The Influence of Curcumin and Ascorbic Acid on Libido and Semen Characteristics of Arsenic-Exposed Rabbit Bucks

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Abstract: Arsenic (As) toxicity, the royal poison is a dangerous pollutant heavy metal. It has a harmful risk on all vital systems of the human, animals and plants. Its oxidative deleterious effect on the male reproductive system has been recorded. The use of natural medicinal plants and their extracts has their talented results in neutralizing the oxidative effects of As. Then the present study aimed to investigate the comparative effect of two natural extracts (ascorbic acid [AA] and curcumin [CMN]) on semen characteristics of the As-exposed rabbit bucks. Twenty-four apparently healthy, sexually mature and fertile New Zealand White (NZW) rabbit bucks were randomly divided into 4 equal groups (n=6). Control negative untreated, control positive (AsO3-exposed), ascorbic acid treated (As-AA) and curcumin treated (As-CMN) were the experimental groups included in the design. Semen was collected via artificial vagina and evaluated through ejaculate volume, motility, sperm concentrations, alive and abnormal sperm %, acrosome integrity and intact plasma membrane of sperm %. Libido was evaluated through the reaction time observation. As-exposed group showed significant decrease in libido, sperm count, mass motility, individual motility, live sperm %, intact acrosome % and intact plasma membrane with a significant increase in abnormal sperm %. As-AA and As-CMN groups took these values back towards normal through their antioxidant activity. Although, the rabbit bucks didn't recover their optimal semen characteristics in comparison to the control group. In conclusion, the AA and CMN have ameliorated the harmful effects of As on libido and semen characteristics of rabbit bucks.

Key words: Rabbit Bucks • Arsenic • Ascorbic Acid • Curcumin • Semen Characteristics

INTRODUCTION

Heavy metals are man made problem that had influenced negatively the health of all life beings. Since the arsenic exploration, the most famous royal toxicant all over the human history, it has induced a broad range of serious environmental problem worldwide [1, 2]. Oxidative stress (OS), one of arsenic-induced response, has influenced male reproductive function as an important factor in disruption of sperm function over 50 years ago. A balance called oxidative stress status normally exists between reactive oxygen species (ROS) production and antioxidant scavenging system in the male reproductive tract [3]. Small physiological levels of ROS are essential for the regulation of normal sperm functions [4]. However, production of excessive amounts of ROS in semen can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma resulting in OS. All spermatozoa cellular components including lipids, proteins, nucleic acids and sugars are potential targets for OS and endanger sperm motility, viability and function [5]. In biological systems, a diversity of antioxidant defense systems operate to control levels of ROS. Some antioxidants synthesized within the cells themselves (endogenous) and others need to be provided in the diet (exogenous). These ROS scavengers have an important protective action on the membrane integrity and lipid stability in both seminal plasma and spermatozoa [6]. Male infertility is reflected by low sperm count, low sperm motility, bad quality of sperms and DNA abnormalities [7]. Arsenic affects the mitochondrial enzymes [8], impairs the cellular respiration and causes cellular toxicity. It can also
substitute phosphate intermediates leading to slow down the rate of metabolism and interrupt the production of energy.

Antioxidant defense systems in vivo are mainly of three kinds [9]: Preventive antioxidants: suppress the formation of free radicals through non-radical decomposition of lipid hydroperoxides and H₂O₂ (such as CAT, GPX and GST), sequester metal ions, iron and copper ions in particular, by chelating (such as Transferrin, Ceruloplasmin, Albumin and Haptoglobin) and quench active O₂ (such as SOD and Carotenoids). Radical-scavenging antioxidants: also called interceptor or chain breaking antioxidant. They scavenge radicals to inhibit chain initiation and break chain propagation (such as Lipophilic: ubiquinol, vit A, vit E, carotenoids and Hydrophilic: uric acid, ascorbic acid, albumin, bilirubin). Repair and denovo enzymes: repair the damage and reconstitute membranes (such as DNA repair enzymes, Protease, Transferase and Lipase). Both Ascorbic acid and Curcumin exert their protective effect by scavenging free radicals and modulating antioxidant defense system [10]. Then, the aim of this study is to examine the comparative effect of ascorbic acid and curcumin on spermatozoa parameters in arsenic exposed rabbit bucks.

