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Ameliorative effects of $MgSO_4$ on Dexamethasone - induced Histo-chemical alterations in the testes of Albino Rats

Inayatullah[1], Mohammad khan[2], Mariya Hidayat[3], Lutfur-Rahman[4]

[1]*Assistant Professor, Department of Anatomy, Gajju khan Medical College, Swabi.*

[2]*Professor, Department of Anatomy, Saidu Medical College Swat.*

[3]*Assistant Professor, Department of Anatomy, Rahbar Medical and Dental College, Lahore.*

[4]*M. Phil Student, Department of Biotechnology, Quaid-I-Azam University Islamabad.*

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ABSTRACT

Background: In experimental animal, Dexamethasone-induced impaired spermatogenesis, causes distortion of the normal architecture of seminiferous tubules along with alteration in male sexual hormone, testosterone. Concomitant administration of $MgSO_4$ preserved the cytoarchitecture of testes as well as hormonal regulation in albino rats. Objective: This study was performed to observe the ameliorative effects of $MgSO_4$ on the histology of testes and correlation with serum testosterone level during dexamethasone administration. Duration of study: twenty days (April 2012).Study design: Prospective experimental study.Place of study: Anatomy department Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. **Methods:** thirty healthy adult albino rats were included in this study and divided equally into three groups. Group-A served as control. Group-B received Dexamethasone (intraperitoneally) at the dose of 4.0 mg/kg body weight/24 hours. Group-C received Dexamethasone in the same dose as given in group-B and additionally received $MgSO_4$ (intramuscularly) at the dose of 20mg/kg/24hours. **Results:** $MgSO_4$ significantly preserved cytoarchitecture of the testes as well as minimized alteration in serum Testosterone level in group-C. **Conclusion:** This study showed that administration of $MgSO_4$ has ameliorated both the histological and biochemical damaging effects induced by dexamethasone in rats' testes.

Keywords: *Dexamethasone, $MgSO_4$, Testicular tissue and testosterone.*

INTRODUCTION

Starting from a self-renewing stem cell pool, male germ cells develop in the seminiferous tubules throughout life from puberty to old age. The complete process of germ cell development is called spermatogenesis.[1].

Name & Address of Corresponding Author
Dr. Inayatullah
Assistant Professor,
Department of Anatomy,
Gajju Khan Medical College Swabi Khyber Pukhtunkhwa Pakistan.
E mail: drinayatswati@yahoo.com

This highly regulated and complex process of germ cell proliferation and differentiation leads to the production and release of mature male gametes, namely spermatozoa from the testes.[2] Normal spermatogenesis represents a precisely regulated balance between continuous cell proliferation and concomitant programmed cell death[3,4], which is mandatory for cellular homeostasis. [5] When the testicular environment cannot support spermatogenesis, specific pathways are accelerated leading to germ cells apoptosis. This abnormal apoptosis of germ cells may lead to an imbalance of cell proliferation and death, resulting in impaired spermatogenesis.[6,7] The causes of impaired spermatogenesis are multifactorial, including environmental, nutritional, behavioral, genetic, hormonal as well as toxin and drugs. [8,9]]
Corticosteroids are a group of steroid hormones synthesize in the adrenal cortex. Three different types of steroid hormones i.e. Glucocorticoids (GCs), Mineralocorticoids, and sex steroids (estrogen and androgens) are synthesize in the zona fasciculate, zona glomerulosa and zona reticularis respectively. [10] In

therapeutic concentration GCs are strong immunosuppressant, anti- inflammatory and anti-allergic, that have made them one of the most frequently prescribed drug worldwide.[11] Prolonged exposure to GCs lead to numerous pathological changes, including a significant redistribution of fat, protein wasting, insulin resistant diabetes mellitus 'steroid diabetes, hypertension, immunodeficiency, poor wound healing and loss of connective tissue leading to easy bruising, impair growth and development, osteoporosis, infertility and other endocrine-related changes.[12]

Dexamethasone (Dexa) a synthetic GC which is thirty times more potent than cortisol has made them an especially important drug for stimulating specific glucocorticoid activity.[13] Experimental studies have shown that excess GCs reduced serum testosterone level, impaired luteinizing hormone signal transduction and steroidogenesis in leydig cells of rats,[14,15] and also suppressed the activity of hypothalamic-pituitary-gonadal (HPG) axis.[16] Dexamethasone acts as testicular toxicant, induces histopathological alterations such as epithelial vacuolization, reduction in seminiferous tubules diameter/height and apoptotic index of germ cells were also increased significantly.[17]

