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Original Article

Potential Role of Ashwagandha [Withania Somnifera] As An Antioxidant On Aluminium Chloride-Induced Testicular Damage In Wistar Rats

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ABSTRACT
Introduction: Aluminium is most prevalent and third most abundant element in earth crust after oxygen. Its exposure in high concentration can accumulate in many organs that damage various organs in living organisms like humans and animals. Although aluminum (Al) is known to induce oxidative damage through various mechanisms including binding to negatively charged phospholipids on the membrane of various tissue cell which are rich in PUFA.Hence,this study was aimed to find the potential role of ashwagandha on aluminum induced testicular toxicity. **Methods:** Animals were segregated into 4 groups of 6 rats in each. The control group, the Ashwagandha treated group, the Aluminum intoxicated group, pretreated with Ashwagandha with Aluminum intoxicity group. Testicular tissue was removed and were stored in 10% formalin saline and histopathological slides were done . A part of the tissues were processed for estimation of MDA and GSH level. **Results:** In the present study administration of aluminum in rats showed a significant decrease in the testicular tissue level of GSH and sperm count, as well as increase in the level of MDA and sperm morphology in aluminum treated group compared to normal control. Treatment with Ashwagandha showed a significant increase in testicular GSH level, sperm count and decrease in MDA level sperm morphology. **Conclusion:** The results of this study revealed that oral Aluminum Chloride administration induced adverse oxidative effects on the exposed animals and treatment with *W. somnifera* reduced the extent of aluminium chloride-induced tissue injury
KEYWORDS: Reactive Oxygen Species, Reduced glutathione, Malondialdehyde, Sperm morphology

INTRODUCTION

Aluminum is an abundant and prevalent metal, element in the earth's crust Aluminum exposure in high concentration can bring about its accumulation in many organs that damages various organs in the living organisms like humans and animals. Some of the toxic effects of Aluminum include neurotoxicity[1,2,3] hematotoxicity[4], hepatotoxicity[5,6], nephrotoxicity[7] and reproductive toxicity[8,9,10]. Oxidative stress is an imbalance between aggressive and defensive system[11,12]. Although aluminum (Al) is known to induce oxidative damage through various mechanisms in the body. Few of them are that Aluminum can bind to negatively charged phospholipids on the membrane of various tissue cell which are rich in PUFA that are targeted by reactive oxygen species (ROS) such as O_2, H_2O_2, OH, and OH[13]. Withania somnifera also called as ashwagandha is an evergreen shrub belonging to Solanaceae family and grows abundantly in India. Winter cherry, Indian ginseng, and Ajagandha, Queen of Ayurveda, called by so many names, Withania somnifera Dunal (Ashwagandha; Family-Solanaceae), is a plant used in ayurvedic medicine in the traditional system[14]. Fresh root of the Ashwagandha herb 'smells like horse' so-called Ashwagandha, because there is the belief that anyone who consumes the herb is given the power and strength of a horse[15,16]. Alkaloids, steroidal lactones, saponins, and withanolides sitoindosides VII-X and *withaferin A are the biological active component of Ashwagangha*[17]. These are known to have antioxidative activities and proved by the activation of antioxidant enzymes superoxide dismutase and GSH, the increase in vitamin C, and decrease in lipid peroxidation. Therefore, this study was aimed to find the potential role of ashwagandha on aluminum-induced testicular toxicity in rats. Hence the present study was conducted to evaluate the damaging consequences of aluminum Chloride on testes and to explore the antioxidant and therapeutic role of Ashwagandha [*Withania Somnifera*] on Aluminum Chloride induced testicular damage

MATERIALS AND METHODS

The Institutional ethics committee approval was secured before starting the experiment (KMC/MNG/IAEC/14-2023). Healthy adult male wistar rats weighing between 150 and 200 grams was taken for this study from our institution's central animal house. They were kept in the laboratory's central animal housing, which had regulated lighting and temperature as well as regular rat food and water. Aluminium chloride was from Sigma company and Ashwagandha was purchased from Saptam veda company.

Experimental design and animal grouping

Animals were segregated into the following groups of 6 rats in each group. Twenty-four rats were randomly allocated into four groups (6 rats each) as followed:

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Group I: Normal rats were given water (2 ml/kg bw) via oral gavage for 3 week and acted as the negative control.

Group II: Rats were Ashwagandha (60mg/kg bw dissolved in distilled water) via oral gavage for 3 weeks and acted as the positive control.

Group III: Rats were treated with aluminium (100mg/kg bw dissolved in distilled water) via oral gavage for 3 weeks

Group IV: Rats treated with Ashwagandha (60 mg/kg bw) & aluminium (100mg/kg bw) via oral gavage for 3 weeks.

