Original Article

# The Protective Role of Magnesium Sulphate on Steroid Induced Liver Damage in Albino Rats

**1. Asma Siddiqui 2. Aisha Qamar 3. Anjum Naqvi**

1. M.Phil Student, Dept. of Anatomy, BMSI, JPMC, Karachi 2. Asstt. Prof. of Anatomy, FJDC, Karachi 3. Assoc. Prof. of Anatomy, BMSI, JPMC, Karachi

## ABSTRACT

**Objective:** Dexamethasone causes metabolic disorders and morphological adverse effects on several organs of the body such as testes, kidney, bone, eye and liver etc. Most commonly it causes damage to liver morphology and its functions. Magnesium is an essential mineral of the body, currently is a subject of interest in medicine. Therefore the present study was designed to observe the ameliorating role of magnesium on dexamethasone induced liver damage and correlate the result with previous studies.

**Study Design:** Experimental Study

**Place and Duration of Study:** This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from 21 April to 10 May 2012.

**Materials and Methods:** Thirty adult albino rats, weighing from 200-300 grams were taken for this study. The rats were divided into 3 groups, Group A served as control , Group B received inj. dexamethasone 4mg/kg and Group C received inj. dexamethasone 4mg/kg with inj. Magnesium sulphate($MgSO_4$) 20mg/kg for 20days at the end of which they were sacrificed and liver tissue sections stained with haematoxylin and eosin.

**Results:** There was marked decrease in weight observed in rats receiving dexamethasone. Haematoxylin and eosin stained sections showed dilated central vein and sinusoids. Moderate fatty infiltration showed in vacuolated hepatocytes with absent or distorted nuclei in dexamethasone group which were protected and reverted to a major extent in Magnesium sulphate along with dexamethasone receiving group.

**Conclusion:** This study has proved that use of Magnesium sulphate along with dexamethasone ameliorates dexamethasone induced damaging effects on liver.

**Key Words:** dexamethasone, fatty infiltration, magnesium sulphate.

## INTRODUCTION

In this era of science, we are standing on the verge of a revolution in medicine, understanding and treatment. The use of extensive drug coverage for treatment or saving life is increasing day by day, regardless of what side effects may occur on other organs.[1]

With all being said, steroids are life saving drugs and recommended in prudent quantities and dosages to save lives. However, these life saving drugs are not free from side effects. Prescribing steroids can be life-saving; however, this treatment can cause considerable hazards. The numerous complications, both major and minor, depend on its dosage & duration of treatment.[2, 3]

Glucocorticoids (GCs) are the major steroid hormones secreted by the adrenal gland. In therapeutics GCs are strongly immunosuppressive and anti-inflammatory; this has made this drug one of the most frequently prescribed drug worldwide.[4, 5]

Excessive glucocorticoids can have deleterious consequences, including increased risk of metabolic syndrome, which may lead to hyperlipidemia, hypertension, impotency, hyperlipidemia, amenorrhea, impaired liver function, and immune suppression.[6-8] Glucocorticoids can also have adverse morphological effects on several organs of the body such as testes, liver, kidney, bone, and eye etc.[9-14]

Dexamethasone, a synthetic glucocorticoid, increases both fatty acid and cholesterol synthesis. Cortisol leads to development of the visceral obesity and its pathologies. Short term effect of dexamethasone, a synthetic cortisol has been on both fatty acid and cholesterol metabolism in rat hepatocytes.[15]

Fatty change or steatosis refers to abnormal accumulation of lipids, mainly triglycerides in liver, since this is the major organ involved in metabolism. Steatosis is relatively benign and reversible. However, with a secondary cellular stress, such as the oxidative stress it may progress to steatohepatitis (NASH), which is characterized by necroinflammation and fibrosis.[16-19]

Since last year, biological role and properties of metal ions have been reconsidered due to greater importance of inorganic bio ions in explanation of numerous biologic processes. Magnesium is an essential mineral of the body. Accumulating evidence suggests that magnesium deficiency is associated with poor metabolic control and plays a key role in pathophysiology of insulin resistance, hypertension, increased cholesterol level in the body, and increased free radical dependent oxidative stress.[21]

Hypomagnesaemia might be a risk factor in the progression of fatty liver to steatohepatitis. Several studies confirmed the hepato-protective role of Magnesium.[22-25]

In the light of above mentioned background, and since no histological study has been done so far to assess the protective role of Magnesium on dexamethasone treated liver, this study was designed to observe the effects of magnesium against dexamethasone induce liver damage..

# MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, for 20 days from 21 April to 10 May 2012.

Thirty healthy, adult young male Albino rats, 90-120 days of age, weighing 200-300gm were taken for this experimental study. The animals were kept under observation for 1 week for the assessment of their health status and diet intake prior to the commencement of the study. After one week they were treated with injection Decadron (OBS pharma), 4 mg/kg[26] and injection magnesium sulphate (Zafa pharma), 20 mg/kg[27], according to experimental dose.

The experimental animals were divided into 3 main groups A, B, and C.

- Group A: animals served as control.
- Group B: were given injection dexamethasone intraperitoneal (IP) daily.
- Group C: were given injection dexamethasone intraperitoneal (IP) along with injection magnesium sulphate ($MgSO_4$) intramuscularly (IM) daily.

The animals were weighed and kept in cages, with twelve hour light and dark cycle under laboratory environment. All the animals were kept on standard laboratory diet and water ad libitum. They were sacrificed at the end of their treatment period. A midline longitudinal incision was given & after carefully exposing abdominal viscera; gross appearance of liver was noted. Liver was removed from the abdominal cavity and weighed. Liver was cut into two halves from the plane dividing into right and left lobes. Then both halves were fixed in the buffer normal saline (BNF) for 24 hours. Then tissue was processed by dehydrating through ascending grades of alcohol (70%, 80%, 90% and two changes 100%) and cleared in two changes of xylene. Tissues were embedded in tissue embedding system at 58 degree centigrade. 4 micron thick sections were made on glass slides and stained with Haematoxylin & Eosin. The stained slides were studied at 40 X objectives of light microscope and results were observed. The results were analyzed by student "t" test.

# RESULTS

Over the course of treatment, dexamethasone administration in group B resulted in rapid and significant loss of body weight in comparison with control group A (P<0.0001). The group C protected by Magnesium sulphate showed minimal weight loss, & significant restoration of final body weight was noticed in this group in comparison with group B (P<0.001) (Table-1).

Inversely to the body weight, group B showed significant increase in mean liver weight (P<0.0001), which is significantly protected by the effect of Magnesium sulphate in Group C (P<0.001) and showed minimal or slightly increase in liver weight. (Table-2)

Microscopic examination of H& E stained section of control animals (Group A) showed normal architecture of liver, composed of hepatic cords which were radiating from the central vein; separated from each other by blood sinusoids. The hepatic lobules appeared almost hexagonal in shape (fig-1). Polygonal hepatocytes appeared normal with eosinophilic cytoplasm and centrally placed rounded nuclei with 1-2 nucleoli. Blood vessels & Periportal areas were seen normal (fig-2).

Dexamethasone treated (Group B) tissue sections showed disrupted lobular architecture with dilated and congested sinusoids. Focal necrosis of hepatocytes was seen. Hepatocytes were swollen & vacuolated. Nuclei had become pyknotic and fragmented. Cytoplasm appeared vesicular. Blood vessels were congested and dilated (fig-3). Portal areas were distorted and dilated along with lymphocytic infiltration (fig-4).

**Table No. 1: *Mean body weight in different groups of albino rats at variable time interval**

| Groups | Initial wt. (gm) | End of 10 days (gm) | End of 20 days (gm) |
|---|---|---|---|
| **A** (n=10) | 233.6±1.86 | 246.4± 3.86 | 244.8±3.67 |
| **B** (n=10) | 247.2 ±3.59 | 215.0±5.00 | 208.8±2.43 |
| **C** (n=10) | 234.0±4.35 | 234.9±2.67 | 221.9±1.45 |

*Mean±SEM

**Statistical analysis of differences in the mean body weight between different groups**

| Groups | End of 10 days P-value | End of 20 days P-value |
|---|---|---|
| **A** vs. **B** | <0.001*** | <0.0001**** |
| **B** vs. **C** | <0.001*** | <0.005** |

Non-significant * Significant**
Moderately-significant*** Highly-significant****

**Table No.2: Mean absolute weight of liver in different groups of albino rats**

| Groups | Mean liver weight (gm) |
|---|---|
| **A** (n=10) | 6.100± 0.218 |
| **B** (n=10) | 10.594±0.405 |
| **C** (n=10) | 7.336±0.301 |

*Mean±SEM

**Statistical analysis of Mean absolute weight of liver between different groups**

| Groups | P-value |
|---|---|
| **A** vs. **B** | <0.0001**** |
| **B** vs. **C** | <0.001*** |

Non-significant * Significant**

Moderately-significant*** Highly-significant****

Magnesium sulphate protected (Group C) liver sections showed preservation of lobular architecture. Hepatic sinusoids were slightly dilated but not congested (fig-5). Hepatocytes appeared less vacuolated. Blood vessels were slightly dilated. Portal areas appeared almost normal (fig-6).

