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AMELIORATIVE EFFECTS OF MGSO4 ON DEXAMETHASONE- INDUCED HISTO-CHEMICAL ALTERATIONS IN THE TESTES OF ALBINO RATS

INAYATULLAH[1], MOHAMMAD KHAN[2], MARIYA HIDAYAT[3], LUTFUR-RAHMAN[4], ASMA SIDDIQUI[5], YASMEEN MAHAR[5]

1. Department of Anatomy, Gajju Khan Medical College, Swabi.
2. Department of Anatomy, Saidu Medical College, Swat.
3. Department of Anatomy, Rahbar Medical and Dental College, Lahore.
4. M.Phil student of Biotechnology, Quaide-I-azam University, Islamabad.
5. Department of Anatomy, Bahria University Medical & Dental College, Karachi.

ABSTRACT

BACKGROUND: In experimental animal, dexamethasone-induced impaired spermatogenesis causes disruption of the normal architecture of seminiferous tubules and alteration in male sexual hormone testosterone. Concomitant administration of MgSO4 preserved the cytoarchitecture of testes as well as hormonal regulation in albino rats.

OBJECTIVE: This study was designed to observe the ameliorative effects of MgSO4 on the histology of testes and there correlation with serum testosterone level during dexamethasone administration in albino rats.

METHODOLOGY: This experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi from 5th April to 25th April 2012. Thirty healthy male adult albino rats were included in the study and divided equally into 3 groups. Each group comprising 10 animals. Group-A served as Control. Group-B received Dexamethasone (intraperitonealy) at the dose of 4mg/kg body weight/24 hours. Group-C received Dexamethasone at the same dose as in group-B and additionally given MgSO4 (intramuscularly) at the dose of 20mg/kg/24hours.

RESULTS: MgSO4 significantly preserved the cytoarchitecture of testes, as well as minimized alteration in serum testosterone level in group-C animals.

CONCLUSION: $MgSO^4$ has restored both the histological and biochemical damaging effects induced by dexamethasone in Rats testes.

KEYWORDS: Dexamethasone, MgSO4, Testicular tissue, Testosterone.

INTRODUCTION

Spermatogenesis is a complex process that involves an array of cellular and biochemical events, collectively culminating in the formation of haploid spermatozoa, from diploid precursor cells known as spermatogonia[1], which is precisely regulated by balance between continuous germ cells proliferation and apoptosis[2,3].When the testicular environment cannot support spermatogenesis, specific pathways are accelerated leading to an imbalance between proliferation and programmed cell death, that impairs spermatogenesis[4,5].

In therapeutic concentration Glucocorticoids (GCs) are strong immunosuppressant, anti-inflammatory and anti-allergic, that have made them one of the most frequently prescribed drug worldwide[6]. Dexamethasone (Dexa) a synthetic GC which is thirty times more potent than cortisol has made it an especially important drug for stimulating specific glucocorticoid activity[7]. Experimental studies have shown that excess GCs reduces serum testosterone level[8], impairs

luteinizing hormone signal transduction and steroidogenesis in leydig cells of rats[9,10], and also suppress the activity of hypothalamic-pituitary-gonadal (HPG) axis[11].

Minerals are required for the normal growth and maintenance of the body. Magnesium (Mg) is an essential cofactor and second abundant intracellular cation after potassium, that activates more than 300 enzyme systems in the body, involved in all phosphorylation processes, necessary for the maintenance of an adequate supply of nucleotides for the synthesis of RNA&DNA[12,13]. It has been shown that Mg controls the activity of Hypothalamic–pituitary-Adrenal Axis (HPA-axis), which is considered to be the main stress response system[14], effectively reduces testicular malondialdehyde (MDA) and increases the activities of superoxide dismutase and glutathione peroxidase[15]. Mg supplementation hasincreased free and total testosterone levels[16]. Significant increase in the activities of testicular Δ 5 3β - hydroxysteroid dehydrogenase and 17β - hydroxysteroid dehydrogenase enzymes and serum testosterone level along with progressive development in the histoarchitecture of genital organs has been observed with the use of Mg [17].

MATERIAL AND METHOD

This experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. Thirty young male albino rats about 90-120 days of age were obtained from the animal house of BMSI, JPMC, Karachi and kept under observation for one week, prior to the commencement of the study for assessment of their health status. The standard laboratory chow and tap water were available at libitum.
Animals were divided into three groups A, B, and C. each group comprised of 10 animals.

- Group–A animals served as control.
- Group–B animals were administered Dexamethasone (OBS pharma Pak) at the dose of 4mg/kg body weight per day intraperitoneally[18].
- Group–C animals were given magnesium sulphate (ZafaPharma Pak) at the dose of 20mg/kg body weight per day intramuscularly [15], with Dexamethasone at the dose as mentioned in group-B.