After the end of the protocol, animals were sacrificed by giving Pentabarbitone (40mg/kg bw). The laparotomy was performed, and the reproductive organs were removed. Testis were removed and were stored in 10% formalin saline and histopathological slides were done. A part of all the tissues were processed for estimation of MDA[18] and GSH[19] level. The tissues were treated to prepare paraffin blocks were as per standard protocols[20]. The epididymis processed for sperm count and sperm morphology[20]. Finally, a drop of Canada balsam in xylol was placed on the slide and covered with a cover slip. Slides were coded for analysis.

Statistical analysis: The data was presented as mean ± SD. The unpaired t-test was used for data analysis using statistical package SPSS version 17.0. P<0.05 was taken as statistically significant.

RESULTS

In the present study administration of aluminum in rats showed a significant(P<0.0001) (Table 1) decrease in the testicular tissue level of GSH when compared to normal control group. There was also a significant (P<0.05) increase in the testicular tissue level of MDA in aluminum treated group compared to normal control (Table 2). Treatment with Ashwagandha showed a significant increase in testicular GSH level (Graph 1) and decrease in MDA level (Graph 2) compared to aluminum intoxicated rats (Group.III). Sperm count (P<0.001) was significantly low in the aluminum treated group compared to normal control group. Treatment with Ashwagandha showed a significant increase(P<0.001) in the sperm count compared to aluminum intoxicated rats In the present study rats intoxicated with aluminum (Group III) showed significant increase in total sperm shape abnormality compared to control groups (P<0.001).But a significant decrease in sperm shape abnormality was observed in rats treated with Ashwagandha.

Table 1. Effect of Ashwagandha treatment on sperm count in aluminum intoxicated rats.

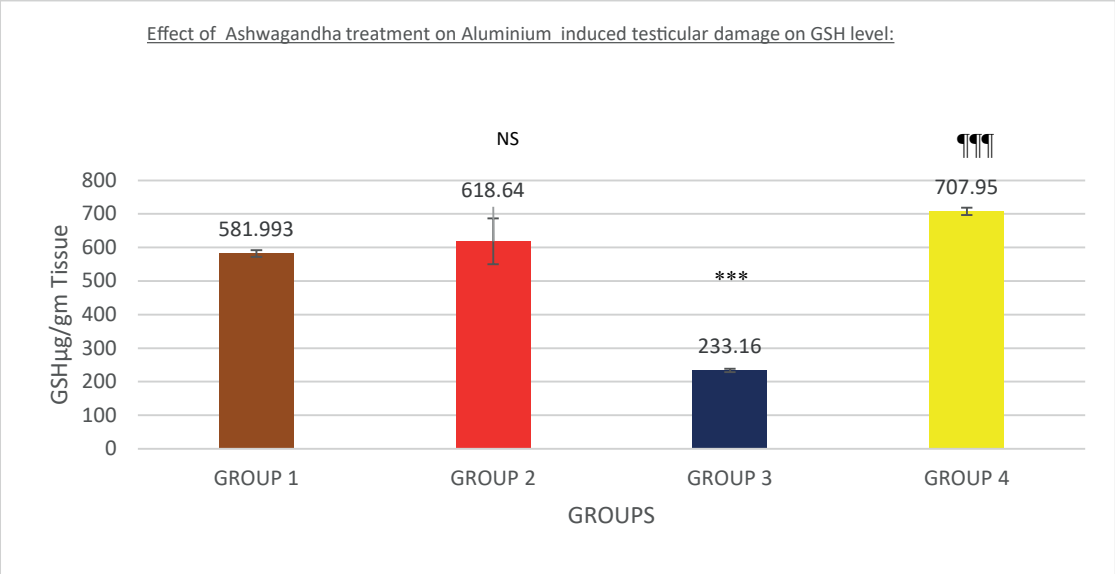
GROUPS	SPERM COUNT
GR.I	824.24±3.14
GR.II	804.24± 9.14^{NS}
Gr.III	516.74± 7.43***
Gr.IV	614.20± 2.78¶¶

Values are express as Mean±SEM. Number of animals (n)=6. P <0.05 is taken as significant,NS(not significant) GR.I versus Gr.II . ***P<0.0001, GR.I versus GR.III¶¶P<0.001, GR.III versus Gr.IV.

