week prior to the study.

All animals were maintained on a stock diet and water ad libitum throughout the adaptation period, sodium arsenate (Na2HASO4.7H2O), was given to rats at dose 100 mg/ L in drinking water.

Antox:-

Contains the three main antioxidant vitamins A,C and E together with very important rare element, selenium.

Each tablet contains selenium 50µg, medical yeast 105 mg, vitamin A acetate 0.554 mg, Ascorbic acid 100mg and vitamin E 30 mg.

The tablets were crushed and suspended in distilled water to prepare daily oral dose of 10 mg Antox/kg body weight.

In the present study, the protective effect of Antox on intoxicated animals with heavy metals has been studied.

For this purpose, a daily oral dosage of (10 mg/kg body.wt) Antox freshly suspended in redistilled water was administrated with a stomach tube to rats.

Groups under investigation:

The rats were divided into main four groups. Each consisted of 12 rats and divided into 2 subgroups, (a) and (b) corresponding to time intervals, 3 weeks and 6 weeks.

Group I: [control]:

Animals of this group served as control. They were given redistilled water ad.libitum.

Group II: [Antox-A]:

Animals of this group received a daily oral dose of Antox.

Group III: [Arsenic+As]:

Lead intoxicated group received lead-Pb as lead acetate in drinking water.

Group IV: [Arsenic + Antox (As+A)]:

Daily oral dose of Antox was administrated to rats receiving Arsenic as sodium arsenate in drinking water.

Serum and Tissues Sampling:

The experimental animals were sacrificed using slight ether anesthesia and the blood of each animal was withdrawn from heart in a clean centrifuge tube. The blood was allowed to coagulate and the tubes were centrifuged at 3000 r.p.m for 15 minutes to separate blood serum which was kept at-5ºC for subsequent analysis. Then, the animals were dissected for histological investigation of the testes according to the method of Carleton. (13).

Determination of Total protein in serum:

Principle:

According to a method described by Doumas et al., Total serum protein was estimated (13)

Electrophoresis:

Technique is one of the simplest techniques for separating serum protein and applied to separate the protein fractions in serum using cellulose acetate paper (14). After separation, permanent fixation of the fraction was applied using special dye. The levels of protein fractions can be measured by densitometer at 530 nm.

Radioimmunoassay technique:

Determination of total testosterone in serum:

Principle:

Total testosterone was estimated according to the coat-A-count procedure which is a solid-phase radioimmunoassay (15; 16 and 17), using a kit supplied from diagnostics Products Corporation (DPC, L.A, CA, USA).

Statistical Analysis:

The Statistical significance of the mean values between control and treated groups was performed using student's t-test at probability level of $P< 0.05$, 0.01 and 0.001. Statistical computation (18; 19 and 20), which was used in the analysis of the collected data obtained in this work, by the aid of Microsoft Office Excel 2003 program.

RESUITS AND DISCUSSION

Serum Total Protein and its fractions:

Data presented in table (1) and figure (1) show the effect of the different treatments on serum total protein contents of adult male albino rats.

The results explained a significantly increase in serum total protein of arsenic (100mg/L) in drinking water receiving groups for 3 and 6 weeks by 14.08% and 24.23% compared to the control group. Moreover, the data revealed that treatment with Antox at dose level of 10 mg/kg body wt. during arsenic exposure caused a highly and very highly significant increase values recording 24.65% and 22.01% after 3 and 6 weeks respectively, table (1) and Fig. (2)

Serum albumin level was more or less comparable to control except in Antox treated groups. Arsenic administration induced a significant increase (24.44%) in serum albumin content of adult male rats after 6 weeks. On the other hand, serum albumin in the groups treated with both arsenic and Antox was increased by (34.75% and 44.00%) after 3 weeks and 6 weeks respectively, table (**1**) and Fig. (3).

Concerning serum α1-globulin in adult male rats, Antox induced percentage decreases of 36.76 and 47.54 after 3 and 6 weeks of treatment. While in the group of rats received arsenic in drinking water (100 mg/L), serum α2-globulin was increased very highly significant by 21.67 and 72.13 percent after 3 weeks and 6 weeks respectively when compared with control. Treatment with Antox plus arsenic very highly significantly decreased serum α1-globulin after 6 weeks, table (**1**) and fig. (4).

