Global Veterinaria 12 (2): 213-218, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.02.82126

Toxic Effects of Arsenic on Reproductive Functions of Male Rabbit and Their Amelioration with Vitamin E

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Abstract: Arsenic commonly contaminates the environment via insecticides and pesticides and seepage through drinking water. The present study was designed to investigate the toxic effects of sodium arsenite on body and testes weights, plasma testosterone, FSH and LH and semen quality parameters which are considered as major potential of fertility. The amelioration of toxic effects of sodium arsenite was carried out with vitamin E. Sixteen adult male rabbits were classified into four groups equally; (A) control, (B) arsenic (10 mg/kg body weight/day), (C) vitamin E (100 mg/kg body weight/day) and (D) sodium arsenite 10 mg/ kg body weight/day + vitamin E 100mg/kg body weight/day group. This treatment was continued for 58 days. After treatment, a significant depletion in weight of testes, plasma level of LH, FSH and testosterone, sperm count, sperm motility, sperm viability, HOS-coiling were recorded in arsenic treated group but there was no difference in the body weights of treated and untreated rabbits against the arsenic. In the sodium arsenite + vitamin E group, vitamin E meliorated (p<0.001) the adverse effects of sodium arsenite toxicity on the reproductive system. In addition, the treatment of rabbits with vitamin E alone significantly (p<0.001) increased the motility, number and HOS coiling of sperms. It was concluded that sodium arsenite caused the toxic effects on reproductive system of male rabbit and vitamin E has ameliorative effects on toxicity of sodium arsenite.

Key words: Sodium Arsenite · Semen Evaluation Parameters and Reproductive System Of Male Rabbit

INTRODUCTION

Arsenic, which has more than 20 valence forms in environment, is continuously contaminating the drinking water. Due to increase in the anthropogenic activities, the sources of polluting the environment with arsenic have increased in recent years. The inorganic arsenic is naturally present in excess amount in ground water through seepage of rocks and millions of people around the world are exposed to arsenic through drinking water and its excess amount was recognized as carcinogenic [1]. Epidemiological studies conducted in West Bengal and have proved that six million people were suffering from arsenic contaminated water and 300,000 people were affected with arsenic toxicity [2]. The exposure of arsenic is associated with more hazards of skin including hyperkeratosis, gangrene and tumors of skin, liver, lung and prostate [3].

Epidemiological studies conducted in Ukraine, Taiwan and Bangladesh have shown that food and water contaminated with arsenic cause the major reproductive disturbances in women[4] and the problems associated with pregnancy outcomes [5]. In another study it is also seen that contaminated food and water also cause the spontaneous abortion in women [6]. Moreover, smelting of copper causes the failure of reproductive system of male workers in industries [7].

Toxicity which is induced by arsenic in reproductive system is also characterized by the reduction of mass of testis along other sex organs [8]. In addition, necrotic changes in testicular tissue [9], massive degeneration of germ cells [10], Leydig cell atrophy [10] and reduction in testicular protein level [11] are also reported. The toxic effects of arsenic are responsible for oxidative stress [12]. The oxidative stress and production of free oxygen species affect the testicular functions [13].

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Vitamin E is identified as strong antioxidant [14], preventing membrane damage mediated by free radicals [15]. This vitamin was reported to reduce oxidative stress in the testis [16].

However, arsenic toxicity was not studied on the reproductive system of rabbits according to our information. This study was designed to examine the effect of arsenic on body weights, testis weights, semen quality parameters and hormonal profile and either vitamin E ameliorates these effects or not.

MATERIALS AND METHODS

Animals: Sixteen adult male rabbits were housed under naturally prevailing climatic conditions in cages in the Department of Theriogenology, University of Agriculture Faisalabad. Acclimatization period of two weeks, the rabbits were randomly assigned into four groups of four animals: group A was kept as control, group B was kept as sodium arsenite (10mg/kg/day), group C was kept as vitamin E (100 mg/kg/day) and group D was treated with sodium arsenite + vitamin E group. The rabbits were treated for the eight weeks. The blood was collected from the ear vein with the help of venofix® 23 gauge butterfly needle and 5 ml Terumo® syringes. The blood was allowed to clot at room temperature for the separation of serum. Serum was stored at –20°C until further analysis.

Body weights of rabbits of all groups were measured in grams before and after completion of the experiment. The rabbits were euthanized after completion of treatment and testes were collected from individual animal.

Weight of Testes: The testes of rabbits buck were removed after sacrifice of animal and extra tissue were excised. The weight of testis was measured in grams.

Epididymal Sperm Count: Epididymis from the testis was removed carefully and kept in 1.0 ml of normal physiological saline at 37°C. The suspension of sperm was prepared and this suspension was used for the examination of sperm count using Neubaeur counting chamber [17].