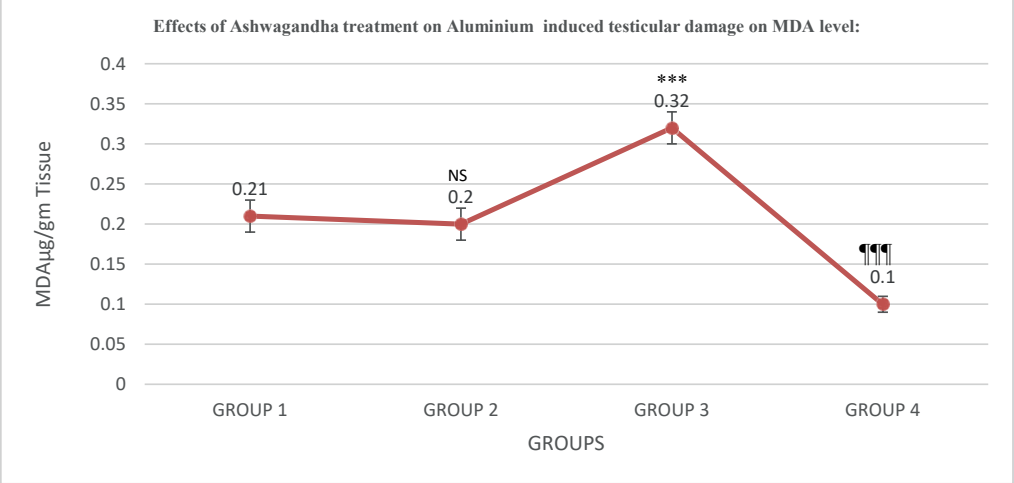
Table 2. Effect of Ashwagandha treatment on sperm morphology in aluminum intoxicated rats.

Groups	Normal	HA	TA	MC	CC	Total
Group I	187±3.22	9.83±0.75	8.83±0.75	0.5±1.22	5±0.40	19.06±2.82
Group II	184.26±3.48	9.83±0.75	9.33±1.03	0.83±1.16	1.1±1.09	19.33±3.46
Group III	134.4±1.33***	34.5±1.51	24.83±1.83	4.16±0.75	3.14±0.63	54.22±2.34***
Group IV	149.17±1.82¶¶¶	26.16±1.16	17.83±0.75	1.83±0.40	3.02±0.63	39.77±1.72¶¶¶

HA – Head abnormality, TA – Tail abnormality, MC – Microcephaly ,CC – Cephalocaudal junction
Mean ± SEM, n=6 in each group.***P<0.0001 , GR.I Versus Gr.III, ¶¶¶P<0.0001 Gr.III versus Gr.IV



Graph 1: Effect of Ashwagandha treatment on testicular tissue level on GSH in aluminum treated rats.Values are express as Mean±SD. Number of animal (n)=6. P <0.05 is taken as significant. ,NS (not significant) GR.I versus Gr.II ***P<0.0001, GR.I versus GR.III,¶¶¶P<0.0001, GR.III versus Gr.IV



Graph 2. Effect of Ashwagandha treatment on testicular tissue level on MDA in aluminum treated rats.Values are express as Mean±SD. Number of animal (n)=6. P <0.05 is taken as significant. NS (not significant) GR.I versus Gr.II ***P<0.0001, GR.I versus GR.III,¶¶¶P<0.0001, GR.III versus Gr.IV

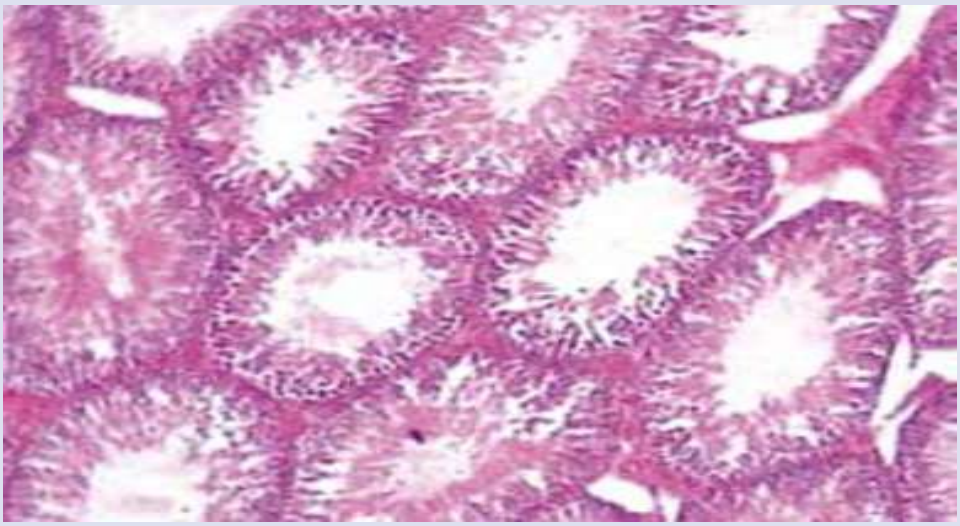


Figure 1. Photomicrograph of rat testis shows normal histological features in normal control group (Group I) (Hematoxylin & eosin).