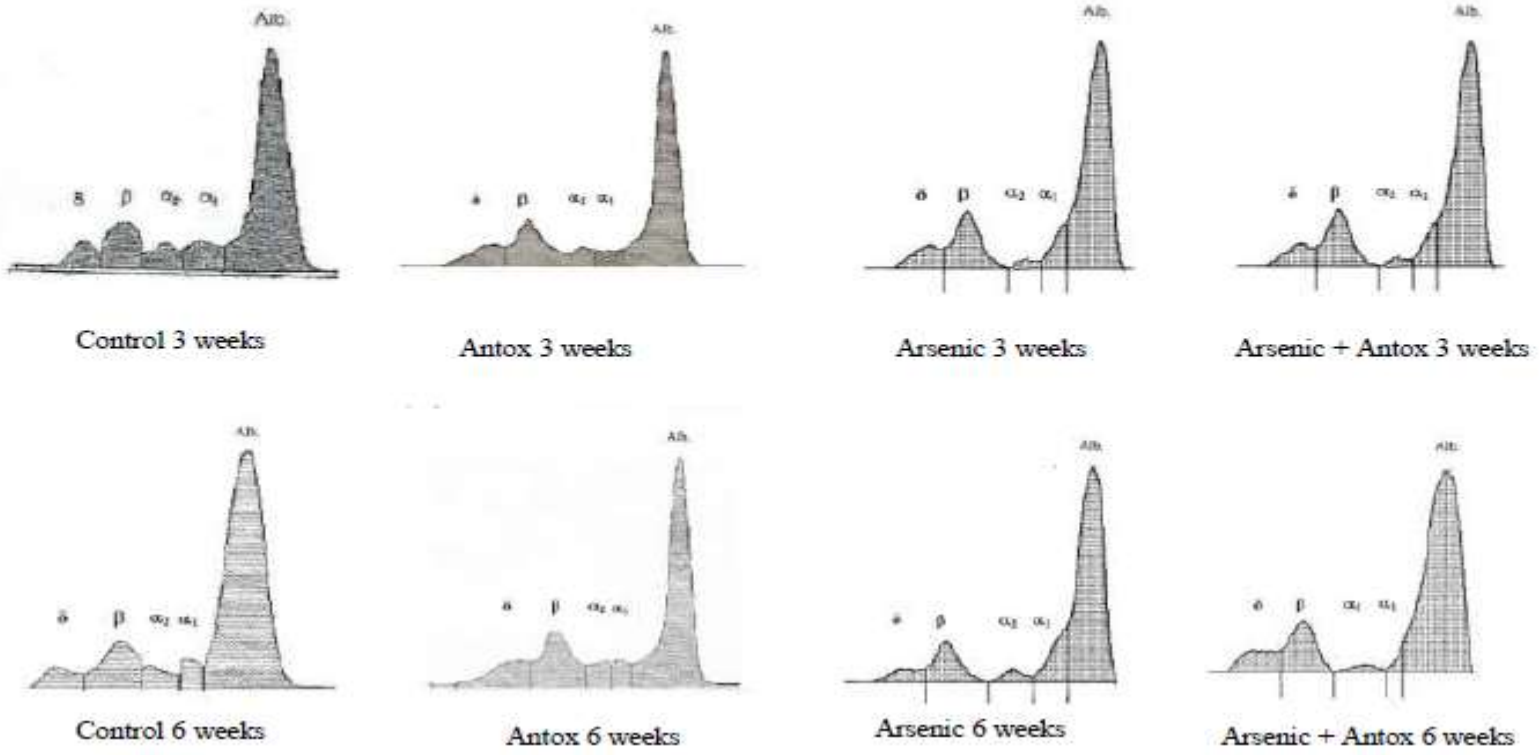
Serum α2-globulin was significantly decreased after Antox, arsenic or Antox along with arsenic treatment after 3 and 6 weeks. The highest percentage decrease was 68.63 recorded after 6 weeks of Antox plus arsenic treatment, table (**1**) and fig. (5).

Regarding serum β-globulin, a very highly significant increase in all groups except in arsenic treated group for 6 weeks, showed a significant increase, table (**1**) and fig. (6).

The results revealed that, serum δ-globulin was affected after arsenic administration in adult male rats drinking water. It recorded a very highly and a significant increase (38.80% and 22.58) after 3 and 6 weeks. Antox treatment along with arsenic exposure greatly improved δ-globulin content revealed by treatment of Antox alone when compared with normal control, table (**1**) and fig. (7).

Total Serum Testosterone:

As shown in table (2) and Fig. (8) Arsenic in drinking water at dose of 100 mg/L caused very highly significant decrease in adult male rat serum total testosterone after 3 and 6 weeks. The percentage decreases were 90.17 and 33.90 respectively. The data also revealed that, treatment of normal control rats with Antox (10 mg/ kg body wt.) resulted in very highly significant decrease by 59.41% and 58.13% after 3 and 6 weeks respectively. On the other hand, upon combined arsenic administration and Antox treatment moderate improvements in serum total testosterone were shown but it was still very highly significantly lower than the control value after 3 weeks. After 6 weeks of Antox treatment together with arsenic exposure the testosterone content was greatly improved.



Alb.: Albumin
α_1: α_1 -globulin
α_2: α_2-globulin
β: β-globulin
δ : δ - globulin

Fig. (1) Variations in serum total protein fractions of normal and treated with Antox (10 mg/kg body wt.) male rats received 100 mg/L of sodium arsenate **added in drinking water for 3 and 6 weeks**

Table (1) variations in serum total protein (U/L) and its fractions (g/dl) of normal and treated with Antox (10 mg/kg body wt.) male rats received 100 mg/L of sodium arsenate added in drinking water for 3 and 6 weeks.

