



International Journal of Pharmacology

ISSN 1811-7775

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The Effects of Sodium Arsenite on the Biochemical Factors in the Blood of Vasectomised Rats

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Abstract: Arsenic salts like sodium arsenite and/or vasectomy may cause variation in sex hormones and enzymes involved in reproductive systems. Four groups of rats (vasectomy + sodium arsenite), (vasectomy), (sham + sodium arsenite) and (sham only) were considered and 8 mg/kg/body weight/day of sodium arsenite was given for a period of 64 days to the treated rats. Hormones (FSH, LH and testosterone), enzymes (aspartate transaminase, alanine transaminase and alkalinephosphatase) and electrolytes (Sodium and potassium) were evaluated using standard methods then the results analyzed using one way ANOVA. The significant reduction of testosterone level was observed in vasectomy + sodium arsenite group compared to vasectomy ($p < 0.05$) and sham only ($p < 0.001$) groups. Level of the LH was decreased significantly ($p < 0.05$) in sham + sodium arsenite and vasectomy groups, when compared to sham only group. In vasectomy group treated with sodium arsenite level of ALP and AST enzymes were decreased significantly ($p < 0.05$) as compared to sham only group. Also level of the AST was decreased significantly ($p < 0.05$) in vasectomy group compared to sham only group. Vasectomy and sodium arsenite toxicity affect the normal level of AST, alkalinephosphatase, LH and testosterone in the rats. A cumulative effect was observed when Vasectomised rats were exposed to sodium arsenite.

Key words: Sodium arsenite, vasectomy, sex hormones, ALT, AST, ALP

INTRODUCTION

Arsenicals are used as herbicides, fungicides and rodenticides and may cause air, soil and water pollution. Exposure with arsenical through drinking water is common in many areas of the world (Nickson *et al.*, 1998; Borzsonyl *et al.*, 1992; Chatterjee *et al.*, 1993). The predominant uses of arsenic are in manufacture of pesticides (including wood preservatives), glass, alloys, electronic components (semiconductors), pigments and pharmaceuticals (Desesso *et al.*, 1998). Arsenite and arsenate oxidation states are the most toxicological form of arsenicals (Desesso *et al.*, 1998). Investigations on arsenate effect are well established and the studies suggest that different forms of inorganic arsenic are rapidly taken up by liver and kidney (Lerman, 1983; Lu *et al.*, 2001). Arsenic exposure cause liver disease (Cui *et al.*, 2004) and also affect reproductive system through imbalance in LH, FSH, estrogen and testosterone level (Chattopadhyay *et al.*, 1999; Sarkar *et al.*, 2003). Testosterone is responsible for normal growth and development of male sex organs and maintenance of secondary sex characteristics where as, luteinizing hormone regulates the production and secretion of

testosterone by the Leydig cells of the testes and FSH stimulates spermatogenesis (Moudgal and Sairam, 1998; Depenbusch *et al.*, 2002). In addition, chronic arsenicosis is a multisystem disorder in which patients show symptoms relating to involvement of the lungs, gastrointestinal system, spleen, genitourinary system, hemopoietic system, eyes, nervous system and cardiovascular system (Rahman *et al.*, 2001). In the other hand vasectomy is a common method of sterilization, though safe and relatively simple, requires a high level of expertise to minimize surgical complications (Awsare *et al.*, 2005). It is also been reported that, Sperm yields/g of testis were significantly decreased in men post-vasectomy (McVicar *et al.*, 2005) and decrease in gonadal weight as well as testicular degeneration have been reported (Lohiya *et al.*, 1987; Schreiber and Schwille, 1995). In case of biochemical factors, no appreciable changes were observed (Lohiya and Tiwari, 1984) but, decrease in testosterone and increase in LH, FSH hormones have been reported (Geierhaas *et al.*, 1991). Still the physiologic significance of the hormone changes after vasectomy remained unclear (Nikkanen and Punnonen, 1982).

From one side, in today's world many people undergo surgical vasectomy base on a population control program and in other side men are exposed to arsenic pollution. Though the arsenate toxicity causes biochemical factors imbalance including enzymes and sexual hormones, what will be the cumulative effect of arsenate and vasectomy is the aim of this investigation. The present study was designed to evaluate the effects of the arsenic toxicity and vasectomy together on biochemical changes like; (SGOT, SGPT, alkaliphosphatase, sodium, potassium, LH, FSH and testosterone) in adult Wistar rats.