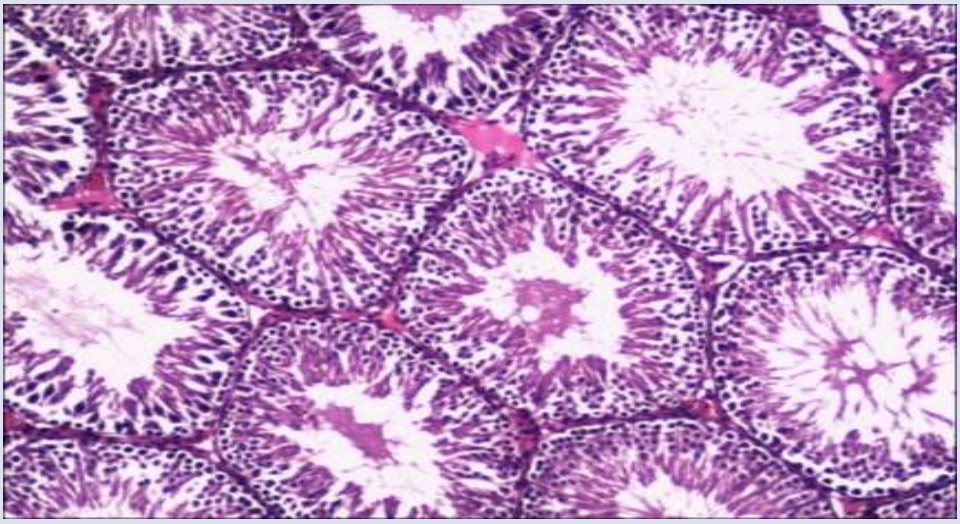


Figure 2. (Group II) (Haematoxylin & eosin). Appearance of normal spermatogenic cells from the basement membrane.The Leydig cells and Sertoli cell is seen to be normal . Normal spermatogenesis is seen.

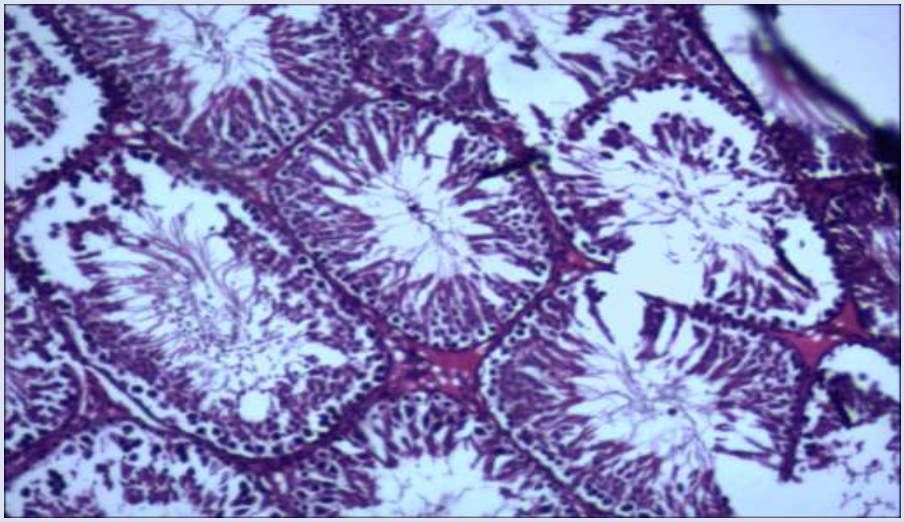


Figure 3. Photomicrograph of rat testis shows sloughing of cells and Leydig cells necrosis, destruction of Sertoli cells.(Group-III)