Groups	Control		Antox		Arsenic		Arsenic + Antox	
Parameter	Time in weeks							
	3	6	3	6	*3*	*6*	*3*	*6*
T.P	7.08 ± 0.24	7.18 ± 0.20	6.92± 0.20	7.17±0.11	*8.10±0.21* **	*8.97±0.63* *	*8.90±0.54* *	*8.80±0.51* *
Change %			-2.26	-0.14	*14.41*	*24.93*	*25.70*	*22.56*
Albumin	4.46± 0.23	4.5 ± 0.23	4.46±0.13	4.30±0.07	*4.76±0.13*	*5.60±0.43* *	*6.01±0.36* **	*6.48±0.38* **
Change %			0.00	-4.44	*6.73*	*24.44*	*34.75*	*44.00*
αı -globulin	0.60 ±0.02	0.61 ±0.01	0.38±0.01 ***	0.32±0.01***	*0.73±0.02* ***	*1.05±0.10***	*0.58±0.03*	*0.24±0.01*
Change %			-36.67	-47.54	*21.67*	*72.13*	*-3.33*	*-60.65*
α2-globulin	0.50 ±0.01	0.51 ±0.01	0.24±0.01 ***	0.40±0.01***	*0.26±0.01* ***	*0.29±0.02* ***	*0.27±0.02* ***	*0.16±0.01* ***
Change %			-52.00	-21.57	*-48.00*	*-43.14*	*-46.00*	*-68.63*
β-globulin	0.85 ±0.03	0.85 ±0.02	1.20±0.03 ***	1.35±0.02***	*1.42±0.04* ***	*1.11±0.08* *	*1.40±0.08* ***	*1.20±0.07* ***
Change %			41.18	58.82	*67.10*	*30.59*	*64.7*	*41.18*
δ - globulin	0.67 ± 0.01	0.62 ±0.01	0.64±0.02	0.80±0.02***	*0.93±0.02* ***	*0.75±0.03* **	*0.64±0.04*	*0.72±0.04* *
Change %			-4.48	29.03	*38.81*	*22.58*	*-4.48*	*16.13*

The values were expressed as mean of 6 rats ± S.E
* Significant at p <0.05
** Highly significant at P < 0.01
*** Very Highly significant at P < 0.001

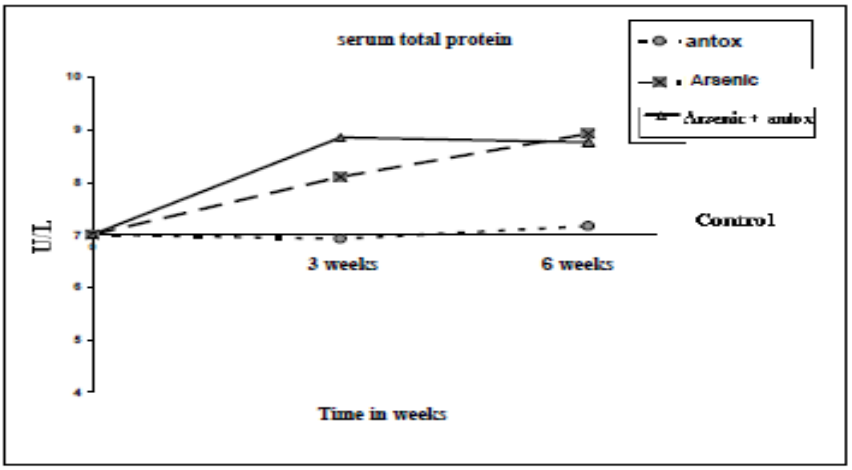


Fig (2) Comparative representation of serum total protein (U/L) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

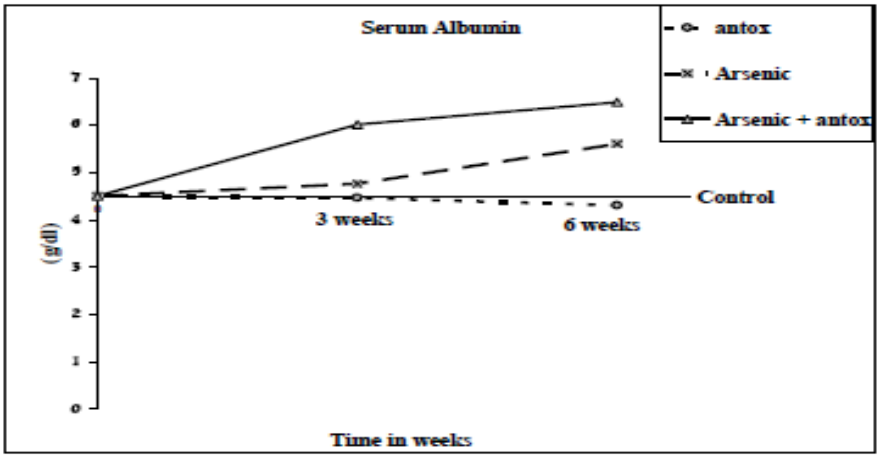


Fig (3) Comparative representation of serum albumin (g/dl) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

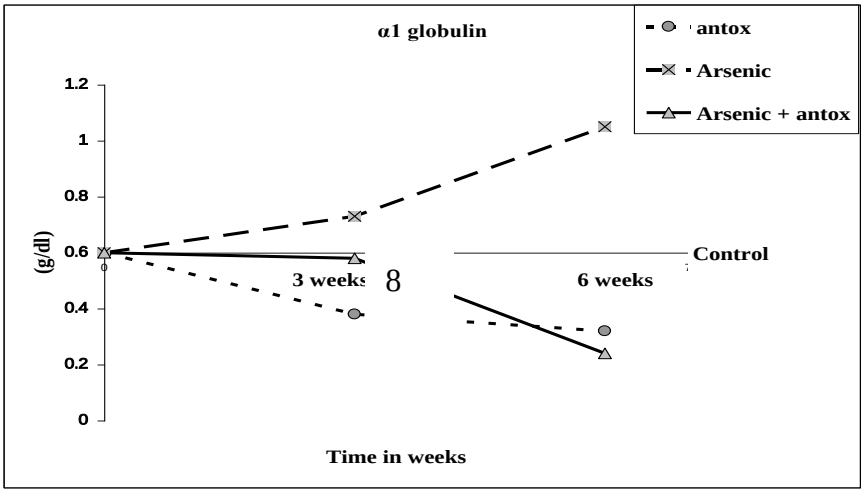


Fig (4) Comparative representation of serum α1-globulin (g/dl) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks

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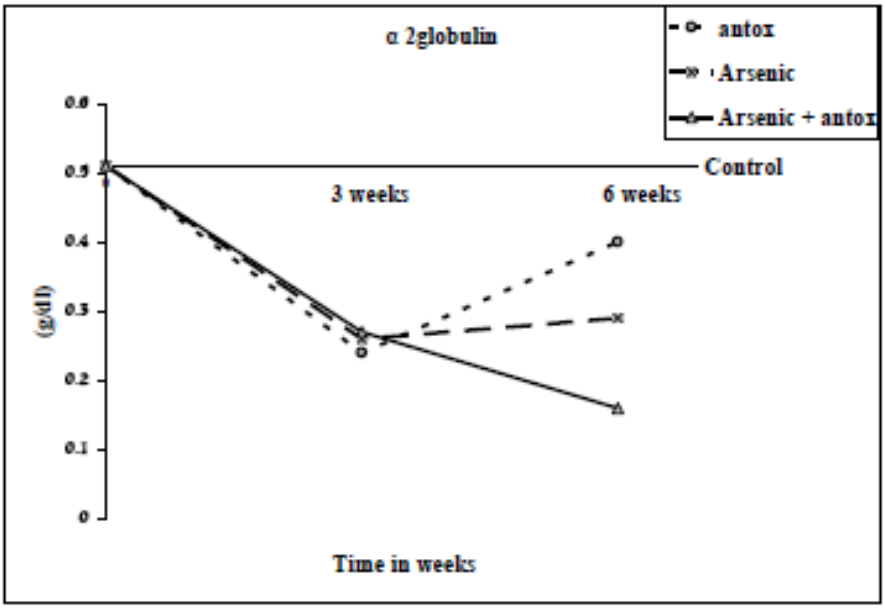

Fig (5) Comparative representation of serum α2-globulin (g/dl) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks

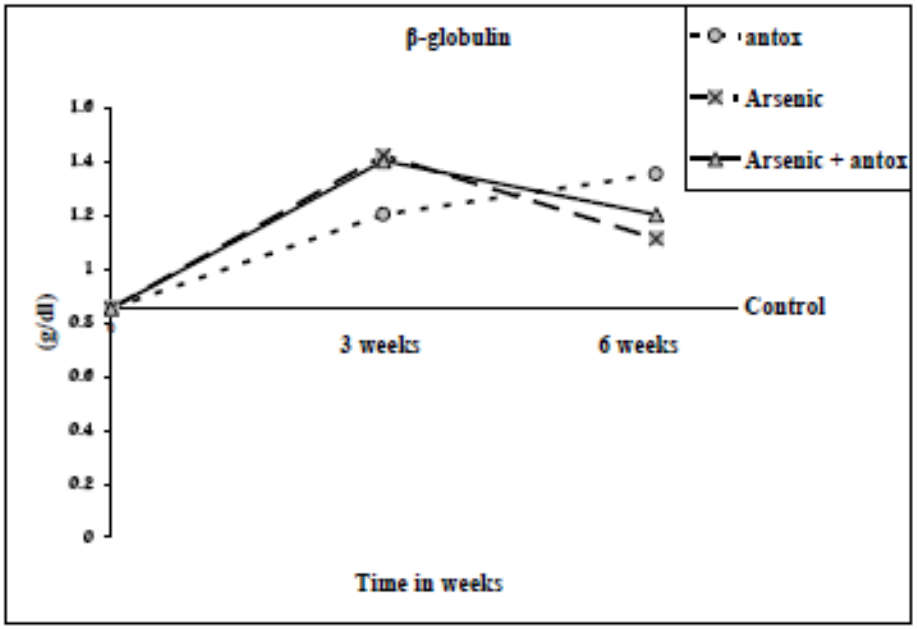

Fig (6) Comparative representation of serum protein β-globulin (g/dl) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

Fig (7) Comparative representation of serum δ-globulin (g/dl) in normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

Table (2) variations in serum Total Testosterone (ng/dl) contents of normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

Groups / Time in weeks	Control	Antox	Arsenic	Arsenic + Antox
3 weeks	406.22±17.12	164.88±4.76 ***	39.95±10.20 ***	225.86±15.43***
% change		-59.41	-90.17	-44.40
6 weeks	410.11±19.40	171.69±3.70 ***	271.10±22.52 ***	341.10±27.14
% change		-58.14	-33.90	-16.83

The values were expressed as mean of 6 rats ± S.E
* Significant at p <0.05
** Highly significant at P < 0.01
*** Very Highly significant at P < 0.001