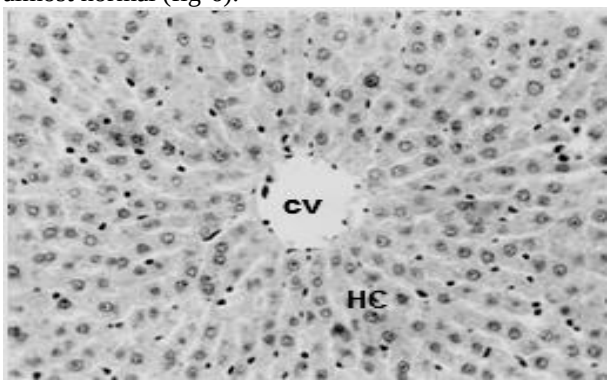


**Figure No. 1: Photomicrograph showing normal hepatic architecture with central vein CV, hepatic cords HC, sinusoids and hepatocytes in control group-A under 40 X magnification**

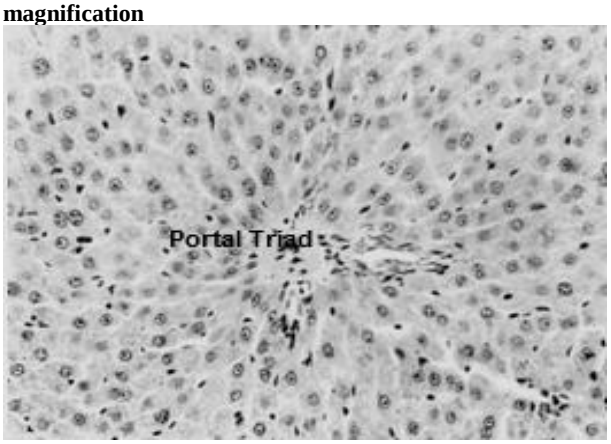


**Figure No. 2: Photomicrograph showing normal hepatic architecture with, Portal Triad in control group-A under 40 X magnification**

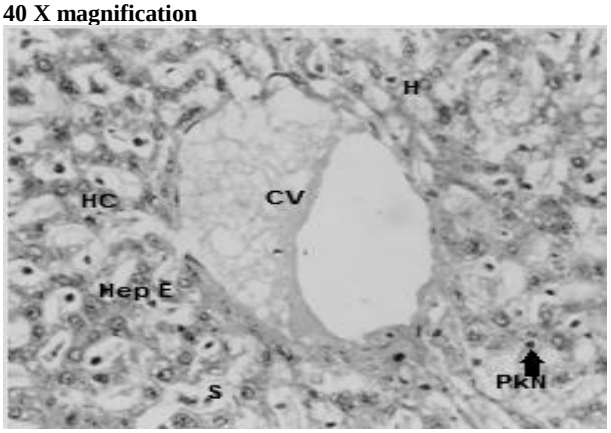


**Figure No. 3: Photomicrograph showing empty, vacuolated hepatocytes Hep E, around a dilated and distorted portal triad , in dexamethasone treated group-B under 40 X magnification**.

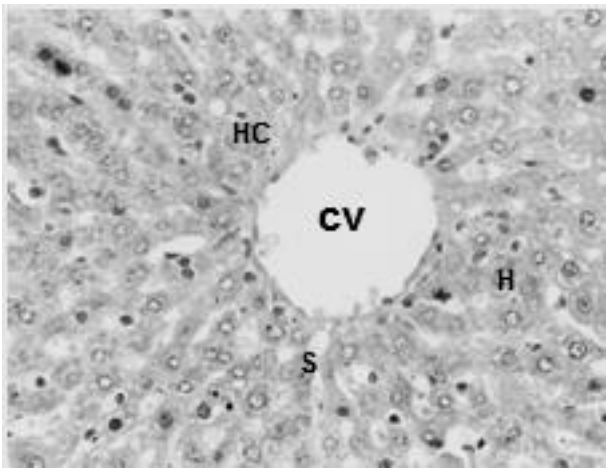


**Figure No. 5: Photomicrograph showing preserved hepatic architecture with less dilated Central vein CV, regular arrangement of hepatocytes H, in hepatic cords HC, with slightly congested sinusoid magnesium protected group-C under 40 X magnification**