MATERIALS AND METHODS

Experimental Animals: Twenty-four apparently healthy, sexually mature and fertile New Zealand White (NZW) rabbit bucks were obtained from a known archived herd at a commercial farm, for the purpose of this study. Bucks aged 26-30 weeks and weighed 2.3-2.9 kg as initial weight. Bucks were individually housed in metal wire mesh cages provided with separate facilities for feeding and water supply. All rabbits were offered a commercial ration pellets (that contain 17.5% crude protein, 14.0% crude fiber, 2.7% crude fat and 2200 kcal/kg diet [11] that provides normal growth and maintains adult body weight. Fresh tap water was supplied ad libitum. All managements care was carefully served.

Chemicals: Arsenic Trioxide (As₂O₃) was purchased from Loba Chemie, VIT, LTD, India (M.W:197.84). Ascorbic Acid (C₆H₈O₆) was purchased from S.D. Fine Chem. Limited, India (M.W: 176.13). Curcumin (C₂₁H₂₀O₆) was purchased from Algomhoria Co. for Chemical Industry, Egypt (M.W: 368.38).

Experimental Design: The experiment was carried out at the Lab Animal House of the National Research Center, Dokki, Giza, Egypt. The experiment was carried out in winter to avoid the effect of heat stress and lasted for 8 weeks. Rabbits were left two weeks for acclimatization before treatment. Daily observation of the experimental animals was conducted in order to record any change in the clinical signs and to overcome it quickly. Rabbits were randomly divided into 4 equal groups of 6 bucks each as follow:-

Control Negative Group: was given distilled water orally and served as untreated animals.

Control Positive (As₂O₃-exposed) Group: was given 1.5 mg As₂O₃/kg BW dissolved in distilled water and given orally [12].

Ascorbic Acid Treated (As-AA) Group: was treated with As₂O₃ (mimic the control positive group) along with 40 mg/kg BW ascorbic acid orally [13].

Curcumin Treated (As-CMN) Group: treated with As₂O₃ (mimic the control positive group) along with 15 mg/kg BW curcumin orally [14].

Oral administration was applied via oral gavages at the base of tongue. The previous dosages were given daily along the experimental period. Control group received the same volume as distilled water.

Semen Samples and Parameters: Adult bucks were trained to serve an artificial vagina (IMV, France) during mounting of a teaser doe two weeks prior to the experimental period (during the acclimatization period). This preliminary period was chosen in order to assure that males were reproductively normal according to their libido and semen characteristics.

Semen was collected once weekly over a period of 8 weeks at 8.00 AM-10.00 AM from all males. Semen sample was used for the evaluation of reproductive efficiency, including:

Libido evaluation interpreted by the reaction time was measured in seconds.

The ejaculate volume (ml) was determined by a graduated tube that directly connected to the artificial vagina. Gel plugs were removed before the volume evaluation.

Spermatozoa concentration (10⁶/ml) was assayed using aqueous eosin solution [15]. Spermatozoa counting were done by a Thomas ruling double counting improved Neubauer hemocytometer slide (Hamburg, Germany).
Total sperm output [TSO] (10⁶/ejaculate) was calculated by multiplying semen ejaculate volume by spermatozoa concentration.

Mass motility was estimated immediately after semen collection by visual examination under low-power magnification (100x) using a warm plate light microscope. Mass motility grades ranged from 0 to 9 according to Petitjean [16] notation scale.

Individual progressive rectilinear motility (%) was assessed within 5 min. after collection using a 37 °C warm plate light microscope at 200x and 400x magnification.