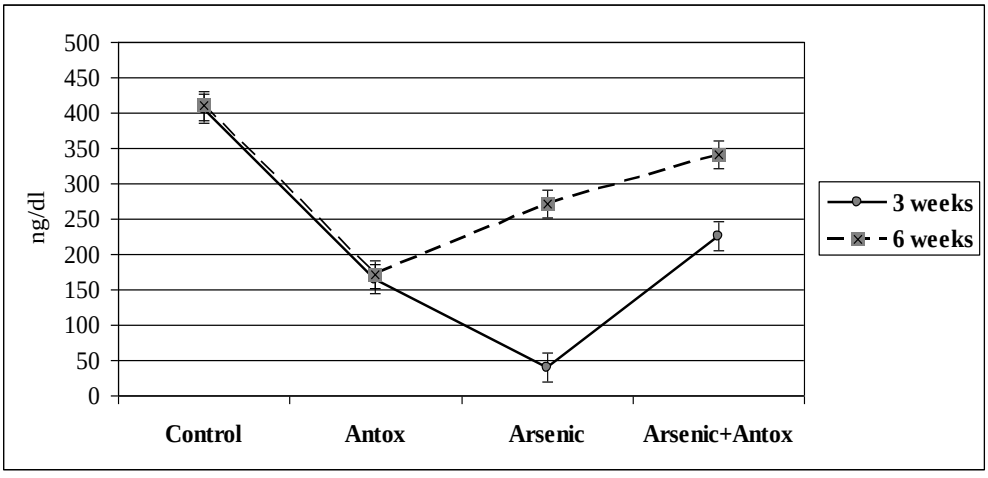

Fig (8) variations in serum Total Testosterone (ng/dl) contents of normal and treated with Antox (10 mg /kg body wt.) adult male rats received 100mg/L of sodium arsenate in drinking water for 3 and 6 weeks.

Testis:

Light microscopic examination of control testis section in adult male rat revealed sminiferous tubules with different stages of spermatogenic cells. All testes had regular structure demonstration normal spermatogenesis, Fig. (9.1).

Supplementation of Antox at dose 10 mg/kg body wt. to testis of normal adult male rats could not induce any change in histological structure of testis after 3 and 6 weeks, Fig. (9.2 and 9.3); sections showed normal appearance

The damage in seminiferous tubules was remarkable observed in the groups treated with arsenic for 3 and 6 weeks, Figs. (10.1 and 10.2). In the groups treated with Antox at the same time with arsenic light microscopic examination revealed improvement in testis section and normal spermatogenesis with only some degrees of degeneration of seminiferous tubules, Fig. (10.3 and 10.4).

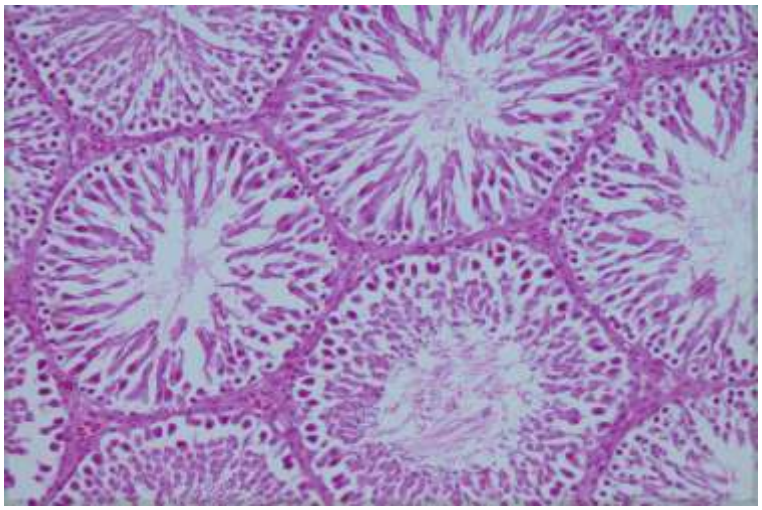

Fig (9.1) photomicrograph of control tetis section in adult male rats showing the different stage of spermatogenic cells in the seminiferous tubules and interstitial cells. (H&E X 100)

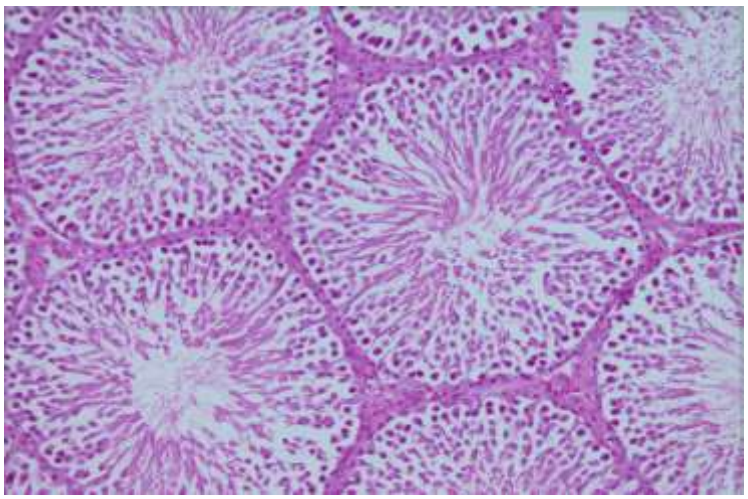

Fig (9.2) photomicrograph of a section of rat testis treated with Antox (10 mg/kg body wt.) for 3 weeks showing the different stage of spermatogenic cells in the seminiferous tubules and interstitial cells. (H&E X 100)

