EFFECT OF ALUMINIUM CHLORIDE ON TESTICULAR FUNCTION UNDER THE INFLUENCE OF MANASAMITRA VATAKAM AN INDIGENOUS DRUG FORMULATION

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Summary

The effect of aluminium chloride on testicular function of rats under the influence of Manasamitra Vatakam (MMV) an indigenous drug has been investigated. The aluminium chloride (AlCl₃) was administered orally at the dose of 100 mg/kg b.wt in experimental group for 90 days. MMV was given orally at the dose of 100 mg/kg b.wt for 30 days in 90 days AlCl₃ treated group. The testis of the AlCl₃ treated group, showed histological changes including severe damage within the seminiferous tubules and vascular degeneration on the spermatogenic and sertoli cells. A marked recovery was noticed in the MMV drug treated group as compared with AlCl₃ treated group, the results are reported in the paper.

Key word: Manasamitra vatakam, Aluminium Chloride

Introduction

Aluminium (Al) has for a long time been considered on an indifferent element from a toxicological point of view. However, it is unclear whether normal environmental levels of Al. Aluminium is known as a neurotoxin that can cause certain diseases such as Alzheimer disease, dialysis dementia, Parkinsonism, and amyotropic lateral sclerosis In addition to its neurotoxicity, Al affects other body structures like the skeletal system, brain tissue, bone, blood cells, liver and kidney. The sources of Al are specially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminium ware and containers. Also, Al is widely used in antacid drugs, as well as in food additives and toothpaste. Environmental pollution with the different aluminium containing compounds, specially those in industrial waste water, exposes people to higher than normal levels of Al.

Investigations in human and experimental animals have supported an association between Al exposure and male reproductive toxicity. High Al contents in human testes, Leydig cells, spermatozoa, seminal plasma, blood and urine, were associated with impaired sperm quality and viability. The suppressed spermatogonial cells, testosterone production, sexual behavior and fertility, and abnormal metabolism of certain trace elements were also observed in Al-treated animals. Furthermore, it has been shown that Al-induced testicular injuries, notably as a result of increased oxidative stress.
The toxic effects of aluminium chloride were reported earlier [4,5]. Chinoy, *et al.* [6] studied the effect of sodium fluoride together with aluminium chloride to male mice for 30 days and found some structural alteration in the testis with formation of giant cells. Krasovskii *et al.* [16] studied the biological effects of lead and aluminium on rats and guinea pigs and observed that the lead and aluminium chloride caused gonad toxicity. Memon *et al.* [18] reported the same toxicity on mice testis after the effect of sodium fluoride and/or aluminium chloride.

The aim of the present study was to investigate the cytoprotective effects of an ayurvedic herbo mineral preparation Manasamitra vatakam (MMV)

(i) A powerful antioxidant against the toxic effects of aluminium chloride on the testes of male Wistar albino rats and

(ii) to investigate the role of MMV as an antioxidant.

**Materials and Methods**

Rats were randomized and divided into four groups with six rats in each group. Group I served as control, group II was treated with aluminium chloride (AlCl₃): orally at the dose of 100 mg/kg b.w for 90 days, group III was treated with aluminium chloride for 90 days followed by 30 days treatment with MMV(100 mg/kg.b.wt). Group IV was given MMV alone for 90 days. All the oral treatment was in a constant volume of 1.0 ml/kg body weight. The doses of aluminium and MMV were calculated according to the animal’s body weight. At the end of the study the testis was cut into Small pieces about 1 mm. thick. The tissues immersed for 3 to 5 mins. In cold (4°C) phosphate buffer then fixed in 10% formalin then placed in fresh cold fixative for 24 hrs. in refrigerator.

**Biochemical analysis.** The tissues of testes of different groups were homogenized in ice-cold 100mM phosphate buffer (pH7.4). The levels of lipid peroxides (LPO) were measured in tissue homogenates as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described by Thayer [24]. GSH levels in tissue homogenates were measured employing 0.04% - 5.5% dithiobis-(2-nitrobenzoic acid) in 10% sodium citrate and recording at 410 nm as described by Dutta *et al.* [8].

**Table: 1 Effect of AlCl₃ treatment for 90 days on GSH and its reversal by MMV treatment in rat testes**

<table>
<thead>
<tr>
<th>Name of the organs</th>
<th>Control (nmols/mg protein)</th>
<th>AlCl₃ (nmols/mg protein)</th>
<th>AlCl₃ +Drug (nmols/mg protein)</th>
<th>Drug (nmols/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis (GSH)</td>
<td>5.96 ± 0.25</td>
<td>2.58 ± 0.19 b$</td>
<td>5.35 ± 0.21 c@</td>
<td>5.94 ± 0.19 a**</td>
</tr>
</tbody>
</table>

n=6; Values are expressed as mean ± S.E.M followed by One Way ANOVA –Turkey’s multiple comparison test. Comparison were between

a – Group I vs. IV, b – Group I vs. group II, c – Group II vs. III

Symbols represent statistical significance, @ - P<0.001, $ - P<0.01, # - P<0.05, ns – Non significance
Table: 2 Effect of AlCl₃ treatment for 90 days on LPO and its reversal by MMV treatment in rat testes