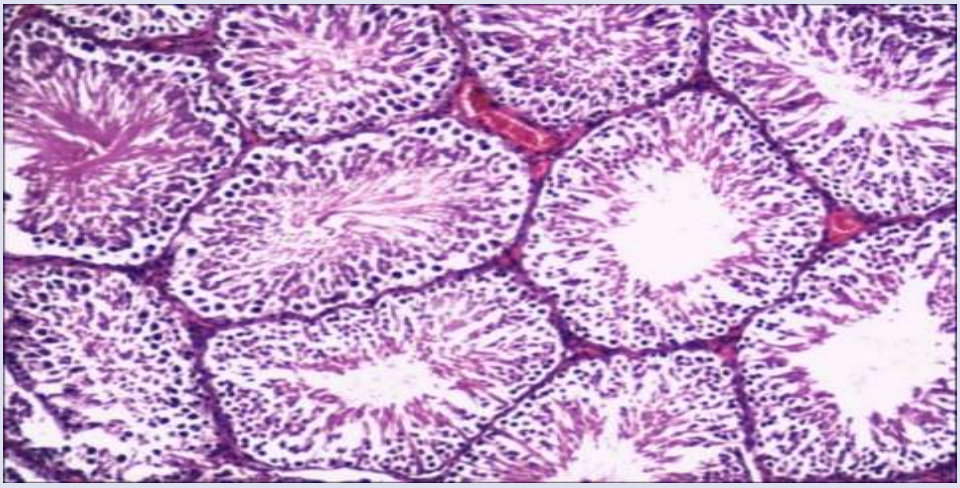


Figure 4. Photomicrograph of rat testis shows resorted spermatogenic cells and matured spermatogenic cells .(Group-IV)

DISCUSSION

Numerous studies have established the neurotoxic nature of Aluminium Chloride[21,22,23]. Eventhough it is considered as a metal with relatively low redox activity, it acts as a causative agent of oxidative stress by various mechanisms. Several clinical investigations suggested that Al could be a factor in the development of neurological illness, including Alzheimer's disease, Parkinson's Disease, and Multiple Sclerosis[24]. The current study unveils aluminum induced oxidative toxicity in testicular tissue of the rats. The toxicity induced by Aluminum Chloride in a dosage of 100mg/kg/bw/day has shown a significant reduction in GSH in testicular tissue. The lower production of GSH following Aluminum exposure might be caused by a reduction in the activation of enzyme the Glutathione Synthetase that might have attributed to lack of elimination of generated ROS[25]. On the other side, MDA level in testicular tissue is increased in Aluminum intoxicated group, which is a well-known LPO indicator. Previous studies have proven that this is associated with reduced SOD activity, thus inhibiting the protection of spermatozoa against elevated O2 toxicity and LPO. Thus AlCl2 acts as an inhibitor of SOD and thereby increases MDA[24,25]. This result has been reflected in the sperm count, sperm morphology, and histological examination. The significant reduction in sperm count and sperm morphology following Al exposure in this study reassures the involvement of Al in oxidative stress and reinforces the role of ROS. Previous studies have documented that declined sperm count and abnormal sperm morphology, can occur as a consequence of decreased cAMP and increased NO production by Aluminum exposure[26]. The testicular examination in the present study has shown visible sloughing of spermatogenic cells and Leydig cell necrosis, which contributes to abnormal spermatogenesis following Al exposure. The results of our study reported that the pretreatment of ashwagandha extract prevented the oxidative stress induced by aluminum. This was indicated by the ability of ashwagandha root extract to cause significant testicular elevation in GSH level. Ashwagandha induced increase in increased GSH level indicates the antioxidant action of *Wthania Somnifera*, and can act as protective agent against cellular damage induced by free radicals. Present study has also shown that ashwaghandha plant extract has also reduced the MDA,which contribute a major proportion of ROS in cells. Thus reduction in ROS indicates the antioxidant action of *Withania Sominifera* in testicular tissue. Results of previous research conducted showed that the maintenance of reduced ROS promotes cell survival in normal cells, while in cancer cells, ROS levels are considerably increased, leading to the accumulation of mutations, aberrant proliferation and an aggressive metastatic phenotype[26].

It has been noticed in current study that action of WS in treatment group has also caused a remarkable increase in sperm count which was declined significantly by Al intoxicated group. Sperm count has been increased significantly along with an appreciable healing in sperm morphology when compared to Al intoxicated group. This finding is validated by the histological examinations in the present study, which showed restoration of spermatogenic cells which was sloughed by the action of Aluminium. Strong anti-apoptotic and antioxidant qualities of WS treatment may be responsible for these preventive benefits[27](Shown in Figure 1,2,3,4).

CONCLUSION

In conclusion, the results of this study revealed that oral Aluminium Chloride administration induced adverse oxidative effects on the exposed animals as evidenced by the recorded abnormalities in the investigated biochemical parameters. In the present study, treatment with root extract of *W. somnifera* reduced the extent of aluminium chloride-induced tissue injury by maintaining the activities of antioxidant enzymes like SOD and GSH and reducing lipid peroxidation indicating that *W. somnifera* has a beneficial role in ameliorating oxidative damages induced by aluminum.

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