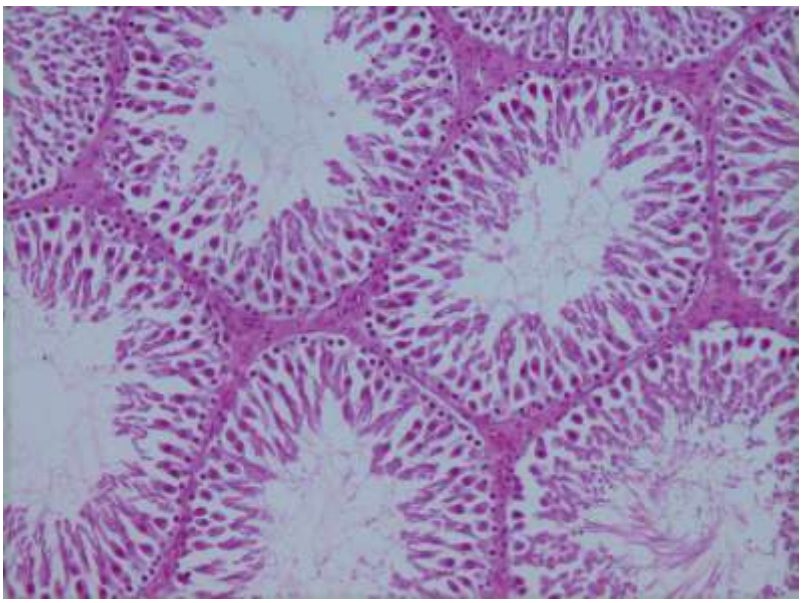

Fig (9.3) photomicrograph of a section of rat testis treated with Antox (10 mg/kg body wt.) for 6 weeks showing the different stage of spermatogenic cells in the seminiferous tubules and interstitial cells. (H&E X 100)

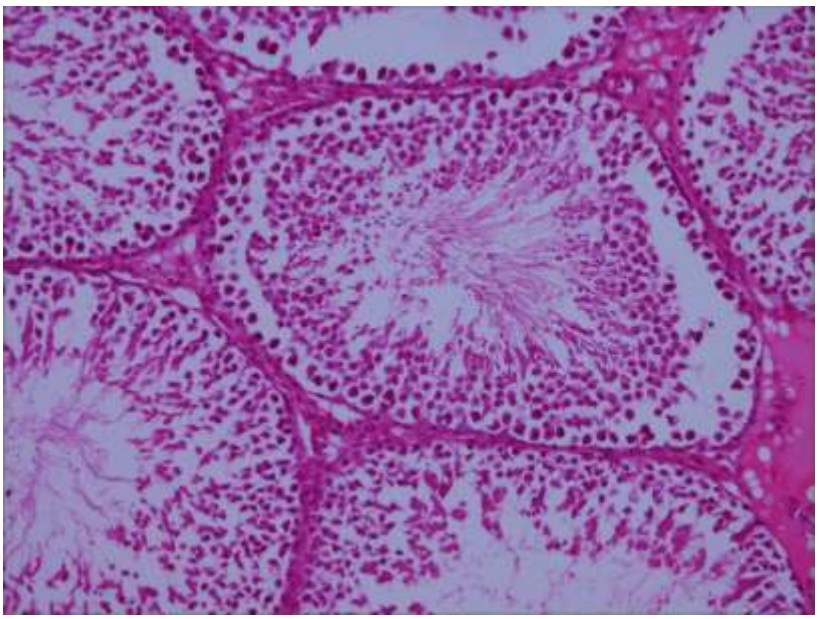

Fig (10.1) photomicrograph of a section of rat testis received arsenic as sodium arsenate in drinking water (100 mg/L) for 3 weeks showing mild degenerative changes in seminiferous tubules (H&E X 100)

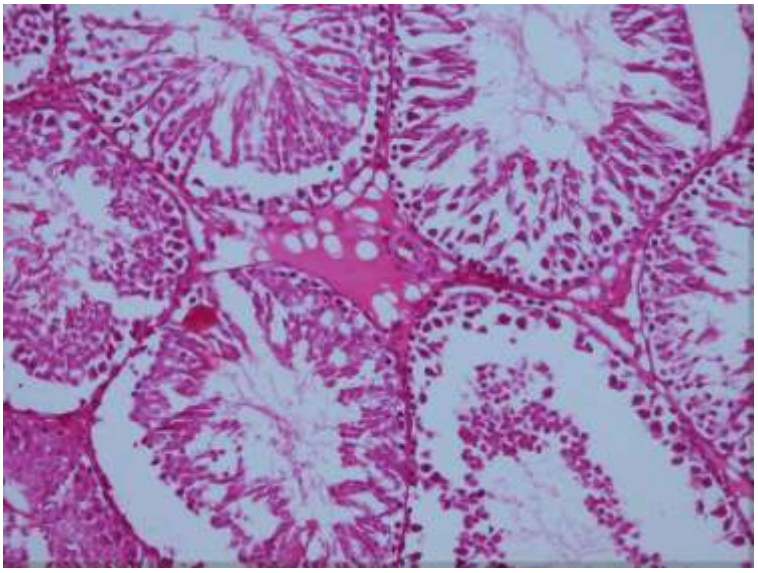

Fig (10.2) photomicrograph of a section of rat testis received arsenic as sodium arsenate in drinking water (100 mg/L) for 6 weeks showing severe degenerative changes in seminiferous tubules (H&E X 100)