<table>
<thead>
<tr>
<th>Name of the organs</th>
<th>Control (µmols/mg protein)</th>
<th>AlCl₃ (µmols/mg protein)</th>
<th>AlCl₃ +Drug (µmols/mg protein)</th>
<th>Drug (µmols/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>82.96 ± 9.64</td>
<td>128.5 ± 6.08</td>
<td>59.51 ± 3.86</td>
<td>64.27 ± 5.49</td>
</tr>
</tbody>
</table>

n=6; Values are expressed as mean ± S.E.M followed by One Way ANOVA –Turkey’s multiple comparison test. Comparison were between

a – Group I vs. IV, b – Group I vs. group II, c – Group II vs. III

Symbols represent statistical significance @ - P<0.001, $ - P<0.01,

Fig: 1 Effect of AlCl₃ treatment for 90 days on GSH and its reversal by MMV treatment in rat testes

Fig: 2 Effect of AlCl₃ treatment for 90 days on LPO and its reversal by MMV treatment in rat testes
Results and Discussions

Biochemical results.
The level of GSH in the tissue homogenate of testes was significantly declined in AlCl₃ group comparing with controls. In the AlCl₃ + MMV (group III), the level of GSH was significantly elevated in comparison with AlCl₃ treated group II (Table 1 and Fig. 1).
The level of LPO in the tissues homogenates of testes was significantly higher in AlCl₃ group than control group. In the AlCl₃ + MMV (group III), the level of LPO in the studied tissue was significantly reduced comparing to AlCl₃ treated group II (Table 2 and Fig. 2).

Histopathological results.

Control group:
The control rats had normal structure and completely enveloped by a thick capsule, tunica albuginea, which is composed mainly of dense collagenous fibrous connective tissue. The structural components of the testis are the seminiferous tubules, interstitial tissues and the spermatogenic cells. These cells are many layers, namely, the spermatogonia, primary and secondary spermatocytes; spermatoids and finally mature spermatozoa. (Fig. 3).

Aluminium induced Testicular toxicity:
After rats treated with high dose (100 mg/kg body weight) of aluminum chloride for 90 days showed more exaggerated features of focal areas of spermatogenesis, arrest at the spermatid level, in the form of degenerative changes in the germinal cells together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane (Figs. 4).

Discussion

The results of this study observed that the oral administration of aluminum chloride in wistar rats, caused testis toxic as indicated by histological changes in the seminiferous tubules of that testis and it was recovered with MMV treatment. Stajn et al [22] and Patra et al [20] reported different dose of Cadmium increase organ lipid peroxidation (LPO) including many organ including male sex organs and brought about changes in the antioxidant defence system. This report supported to our work, the testes of male albino rats intoxicated with AlCl₃ for 90 days alone showed more exaggerated features of focal areas of spermatogenesis, arrest at the spermatid level, in the form of degenerative changes in the germinal cells together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane damage of testicular tubules and spermatogenesis.

AlCl₃+ Drug:
The group III animals treated with AlCl₃+ MMV showed mid degenerative changes, mild edema and congestion (Fig: 5). The group IV animals were treated with MMV alone for 90 days, the testes of animals were showed normal architecture of the testes. No detectable histological alterations showed in the testes of rats treated with MMV (Fig:6).
The Kamboji & Kar [15] reported that, the seminiferous tubules are shrinkage and spermatogenic arrest at the primary spermatocytes or spermatogonial stage when treated mice with daily
subcutaneous injection of 27.4 mg/kg of aluminium sulphate for 30 days. Furthermore, \cite{2,16,3} have reported that, short term aluminium chloride exposures to rats and guinea pigs caused gonadal toxicity whereas in chronic exposure, the sperm density and motility were affected in agreement with the work of Chinoy, \textit{et al.} \cite{6}, who showed that the administration of sodium fluoride (NaF, 10 mg/kg. body weight) together with aluminium chloride (AlCl$_2$, 200 mg/kg body weight) to mice for 30 days, caused degenerative in structure of spermatogensis and formation of giant cells. These resulted agree with our results obtained after the treatment with aluminium chloride. The same results were recorded by, Libet \textit{et al.} \cite{18}, after the effect of aluminium nitrite on mice and found that the spermatocytes and spermatids are necrosis. Guo, \textit{et al.} \cite{10} have reported that aluminium caused suggested that ACE activity had a role in oxidative damage of Al-induced testicular toxicity in mice, after intraperitoneally exposed to 0.13 or 35 mg. Al./kg. Body weight for a period of 14 days. Mayyas, \textit{et al.} \cite{17}, reported the same resulted after the mice treatment with aluminium chloride, and found that destruction of the seminiferous tubules with large necrotic areas and degenerative cells.

The present study has established the ameliorating effect of MMV in the testicular degeneration caused by chronic AlCl$_3$ treatment. The therapeutic efficacy of MMV may be attributed to its antioxidant activity against free radicals. The anti peroxidative activity of MMV may also be accounted for its protective activity against testicular degeneration.
References