Minerals are required for the normal growth and maintenance of the body.[18] Magnesium (Mg) is the second abundant intracellular cation after potassium. Many enzymes require the presence of magnesium ions for their catalytic action, especially enzymes utilizing ATP or those, which use other nucleotides to synthesize DNA & RNA. [19]

Mg is an essential cofactor that activates more than 300 enzyme systems in the body, involved in carbohydrate, lipid and protein metabolism. Due to high affinity of Mg to phosphate, involved in all phosphorylation processes where ATP (adenosine triphosphate), primarily exists as an ATP-Mg complex, therefore plays a predominant role in energy metabolism,[18] and has also been shown, control on the activity of Hypothalamic–pituitary-Adrenal Axis, which is considered to be the main stress response system. [20]

The pathological changes induced by ethanol such as, seminiferous epithelium disorganization and degeneration of Sertoli and germ cells, decreased sperm count and motility are effectively prevented by magnesium isoglycyrrhizinate . The drug exhibits the ability to counteract ethanol induced oxidative challenges as it effectively reduces testicular malondialdehyde (MDA) and increases the activities of superoxide dismutase and glutathione peroxidase.[21] Magnesium increases free and total testosterone levels in sedentary and athletes; the increase is higher in those who exercise than in sedentary individuals. [22]

Factors decreasing Mg reabsorption in the renal tubules or increasing renal excretion have been reported that include, hyperthyroidism, corticoids, anti-diuretic hormones (ADH), increased dietary calcium intake and chronic alcohol ingestion. [18]

Androgens are a group of hormones derived from cholesterol, Testosterone is so much abundant than the others that it is to be considered as the primary testicular hormone. In the target tissues most of the testosterone is eventually converted into the more active and potent form, dehydrotestosterone.[13,18] Testosterone is synthesizes by leydig cells and in small amount by the ovary and adrenal gland and secretion is under the control of hypothalamic-pituitary-gonadal axis.[13] Magnesium supplementation increases free and total testosterone levels. [22]

This study has been undertaken to evaluate the protective role of magnesium sulphate on dexamethasone induced histological and biochemical changes in albino rats.

MATERIALS AND METHODS

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.

- 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.[17]
- Group–C animals were given magnesium sulphate (Zafa Pharma Pak) at the dose of 20mg/kg body weight per day intra-

muscularly [21], with Dexa 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.

The results were based on histological findings and correlation with serum testosterone.

Tissue Treatment

The testes were 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 and then tissues were processed in ascending grades of alcohol, infiltrated, embedded in paraffin and 5µm thick longitudinal sections were cut on microtome and stained with PAS-Iron Haematoxylin Technique for study of Histo-architecture of seminiferous tubules. [23]

RESULTS

The mean values of serum testosterone (ng/dl) in group-A were 6.28±0.47 (Graph-1) & 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/ml) in groups 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. The data also showed significant increase ($P<0.05$) in mean serum testosterone level in group C when compared with dexamethasone treated [Figure 1].

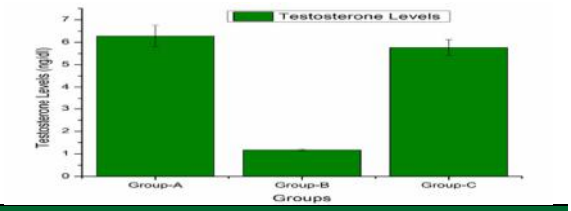

Figure 1: Graph shows Control group (A), Dexamethasone treated Group (B) and Magnesium sulphate and dexamethasone treated group (C). Further represent Testosterone level in ng/dl in each group with standard deviation.

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

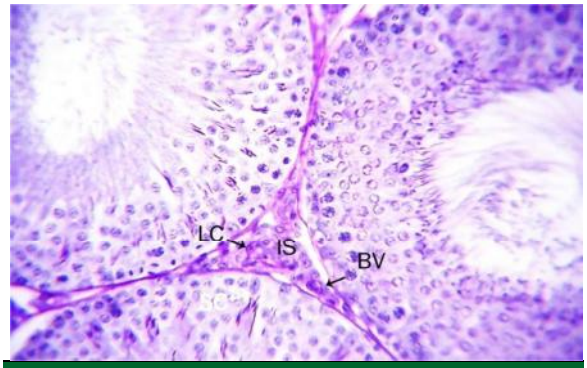

Figure 2: PAS-iron Heamatoxylin stained, 5µm thick section of testis of control albino rat, showing Germinal epithelium, Interstitial space (IS), Leydig cells (LC), and blood vessel (BV).