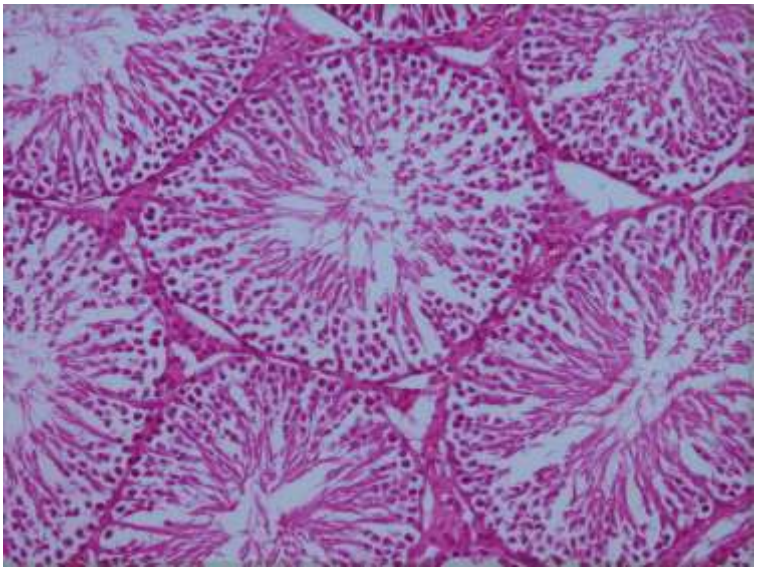

Fig (10.3) photomicrograph of a section of rat testis received arsenic as sodium arsenate in drinking water (100 mg/L) and treated with Antox (10 mg/kg body wt.) for 3 weeks showing the different stage of spermatogenic cells in the seminiferous tubules and interstitial cells (H&E X 100)

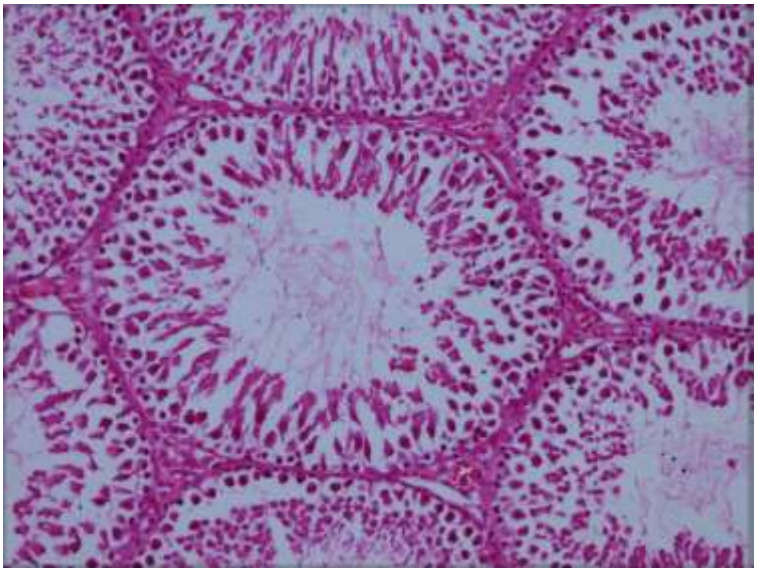

Fig (10.4) photomicrograph of a section of rat testis received arsenic as sodium arsenate in drinking water (100 mg/L) and treated with Antox (10 mg/kg body wt.) for 6 weeks showing the different stage of spermatogenic cells in the seminiferous tubules and interstitial cells (H&E X 100)

DISCUSSION

Effect of Arsenic as sodium arsenate added in drinking water (100 mg/L) of normal and treated adult male rats with Antox (10 mg/ kg body wt.)

Serum total protein and its fractions:

In the present study, a significant increase in serum total protein was noticed in rats received sodium arsenate (100 mg/L) in drinking water. The increase of serum total protein maybe attributed to synthesized proteins which were liberated as a result of cyctolysis and other pathological changes in the liver tissue associated with progression of the toxicity condition.

In disagreement with the present study, are the reports of other investigates (21; 22; 24; 25; 26 and 27), who reported decreases in serum total protein due to treatment with sodium arsenite and attributed such decrease to the decrease in albumin fraction and the ability of arsenic to inhibit the ability of glucocorticoid and its receptor to turn on genes normally. The glycocorticoid hormonal system plays an important role in protein metabolism. The reduction in plasma protein in animals exposed to environmental pollutants could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. In addition, the protein depression in the blood was also reported to be mainly due to excessive loss through nephrosis. Also, it maybe due to reduced protein synthesis or increased proteolytic activity or degradation. The observed decrease in plasma proteins could be also attributed in part to the damaging effect of sodium arsenate on liver cells as confirmed by the increase in the activities of plasma AST, ALT.