Total motile sperm [TMS] (10⁶/ejaculate) was calculated by multiplying percentage of individual progressive rectilinear motility and total sperm output.

Abnormalities and Live sperm (%): Stained smear of the collected semen was prepared as soon after ejaculation using an eosin-nigrosine staining mixture at 1:4 dilution rate [17]. Two hundred spermatozoa per sample were examined for morphology and viability in stained smear at 1000x magnification (oil immersion).

Total functional sperm fraction [TFSF] (10⁶/ejaculate) was calculated as the product of multiplying total sperm output (10⁶) by individual motility (%) by normal morphology (%) [18].

Acrosome integrity %: In the present study, Giemsa was used to stain the acrosome dark purple. One hundred spermatozoa per sample were examined at 1000x for acrosome integrity in each stained smear [19].

**Functional Membrane Integrity %**: Hypo-osmotic swelling test (HOST %) was used to evaluate the functional integrity of the spermatozoa membrane for rabbit spermatozoa [20]. A total of 200 spermatozoa were counted in at least five different fields and examined for swollen coiled tail at 1000×magnification.

### Table 1: Effect of Ascorbic acid and Curcumin treatment on libido and semen characteristics for As₂O₃-exposed rabbit bucks

<table>
<thead>
<tr>
<th>Group</th>
<th>Reaction time (second)</th>
<th>Sperm concentration (10⁹/ml)</th>
<th>TSO (10⁶/ejaculate)</th>
<th>Sperm mass motility</th>
<th>Sperm individual motility (%)</th>
<th>TMS (10⁶/ejaculate)</th>
<th>Live sperm (%)</th>
<th>Abnormal sperm (%)</th>
<th>TFSF (10⁶/ejaculate)</th>
<th>Intact acrosome (%)</th>
<th>HOST (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.00±0.10</td>
<td>260.62±1.96</td>
<td>222.88±5.65</td>
<td>7.75±0.10</td>
<td>69.92±0.38</td>
<td>156.02±4.22</td>
<td>68.73±0.48</td>
<td>18.95±0.30</td>
<td>126.67±3.66</td>
<td>41.25±0.98</td>
<td>58.20±0.53</td>
</tr>
<tr>
<td>As₂O₃</td>
<td>10.46±0.11</td>
<td>200.73±1.29</td>
<td>158.62±4.37</td>
<td>6.71±0.12</td>
<td>66.90±0.38</td>
<td>106.80±3.67</td>
<td>64.85±0.67</td>
<td>21.06±0.42</td>
<td>84.65±3.14</td>
<td>31.17±0.85</td>
<td>43.27±0.71</td>
</tr>
<tr>
<td>As₂O₃ + AA</td>
<td>10.08±0.13</td>
<td>211.15±1.35</td>
<td>183.51±4.92</td>
<td>7.10±0.12</td>
<td>67.02±0.75</td>
<td>123.57±4.02</td>
<td>68.42±0.74</td>
<td>20.29±0.38</td>
<td>98.87±3.50</td>
<td>34.46±0.83</td>
<td>56.23±0.58</td>
</tr>
<tr>
<td>As₂O₃ + CMN</td>
<td>9.85±0.12</td>
<td>208.85±2.15</td>
<td>176.61±4.41</td>
<td>6.96±0.11</td>
<td>65.62±0.67</td>
<td>115.98±3.25</td>
<td>67.60±0.76</td>
<td>20.06±0.31</td>
<td>92.81±2.72</td>
<td>34.00±0.91</td>
<td>52.27±0.80</td>
</tr>
</tbody>
</table>

Means with different superscripts A, B, C, D, within rows are significantly different at p =0.05.

**Statistical Analysis**: All data were subjected to statistical analysis including the calculation of the mean (M), standard error of the mean (SE) and F-test (one way ANOVA) at a confidence limit of 95% (P<0.05). Statistical analyses were conducted according to the method of Armitage [21] using practicing statistical analysis program (SPSS, Edition 11). Duncan’s multiple range test was used for testing pairs of means for comparison at a probability of 5% [22, 23].