Sperm Motility: The tissue of cauda Epididymis was cut into small pieces in a 2.5 ml physiological saline to release the spermatozoa. Percentage motility was assessed at 37°C with the help [17] within 4 minutes after their separation from the tissue of cauda Epididymis.

Sperm Morphology: The morphology of spermatozoa was determined with the Eosin stain. The staining of spermatozoa is based on the principle that dead spermatozoa will stain according to colour of stain whereas live will remain colorless on staining. The 30ul of sperm suspension was mixed with 5ul of eosin stain. A total of 200 spermatozoa were counted for each rabbit at the magnification power of 400X. The abnormalities of morphology were classified as detached head, bent neck and curling of tail.

Hypo-Osmotic Swelling Test: Sperm functional was carried out by method of HOST [18]. This test based on the principle of osmotic differences. The base of this test is when sperm are held in hypo osmotic solution, the influx of water causes the tail to coil. Thus viable sperms were exposed to hypo-osmotic medium and observed with the help of phase contrast microscope. The coiling percentage of sperm under the hypo osmotic conditions was estimated.

Hormonal Assay: The level of FSH, LH and testosterone in serum was measured by using Radioimmunoassay (RIA). Rabbits kits of FSH, LH and testosterone were obtained from immunotech Beckman Coulter Company-USA, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2 IU/L, 0.2IU/L and 0.025ng/ml for FSH LH and testosterone respectively.

Data Analysis: The data of present study was presented as means ± SEM. The analysis of data was performed using the analysis of variance (ANOVA).

RESULTS

- Body Weights of Animal There was no significant difference in the body weights of all animals in four groups (Table 1).
- Weight of testes The sodium arsenite significantly reduced (p <0.05) the weight of testis rabbits bucks as compared to weight of control as in (Table 3). However the vitamin E compensated the harmful effects of sodium arsenite and there was no reduction in the weight of testis. Whereas in those animals which were treated only with vitamin E, the significant increase in weights of testis of these animals were reported.

Table 1: Analysis of variance table of body weights

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	1	922	922	0.20^{NS}
Group	3	7312	2437	0.53^{NS}
Treatment x Group	3	720	240	0.05^{NS}
Error	32	148328	4635	
Total	39	157282		

NS = Non-significant (P>0.05)

Comparison of means of body weights of rabbits in (gms)

	Treatment		
Group	Before	After	Mean±SD
Control	929.80±53.61	915.60±44.09	922.70±32.81
Arsenic	934.00±29.14	937.40±16.05	935.70±15.69
Arsenic+Vit.E	950.20±11.01	942.00±24.78	946.10±12.86
Vit.E	969.20±12.93	949.80±24.27	959.50±13.36
Mean±SD	945.80±14.96	936.20±13.70	

Table 2: Analysis of variance semen parameters

		Mean squares			
Source of variation	Degrees of freedom	Count	Motility	Morphology.	HOS coiling
Treatments	3	8212.50**	980.750**	501.083**	856.396**
Error	12	282.29	29.792	25.042	30.146
Total	15				

** = Highly significant (P<0.01)

Comparison of means of semen parameters (%age)

Treatments	Count	Motility	Morphology.	HOS coiling
Control	265.0±6.45A	55.25±2.06B	46.25±2.06A	50.00±2.04A
Sodium arsenite	178.7±6.57C	27.50±3.23C	25.75±2.17B	21.25±2.39B
Sodium arsenite+vit.E	215.0±6.45B	47.50±3.23B	47.50±3.23A	47.50±3.23A
VitaminE	276.3±12.5A	64.25±2.17A	50.00±2.04A	53.00±3.14A

Means sharing similar letter in a column are statistically non-significant (P>0.05).

Table 3: Analysis of variance (mean square) hormones and testis weights

		Mean squares			
Source of variation	Degrees of freedom	Testosterone Ng/ml	LH IU/L	FSH IU/L	Testis weight (gms)
Treatments	3	0.66614**	0.19730**	7.61652**	2.04585**
Error	16	0.00380	0.00057	0.03528	0.02370
Total	16				

** = Highly significant (P<0.01)

Comparison of means of hormones and testis weights

Group	Testosterone Ng/L	LH IU/L	FSH IU/L	Testis weight (gms)
Control	0.850±0.029 A	1.026±0.009 A	6.024±0.029 AB	1.988±0.083 A
Arsenic	0.124±0.005 B	0.634±0.007 B	3.570±0.125 C	0.706±0.034 B
Arsenic + vit.E	0.836±0.029 A	1.046±0.016 A	5.856±0.083 B	1.958±0.076 A
Vit.E	0.874±0.036 A	1.020±0.008 A	6.190±0.070 A	2.008±0.072 A