MATERIALS AND METHODS

Animals and treatment: Thirty two male adult Wistar rats weighing 250 ± 20 g obtained from pasture institute, Iran, were maintained under standard laboratory conditions (12 h light and 12 h dark and 20 ± 2 °C) with free access to food. The rats were divided in four groups (n = 8), I (vasectomy + sodium-arsenite), II (vasectomy), III (sham + sodium arsenite) and IV (sham only).

Vasectomy procedure: Rats were injected with 50 mg ketamine and 10 mg xylosine and under anesthetic condition, a small vertical midline lower abdominal incision was made. The vas deferens were ligated twice with the 4-zero silk sutures and then transected between the ligatures. The spermatid vessels were carefully dissected. The wound was closed in 2 layers: 4-zero absorbable sutures were used for the musculature and 4-zero silk sutures for the skin. Sham operation was performed in the same manner except that the vas deferens was neither ligated nor divided (Ikeda and Sofikitis, 2000).

Oral treatment of sodium arsenite: Sodium arsenite was purchased from Merck Company (Germany). One week after vasectomy, vasectomy + sodium arsenite and sham + sodium arsenite groups were treated orally with sodium arsenite (8 mg/kg/body weight) prepared freshly in distilled water just before used. Vasectomised and sham only groups were given only water in a period of 64 days.

Serum sample collection: Blood samples were collected after the period of treatment (64 days) from the hepatic vein under ether anesthesia. Heparinized plasma was separated using centrifugation (3000 rpm) and stored at -20 °C to carry out the biochemical-assays.

Hormones (LH, FSH and testosterone) assay: Plasma follicle-stimulating hormone (FSH), lutoneizing hormone (LH) and Testosterone concentration were measured by enzyme linked immunosorbent assay (ELIZA) using eliza

reader statfax-303 (Awareness comp. USA). LH and FSH concentrations were measured as described in the instructions provided with the kits (RADIM S.p.A. Roma, Italy), where as Testosterone concentration was measured as described in the instruction provided by kits (DRG Diagnostics GmbH, Germany) in which endogenous testosterone of the rats plasma competes with a testosterone horseradish peroxides conjugate for binding to the coated antibody.

Enzymes (Alkaline phosphatase and Transaminases) assay: Serum aspartate transaminase (AST), alamine transaminase (ALT) and alkaline phosphatase (ALP) were measured described in the instructions provided with the kits (parsazmon co. reagents Boheringer Manheim, Germany) and the absorption of the test samples were read at 340 nm with the help of (COBAS-MIRA ROCHE DIAGNOSTIC INSTRUMENT) automated reader.

Electrolytes (sodium and potassium) concentration assay: Serum sodium and potassium concentration was determined using FLAM PHOTOMETER CIBA CORNING INSTRUMENT USA. Serum was diluted by lithium solution (50 μ L/10 mL DILEUENT) and their concentration was determined against standard solution of sodium and potassium.

Statistical analysis: The data was statistically analyzed using one way ANOVA by Tukey test and statistical significance was defined as $p < 0.05$.

RESULTS

Testosterone reduction was significant in vasectomised group treated with sodium arsenite ($p < 0.001$ and $p < 0.05$), respectively when compared with the results of the sham only and vasectomised groups. Also, no difference ($p > 0.05$) was observed between the testosterone level in sham group and vasectomy group treated with sodium arsenite. In addition, this study showed that there was no significant difference ($p > 0.05$) in the level of FSH in experimental groups compare to sham only group. A significant LH level reduction ($p < 0.05$) were observed in all the experimental groups with compared to sham only group, although no significant difference ($p > 0.05$) in the LH hormone level was detected among the groups (vasectomy + sodium arsenite, vasectomy and sham + sodium arsenite) as shown in Table 1.