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

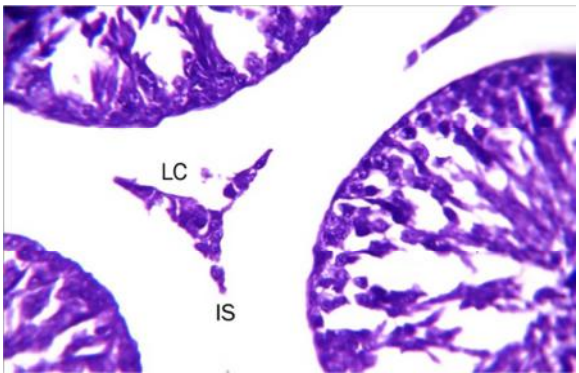

Figure 3: PAS-iron Heamatoxylin stained, 5µm thick section of testis of Dexamethasone treated albino rat, showing extensive vacoulation, widened interstitial space (IS), scanty Leydig cells (LC).

Organized developing germ cell series in group C, with some vacuoles observed. Slightly detached basement membrane and slough seen in the lumen. The feature showed marked improvement as compared to Dexamethasone treated animals. Leydig cells were comparatively less in number as compared to control group, but highly significant improvement was observed when compared to group-B [Figure 4].

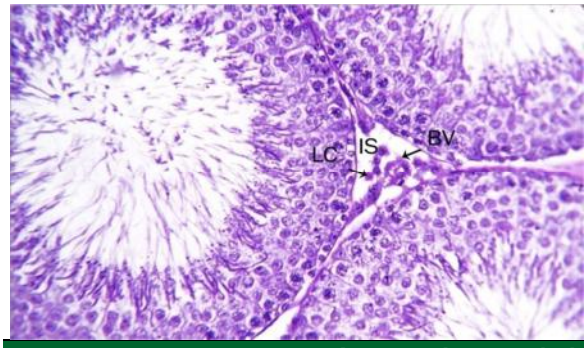

Figure 4: PAS-iron Heamatoxylin stained, 5µm thick section of dexamethasone with magnesium sulphate treated albino rat, showing all series of germ cells, vacuoles (V), and detached basement membrane (BM).

DISCUSSION

Normal spermatogenesis represents a precisely regulated balance between continuous cell proliferation and concomitant programmed cell death. [3,4] The causes of impaired spermatogenesis are multifactorial, including environmental, nutritional, behavioral, genetic and hormonal factors, as well as drugs. [24-26]

The observations and results of the present study has clearly demonstrated that dexamethasone has damaging effects on spermatogenesis, while the simultaneous use of magnesium sulphate has shown ameliorative 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 Dexamethasone on Leydig cells via glucocorticoid receptors. Guilliams & Edwards, 2010,[27] stated that suppression of axis is variable among patients and depend on the dose and duration of drug. The findings of the present study are in conformity with the study done by Maeda & Tsukamura, 2006,[16] who reported that, acute or chronic administration of glucocorticoid suppresses 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., 2012 [20] 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 et al., 2005 and Abeyagunawarden et al., 2007. [15-28]

The results of present study are in agreement with the findings of Orazizadeh et al., 2010, [17] who observed varying degrees of germ cell degenerative changes, disorganized germ cell layers, sloughing to vacoulation within the seminiferous tubules in mouse testicular germ cells exposed to injectable dexamethasone.

GCs induced apoptosis in rat leydig cells was observed by Gao et al 2002 [29] 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 effect of magnesium sulphate on the regulation of HPA-axis, and elevated level of testosterone as suggested by Cinar et al., 2011. [22]

Chandra et al 2013, [30] had given magnesium sulphate with standard diet at diverse doses for one and two consecutive spermatogenic cycles. They found significant increase in the activities of testicular 3β-hydroxysteroid & 17β-hydroxysteroid dehydrogenase enzymes and serum testosterone level along with progressive development in histoarchitecture of genital organs.

CONCLUSION

Based on the results it is concluded that administration of $MgSO_4$ has ameliorated both the histological and biochemical damaging effects induced by dexamethasone in rats' testes.

The present study may act as a baseline for extension in humans.

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