The present results demonstrated that serum total protein did not decrease in plasma of treated rats with arsenic for 3 and 6 weeks may be attributed to short period of exposure to arsenic in drinking water. While Santra et al., ; treated mice for 15 months and Biswas et al., administrated arsenic orally to goats for 12 weeks. On the other hand, Wang et al., investigated the effect of arsenic for 78 days in fishing pigs. Moreover, Nandi et al., exposed rats to arsenic for 12 weeks.(21, 22, 26, 24 and 25)

A significant increase in α-1 globulin is observed after 3 and 6 weeks of arsenic administration may be attributed to inflammation and defect in the liver. Furthermore α-2 globulin was markedly decreased at all intervals and these results may be indicate sever hypatocellular damage or hemolysis. Moreover, β-globulin was significantly increased maybe due to iron deficiency like anemia or nephrosis.

While gamma-globulin was increased 3 weeks after arsenic intoxication maybe due to chronic inflammatory disease or chronic liver disease and the increased gamma-globulin synthesis against the toxin.

Serum Total Testosterone:

The present data elucidated that serum testosterone was significantly reduced in male rats exposed to sodium arsenate in drinking water. These data are in accordance with the finding of Sarkar et al., and Jana et al., who reported a reduction in testosterone level which may be due to a reduction in serum LH level, the main regulating factor in testosterone production by leyding cells of the testis (28, 29, 30 and 31).

Furtherance, arsenic causes testicular toxicity by germ cell degeneration and inhibits androgen production in adult male rats probably by affecting pituitary gonadotrophins (30)

Antox plus arsenic treated rats causes serum total testosterone arise toward normal values of control group. As Antox has antioxidants properties of scavenging free radicals induced by arsenic toxicity.

The effect on testes:

The damage in seminiferous tubules was remarkable observed in the groups treated with arsenic for 3 and 6 weeks in the present study.

In agreement with earlier studies, Omura et al., who reported that arsenic cause a necrotic change in the testis and disturbed the spermatid head transformation at the late spermatogenic phases and caused spermiation failure. On the other hand, das Neves at al., found that the testes of arsenite exposed animals showed vacuolation and atrophy of seminiferous tubules(32 and 33).

Jana et al., reported histological damage revealing extensive degeneration of different varieties of germ cells at spermatogenic cycle in arsenic exposed rats. While, Mehranjani and Hemadi, showed that spermatogenic cells had become vacuolated as a result of sodium arsenide treatment which also led to a less compact arrangement of them, it also reduced the number of spermatides (30 and 34).

In the groups treated with Antox at the same time with arsenic light microscopic examination revealed an improvement in testis section and normal spermatogenesis with only some degrees of degeneration of seminiferous tubules. The improvement reflected the antioxidant properties of Antox as it includes vitamins with selenium.

CONCLUSION

It is concluded that oral pretreatment with Antox was effective in attenuating heavy metals – induced biochemical and histopathological damage in adult male albino rats and also success in protecting from arsenic toxicity. These facts present a novel approach to strategies for treating arsenic

poisoning by supplementation of Antox. The present study, so far suggest that antioxidants can play an extremely important role in abating some toxic effects of arsenic-induced subchronic toxicity in rats' organs.

RECOMMENDATIONS

We recommend that exposed people to heavy metals via contaminated surrounding environment or occupationally exposed workers to administrate antioxidants beside their food. Administration of Antox alone to (normal) healthy male albino rats caused some side effects which maybe due to vitamins overloading because the body doesn't need these vitamins except in a very rare amount which could be taken via natural vegetables and fruits. So we recommend people not to take Antox except if there is a problem that Antox can help in treatment or protection from these problems such as heavy metals toxicity. It is important for us to inform ourselves about the heavy metals and to take protective measures against excessive exposure as they are called silent killers.

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دور مضادات الأكسدة على التغيرات البيوكيميائية الناجمة عن تعرض ذكور الفئران اليافعة لعنصر الزرنيخ.

نظرا لانتشار الواسع للعناصر الثقيلة سواء بصورة طبيعية او بسبب استخدامات الانسان المختلفة في مختلف المجالات تم دراسة الزرنيخ ومعاملة الفئران به كزرنيخات الصوديوم فى مجموعتين بجرعة مقدارها 100مجم/لتر فى ماء الشرب لمدة ثلاثة وستة أسابيع متتالية لدراسة تأثير سميته علي البروتين ومكوناتة بإستخدام تحليل الفصل الكهربى وتقدير هرمون التيستوستيرون بإستخدام طرق المناعة الإشعاعية. وبالإضافة إلى الفحص النسيجى للخصية تشير نتائج هذه الدراسة إلى الآتى

وبعد إعطاء الفئران 100 مجم زرنيخات الصوديوم أدى إلى تغير فى قيمة البروتين الكلى عن المجموعة الضابطة وأيضا إلى تفاوت فى قيم مكونات البروتين. كما لوحظ نقص معنوى فى هرمون التيستوستيرون عن المعدل الطبيعى.

وأثبتت الدراسة التشريحية التأثير الضار للزرنيخ على نسيج خصية الفأر حيث لوحظ خلل وتلف فى الأنابيب المنوية بعد ثلاثة وستة أسابيع من إعطاء الرصاص وبعد المعالجة بالأنتوكس أثناء فترة التجربة أدى إلى تحسن ملحوظ فى البروتين ومكوناته وكذلك مستوى التيستوستيرون ولوحظ تحسن واضح فى نسيج الخصية.