A significant decrease ($p < 0.05$) of ALP level was observed in the vasectomised group treated with sodium arsenite when compared to sham only and vasectomised groups, although the value of the ALP enzyme was not

Table 1: Comparison between sex hormones (LH mLU mL⁻¹, FSH mLU mL⁻¹ and Testosterone ng mL⁻¹) in different groups of rats, 64 days after vasectomy and sodium arsenite treatment (8 mg/kg/body weight/day)

Group of rats	LH	FSH	Testosterone
Vasectomy + Sodium arsenite	0.59±0.29 ^a	0.71±0.4 ^a	0.94±0.31 ^a
Vasectomy	0.61±0.25 ^a	0.36±0.16 ^a	3.05±1.93 ^{ab}
Sham + Sodium arsenite	0.69±0.17 ^a	0.68±0.48 ^a	2.26±0.99 ^a
Sham only	2.08±1.93 ^b	0.60±0.32 ^a	5.01±2.18 ^b

Values are means±SD. Means with the same letter code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

Table 2: Comparison between enzymes (IU L⁻¹); aspartate transaminase (AST), alanine transaminase (ALT) and alkalinephosphatase (ALP) in different groups of rats, 64 days after vasectomy and treatment with sodium arsenite (8 mg/kg/body weight/day)

Group of rats	AST	ALT	ALP
Vasectomy + Sodium arsenite	295.83±126.27 ^a	90.00±38.19 ^a	225.83±42.07 ^a
Vasectomy	210.00±52.12 ^{ba}	62.86±18.45 ^a	338.57±100.32 ^b
Sham + Sodium arsenite	230.00±66.52 ^{ba}	71.66±6.24 ^a	333.57±46.07 ^{ab}
Sham only	149.00±20.74 ^b	70.00±23.45 ^a	393.75±100.58 ^b

Values are means±SD. Means with the same letter code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

Table 3: Comparison between electrolytes (mEq L⁻¹); sodium and potassium ions in different groups of rats, 64 days after vasectomy and treatment with sodium arsenite (8 mg/kg/body weight/day)

Group of rats	Sodium (Na ⁺)	Potassium (K ⁺)
Vasectomy + Sodium arsenite	139.29±2.29 ^a	5.54±1.54 ^a
Vasectomy	151.43±10.64 ^a	6.34±1.94 ^a
Sham + Sodium arsenite	149.86±15.84 ^a	5.97±2.25 ^a
Sham only	144.60±2.79 ^a	4.40±0.39 ^a

Values are means±SD. Means with the same letter code do not differ significantly from each other (ANOVA, Tukey test, p>0.05)

significant (p>0.05) in the vasectomy + sodium arsenite group when it compared to sham + sodium arsenite. These results also indicated that there was no variation in the level of the ALP enzyme among vasectomised, sham + sodium arsenite and sham only groups. On the other hand in this study, no significant difference (p>0.05) in alanine transaminase (ALT) was detected in all the experimental groups compared to sham only group. AST level was significantly increased (p<0.05) in vasectomised + sodium arsenite, vasectomised and sham + sodium arsenite groups when it compared to sham only group although there is no significant difference (p>0.05) in AST enzyme level among the vasectomised + sodium arsenite, vasectomised and sham + sodium arsenite groups as shown in Table 2. In addition, this study indicated that there is no significant difference (p>0.05) in the level of both sodium and potassium electrolytes when all the groups were compared together (Table 3).

DISCUSSION

Arsenite and arsenate have different fates in the body and the former is chemically more reactive

(Radabaugh and Aposhian, 2000; Radabaugh *et al.*, 2002). In the present study as the results showed treatment with the sodium arsenite caused reduction in the testosterone and LH level in the rats. Previous investigations report, sodium arsenite causes the reduction of sex hormones level including testosterone, LH and FSH (Sarkar *et al.*, 1991; Chattopadhyay *et al.*, 1999) conform the results of this study. The variation of FSH level was not significant in relation to 64 days of sodium arsenite treatment, Chattopadhyay (1999), discusses the reduction of FSH level after 28 days of arsenic treatment and reported that the sodium arsenite has demolishing effect on the delta 3-5 beta HSD and 17-beta HSD enzymes responsible for steroid hormones synthesis (Chattopadhyay *et al.*, 1999), also, arsenite partially block protein and enzymes involve in sex hormone synthesis and interfere with protein phosphorylation (Tamaki and Frankenberger, 1992). Reduction in the activity of these enzymes brings about the imbalance of the sex hormones following sodium arsenite treatment (Dupont *et al.*, 1992). Therefore non significant reduction of FSH in this study might have been compensated by extension of the treatment period from 28 days to 64 days. Production of Gonadotropin (LH and FSH) hormones from pituitary gland is under direct control of hypothalamus which releases gonadotropin releasing hormone (GnRH) and gonadotropin hormones control the synthesis and secretion of the testosterone from leydic cells in the testes. Testosterone itself through negative feed-back controls the release of LH hormone (Zwat *et al.*, 1997). In our results sodium arsenite treatment caused decrease in the level of testosterone as well as LH, it seems the reduction of LH by sodium arsenite is due to the direct effect of this agent on the hypothalamic-pituitary axis. Thus it is suspected that sodium arsenite effect on testosterone and LH would be base on the separate mechanisms.