Means sharing similar letters are statistically non-significant (P>0.05)

Semen Quality: The reproductive functions of rabbit including sperm count, motility, morphology and HOS curling were significantly reduced with arsenic induced toxicity (Table 2). A significant (p<0.001) reduction of motility was recorded in experimental rabbit which were treated by arsenic as compared to control whereas rabbits

treated sodium arsenite along with Vitamin E has significant (p<0.001) effects in the restoration of semen motility but the animals which were treated with vitamin alone E alone showed highest sperm motility. A similar trend was seen for sperm count. When sperm morphology was considered, rabbits treated with sodium- arsenite had lowest %age of normal sperm compared to rabbits of other three groups; the difference in normal sperm among rabbits of other three groups were non-significant. A similar trend was seen for HOS coiling of sperms.

Hormonal Assay: Plasma levels of FSH, testosterone and LH were significantly (p>0.05) decreased in the arsenite treated rats when compared with the control as represented in (Table 3). The co administration of sodium arsenite with vitamin E ameliorated the effect of sodium arsenite and the plasma levels of FSH, LH and testosterone were restored.

DISCUSSION

In the present study, body weights of arsenic treated rabbits were not significantly different from the control rabbits and these results were in close harmony to previous findings about the effects of sodium arsenite on the weights of body [10]. However, another study found the decrease in weights of body in the arsenic exposed animals [19]. These different results might be due to the duration of treatment or administrated dosages [20]. The mass of testis is an important index of toxicity of reproductive system in the male [21] and the reduction in mass of testes was dependable with removal of germ cells [22].

The potential of reproductive system of male rabbit was calculated by measuring sperm evaluation parameters like motility, viability, sperm count and hypo osmotic swelling. These parameters are considered as key indices of spermatogenesis and maturation. The dose which was selected for present study caused many symptoms of toxicity like shivering and diarrhea in rabbits. Two rabbits died with signs of trembling, diarrhea and weakness in experimental rabbits which indicate the toxicity of sodium arsenite at selected dose. The present study investigated the harmful effects of sodium arsenite by significantly decreasing the count of sperm and sperm motility which were observed in rabbits exposed to arsenic. The reduction of these semen parameters by sodium arsenite are in close with the results of others, who reported the reduction in sperm count, motility and increased morphology abnormalities [23]. Reduction in livability of sperms was reported in rats following contact to arsenic [24]. No harmful effect was produced on sperm count, motility and morphological abnormalities by arsenic through drinking water for 35 days at low doses [25]. But at higher doses, significant reduction in sperm count, motility and increased in number of atypical sperm were observed [26].

Thiol-rich proteins are present in the flagellum and chromatin of sperm and these proteins are important in the protection of sperm motility and stability [27]. Arsenic is a well known thiol-inhibiting metalloid [23]. The reduction of motility of sperm during the present study might be due to gathering of arsenic in epididymis where the sperm mature and acquire motility. Due to electrophilic affinity of the arsenic, it binds to sulphydryl groups on proteins and in that way inhibits enzyme action [28].

Evidence also suggests that reactive oxygen species produced due to arsenic causes the interaction with polyunsaturated fatty acids of spermatozoa which creates the peroxidation causing the deformities like reduction of motility and livability [29]. In the present study, significant increases in morphological abnormalities of spermatozoa are reported due to sodium arsenite which are similar to previous findings [9]. The decrease in plasma level of testosterone, FSH and LH hormones in arsenic treated rabbits were similar to previous studies [30]. Reduced level of testosterone causes reduction of semen parameters as testosterone is responsible for the normal physiology of male reproductive system [31, 32]. The reduced level of testosterone in arsenic treated rabbits can be attributed to the less level of LH [33, 34] as the concentration of LH controls the normal level of testosterone in blood.

In addition to the above mechanisms, it is also reported that arsenic causes infertility by creating the oxidative stress in testis [13]. Vitamin E which is identified as strong antioxidant [14] ameliorated the harmful effect of sodium arsenite on semen parameters. It is therefore assumed that sodium arsenite decreased the motility, viability and HOS by creating oxidative stress. Interestingly, we showed that in sodium arsenite+ VitaminE group, Vit.E significantly ameliorated sodium arsenite-mediated decrease in sperm number, motility, HOS coiling, increase in morphological abnormalities and plasma level of male hormones.

CONCLUSION

From the present study it is concluded that arsenic causes the male infertility by decreasing the functions of reproductive system and vitamin E has ameliorating effects against arsenic. Arsenic which is continuously

used as insecticide and pesticides and livestock is exposed indirectly, so there is need in future to study its effects on livestock health and production.

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