EXPERIMENTAL PROCEDURE

At the end of experimental period, all the animals were sacrificed under deep ether anesthesia. A mid line incision was made, extended downward up to the scrotum and upwards to the thoracic region. Blood samples were taken from each animal by intra-cardiac puncture with the help of disposable syringe, for the detection of serum testosterone levels on Elisa kit.

The results were based on estimation of serum testosterone, and there correlation with histological findings.

TISSUE TREATMENT

Both the testes were excised and fixed in Bouin's fluid for 24 hours. After 24 hours each testis was cut longitudinally into two equal halves and again post fixed in fresh Bouin's fluid for next 24 hours. After fixation, tissues were processed in ascending grades of alcohol, infiltrated and embedded in paraffin. 5µm thick longitudinal sections were cut on microtome and stained with PAS-Iron Heamatoxylin Technique and observed under light Microscope[19].

RESULTS

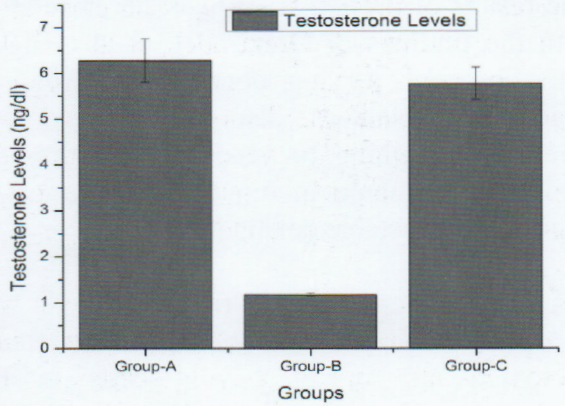
Biochemical parameter (Testosterone)

The mean values of serum testosterone (ng/dl) in control group-A were 6.28 ± 0.47, while the mean values of testosterone (ng/dl) in group-B were 1.17±0.04. There was significant decrease ($P<0.05$) in the mean serum testosterone level in group-B, when compared with control (Graph-1).

The mean values of testosterone (ng/dl) in group-C were 5.77±0.35. There was insignificant decrease ($P>0.05$) in the mean serum testosterone level in group-C,when

compared with control group. The data also showed significant increase ($P<0.05$) in mean serum testosterone level in group-C, when compared with group-B (Graph-1).

Figure 1: Graph shows Control group (A), Dexamethasone treated Group (B), Magnesium sulphate and dexamethasone treated group (C).



HISTOLOGICAL PARAMETER

Periodic Acid Schiff (PAS)-Iron Haematoxylin stained 5µm thick sections in group-A revealed that, the parenchyma of testes are formed of round seminiferous tubules and narrow Lumina. The tubules are regularly arranged and lined by stratified germinal epithelium with different types of spermatogenic cells and intact basement membrane. The tubules are separated from each other by interstitial spaces, containing Leydig cells and blood vessels, Sertoli cells are interposed between the developing spermatogenic cells (Figure-2).

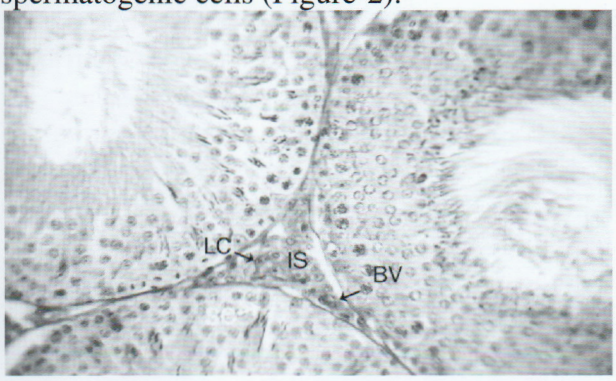


Figure-2 (Group A):PAS-iron Heamatoxylin stained, 5µm thick section of testis of control albino rat, showing Germinal epithelium, Interstitial space (IS), Leydig cells (LC), and blood vessel (BV).

Histological details of group-B, showed, distorted and disorganized germinal epithelium. Marked vacuoles and the lumen is full of slough materials with no visible spermatozoa. The leydig cells are scanty and difficult to differentiate due to their darkly stained basophilic appearance. The interstitial spaces are markedly widened.Sertoli cells are not detected and most of the nuclei are pyknotic (Figure-3)

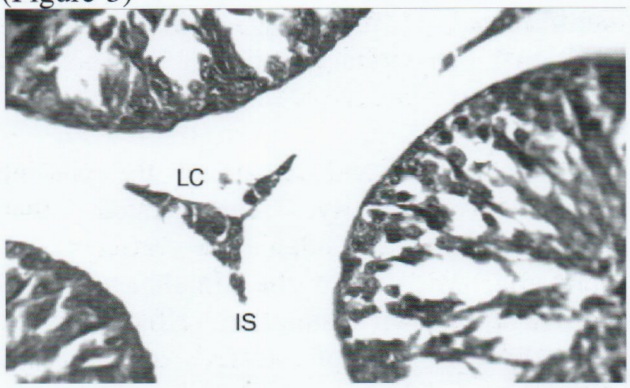


Figure-3 (Group B)PAS-iron Heamatoxylin stained, 5µm thick section of testis of Dexamethasone treated albino rat, showing extensive vacoulation, widened interstitial space (IS), scanty Leydig cells (LC).