In the other hand, current study shows vasectomy caused significant reduction of LH in the rat while the other hormones level were not affected by vasectomy. In this respect the other studies have reported that testosterone, LH and FSH level variation do not follow the same pattern always. In some, the level of sex hormones reduces (Pant *et al.*, 2004) where as in other an increase (Geierhaas *et al.*, 1991) of them is reported. In addition, it is documented that the imbalance of sex hormone after longer term vasectomy operation will be automatically adjusted to normal level (Nikkanen and Punnonen, 1982; Glavind *et al.*, 1990) but it depends on the condition of operation (Awsare *et al.*, 2005). Present results indicated that the addition of sodium arsenite on the post

vasectomy condition lead to a dramatic reduction of the testosterone level in the rats. So this study suggests that the cumulative effect of vasectomy and sodium arsenite exposure might be a consideration point to alert the vasectomised men of hazardous effect of sodium arsenite on reproductive system.

In present study, vasectomy and sodium arsenite treatment caused significant elevation of AST enzyme. However the effect of sodium arsenite on treated rats was not considerable on the AST and also ALT while sodium arsenite treatment decreased ALP. Results of previous study shows arsenic exposure caused elevation of transaminases activity, fatty liver and ultimately damages the liver tissue (Guha Mazumder, 2001). Cui *et al.* (2004), report that the arsenic accumulated in the rat liver is dose-dependent and caused hepatic histopathological changes, such as disruption of hepatic cords, sinusoidal dilation and fatty infiltration (Cui *et al.*, 2004). Liver carries out hydroxylation, methylation and glycosylation of the toxic compounds which detoxify them (Mckinney, 1992) although the mono/di methylated metabolites are more toxic than the original compound and these metabolite are more readily excreted in the urine (Aposhian *et al.*, 2004; Kitchin and Wallace, 2005). Also liver plays important role in metabolic processes like lipoprotein synthesis which is vital in the transport of the cholesterol to the tissues. Thus, if the cholesterol metabolism is affected, sex hormone level also will be altered. It is reported that arsenic (+3) administration markedly increased the activities of ALP in the liver and kidneys (Tripathi and Flora, 1998) and decrease acid phosphatase enzyme in testes tissue (Pant *et al.*, 2004), therefore, sodium arsenite not only causes liver damage but also testes tissue will get affected too and as a result it will affect the reproductive system of animal.

Long term vasectomy in langour monkey causes no significant difference in AST, ALT and ALP level (Lohiya and Tiwari, 1984; Lohiya and Tiwary, 1983) which confirm our results on ALT and ALP. Elevation of AST enzyme in vasectomy group might be of cellular and molecular difference of the races or may be because of shorter period of vasectomy in our investigation. In this investigation there was no significant differences in the level of electrolytes as result of other investigators also confirm the same result (Lohiya *et al.*, 1987; Lohiya and Tiwari, 1984).

In conclusion, sodium arsenite alone decreased testosterone and LH level significantly as compare to sham only group but no significant changes were observed in enzymes. The changes was more effective when sodium arsenite was administered to vasectomised rats and results showed significant decreased in testosterone, LH and ALP where AST was increased significantly. Thus, sodium arsenite and also imbalances in the testosterone, LH, ALP and AST would induce

metabolic and tissue damage. Therefore, this agent should be very cautiously handled, from the view point of its possible male reproductive toxicity specially in vasectomised man.

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