**RESULTS**

Data output in table (1) revealed that As₂O₃ significantly (P<0.004) delayed sexual eager of rabbit bucks represented in reaction time concurrent with significant decrease in sperm concentration, TSO, mass motility percent, individual progressive motility %, TMS, live sperm %, TFSF, acrosome integrity %, functional membrane integrity [HOST %] and significant increase in sperms’ abnormal morphology %. On the other hand, AA and CMN had attenuated the effect of As₂O₃ on the male reproductive system through preventing the sequel of harmful changes. They improved the reaction time, sperm concentration, TSO, sperm mass motility, TMS, live sperm %, TFSF, Intact acrosome %, HOST %, while, they decreased the percentage of abnormal sperm %.

**DISCUSSION**

Despite all the investigations done about As and their hazardous risks for human, animal and plant health, still the treatment against As toxicity opened new ways of trials. The present study was designed to investigate the influence of arsenic on reproductive performance of rabbit bucks and attempts to alleviate its harmful effect through
repairing their deleterious consequences on the male reproductive system using selected antioxidants natural extracts. Arsenic trioxide (As$_2$O$_3$) exposed rabbit bucks showed significant harmful changes in semen characteristics compared to the control. Sarkar et al. [24] and Jana et al. [25] reported that exposure to sodium arsenite led to pathological damage in the structure of testes and imbalance of sex hormones including testosterone, LH and FSH. Reduction in testosterone level suppressed spermatogenesis and inhibited testicular enzymes and functions. This affects libido and the majority of all semen characteristics assessed in the present study. Lebas et al. [26]; Sarkar et al. [24]; Mukherjee and Mukhopadhyay [27]; Pant et al. [7, 28]; Chang et al. [29] and Yousef [13] reported that As$_2$O$_3$ had affected deleteriously libido, sperm count, viability, motility, TMS and TFSF of male in different animal species. In addition, arsenic targets vicinal thiol groups in tubulin (the main component of axonemal microtubules) with high specificity. Since sperm flagellar motility is the result of specific interactions between axonemal microtubular proteins and the dynein motors, so the loss of sperm motility may be due to binding of arsenic to functional SH groups of the sperm membrane as well as to axonemal microtubules essential for flagellar motility [30]. Also, the decrease of intact acrosome percentage may be attributed to the high lipid peroxidation in epididymes as a result of elevated oxidative stress. This alters the stability of plasma membrane that surrounds the acrosome through the effect on its content of polyunsaturated fatty acids and lipoproteins [20, 31-33]. On the other hand, the spontaneous treatment with AA or CMN had attenuated the hazard risk of As on semen characteristics especially libido, sperm concentration and TSO, sperm mass motility, TMS, live sperm, TFSF, intact acrosome and functional membrane integrity of rabbit bucks. AA is a powerful antioxidant having ramified paths in the organism to manage several functions and neutralizing free radicals hazard. It works both inside and outside the cells to combat free radicals damages through offering an electron pair for free radicals to recover their stability such as hydroxyl and superoxide radicals and quench their reactivity [34]. In consent with its previous role, AA protects the DNA of the cell from damage caused by free radicals and mutagens [35]. CMN (commonly called diferuloyl methane) is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. Its chemical formulae is a bis-a, b-unsaturated b-diketone that exhibits keto-enol tautomerism [36]. The curcuminoids have been shown to be scavengers of free radicals and reactive oxygen species (ROS), such as hydroxyl radicals, superoxide radicals, singlet oxygen, peroxyl radicals and peroxynitrite, whose production is implicated in the induction of oxidative stress [37-40]. They efficiently neutralized the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and this reaction is often used in comparing the antioxidant activities of different compounds [38, 39].

It is concluded that the AA and CMN ameliorated the harmful effects of As on libido and semen characteristics of rabbit bucks.

**REFERENCES**