In group-C, Organized developing germ cell series, with some vacuoles are observed. Slightly detached basement membrane and slough seen in the lumen. The feature showed marked improvement as compared to dexamethasone treated sections. Leydig cells are comparatively less in number as compared to control group, but highly significant improvement is observed when compared to group-B (Figure-4).

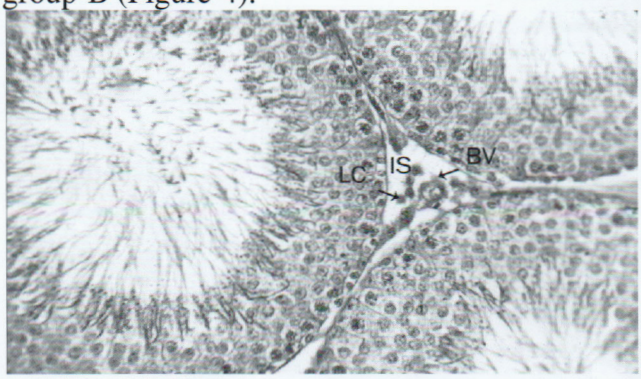


Figure-4 (Group C):PAS-iron Heamatoxylin stained, 5µm thick section of dexamethasone with magnesium sulphate treated albino rat, showing all series of germ cells, Leydig cells(LC), Interstitial space(IS)&Blood vessels(BV).

DISCUSSION

The causes of impaired spermatogenesis are multifactorial, including environmental, nutritional, behavioral, genetic and hormonal, as well as drugs[20,21,22].

The observations and results of the present study have clearly demonstrated that dexamethasone has damaging effects on spermatogenesis, while the simultaneous use $MgSO_4$ has shown restorative effects on the cytoarchitecture of testes and serum testosterone level.

The results of present study indicate that dexamethasone treated animals showed decrease in serum testosterone level, which is due to the suppression of hypothalamic-pituitary-adrenal and gonadal axis (HPA&HPG-axis) and the direct effect of Dexa on Leydig cells via glucocorticoid receptors. Guilliams& Edwards (2010)[23] stated that, suppression of axis is variable among patients and depends on the dose and duration of the drug. The findings of our study are in conformity with the study done by Maeda&Tsukamura (2006)[11], who reported that, acute or chronic administration of glucocorticoid had suppressed the activity of hypothalamic-pituitary-adrenal and gonadal axis.

The mean level of serum testosterone was high in group–C animals as compared to dexamethasone treated group. That might be due to the effect of magnesium sulphate on modulation of HPA-axis, as suggested by Sartori et al (2011)[14], that magnesium deficiency induces anxiety and HPA-axis dysregulation.

The reason for impaired spermatogenesis by dexamethasone include, direct inhibition of germinal epithelium via glucocorticoid receptors or indirectly by influencing the axis between hypothalamic-pituitary and gonads as suggested by Ge (2005)[10] and Abeyagunawardena et al (2007)[24].

The results of present study were in correlation with the findings of Orazizadeh et al (2010)[18] who observed varying degrees of germ cell degenerative changes, disorganized germ cell layers & sloughing to vacoulation within the seminiferous tubule jn mouse testicular tissue exposed to injectable dexamethasone.

GCs induced apoptosis in rat leydig cells was observed by GAO et al (2002)[25] and found decreased number of Leydig cells in the interstitium. This was due to the direct stress effect of dexamethasone via GC-receptor mediated process and indirectly by the inhibition of LH signal transduction.

Reason for preserving the histoarchitecture of seminiferous tubules is due to the role of magnesium sulphate in the regulation of HPA-axis, and elevated level of testosterone as suggested by Cinar et al (2012)[16].

Chandra- Amar et al (2012)[17], have given magnesium sulphate with standard diet at diverse doses for one and two consecutive spermatogenic cycles. He found significant increase in the activities of testicular 3β-hydroxysteroid & 17β-hydroxysteroid dehydrogenase enzymes and serum testosterone level along with the progressive development in histoarchitecture of genital organs.

CONCLUSION

Based on the results it is concluded that damaging effects of dexamethasone on rat testes can be minimized by the concomitant use of magnesium sulphate.The present study may act as a baseline for extension in humans.

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CORRESPONDENCE ADDRESS

Name: Dr. Inayatullah
Address: Assistant Professor of Anatomy,
Gajju Khan Medical College Swabi.
Email: drinayatswati@yahoo.com
Cell No: 03005746113.