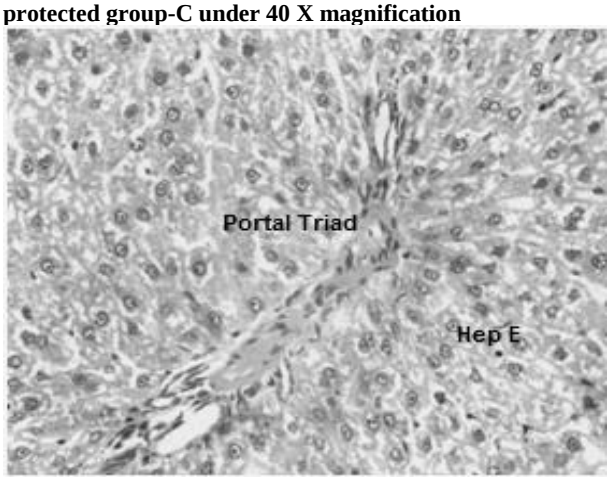


**Figure No. 6: Photomicrograph showing preserved dilated Portal Triad and normal hepatocytes arranged in hepatic cords. Less empty & vacuolated hepatocytes Hep E were seen in Magnesium protected group-C under 40X magnification**

# DISCUSSION

Administration of dexamethasone has been reported to induce weight loss, insulin resistance, vascular disruption, altered lipid metabolism and fatty change in organs in several studies. Hepatic steatosis is very common finding in clinical practice.[8]

Magnesium is an obligate ion that is essential for activation of many enzymes involved in different metabolisms and functions, such as glucose metabolism, fatty acid synthesis and breakdown and DNA protein metabolism. [25]

In the present study, marked decrease in body weight was seen in dexamethasone treated animals (Group B) in comparison to control and Magnesium protected animals (Group C), this result is in accordance with Shafagoj et al. (2008)[8] who also observed decrease in

body weight in response to dexamethasone in albino rats.

There was a significant increase in liver weight in dexamethasone treated group-B, with grossly pale appearance of liver & signs of hemorrhage and congestion. This is in accordance to Micuda et al. (2007)[5] who also found increase in liver weight due to dexamethasone treatment, inversely to body weight of animals.

In the present study dexamethasone had disrupted the liver cytoarchitecture. It increased fat accumulation in the liver, which induced severe microvesicular steatosis with large vacuolated hepatocytes. This finding is in accordance to Matsunaga et al. (2008)[14] who also observed the vacuolation in liver cells due to dexamethasone administration. The central vein was congested and dilated. Sinusoids were dilated and leucocytic infiltration was seen. Similar findings were reported by Chaweeborisuit et al. (2009)[13].

This study also showed that Magnesium significantly ameliorated the effects of dexamethasone induced hepatic injury. Magnesium markedly improved the cytoarchitecture of liver. These findings are in agreement to Bao et al. (2008)[22] who also observed that magnesium reduced steatosis and relieved the congestion and dilatation of central vein and sinusoids

# CONCLUSION

The present study concluded that dexamethasone causes moderate to severe hepatic damage and magnesium ameliorates the effects of dexamethasone on liver. Therefore it is suggested to avoid the irrelevant & long term use of dexamethasone and the concomitant use of magnesium prevents as well as reverts the liver damage.

# REFERENCES


1. Pauly MV. Medicare drug coverage and moral hazards. Health affairs 2004; 23(1):113-122.
2. Priti D, Asawari R, Arundhati D, Kushal G, Aeyna T. A prospective study of steroid induced hyperglycemia. J Med Sci & Res 2011;2 (1): 46-49
3. Hurrell GD. The prescription and side-effect of steroids. J the Royal College of General Practitioners 1972; 22: 387-392.
4. Orazizadeh M, Khorsandi LS, Hashemitabar M. Toxic effects of dexamethasone on mouse testicular germ cells. Andrologia 2010; 42(4): 247-53.
5. Micuda S, Fuksa L, Mundlova L, Osterreicher J, Morky J, Cermanova J, et al. Morphological and functional changes in p-glycoprotein during dexamethasone-induced hepatomegaly. Clin Exp Pharmacol Physiol 2007; 34(4): 296-303.
6. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000; 57(10): 925-35.
7. Sivabalan S, Renuka S, Menon VP. Fat feeding potentiates the diabetogenic effect of dexamethasone in Wistar rats. Int Arch Med 2008; 1(1): 1- 7.
8. Shafagoj Y, Opoku J, Qureshi D, Regelson W, Kalimi M. Dehydroepiandrosterone prevents dexamethasone-induced hypertension in rats. Am J Physiol 1992; 263 (2): 210-3.
9. McDonald AT, Crocker JF, Digout SC, McCarthy SC, Blecher SR, Cole DE. Glucocorticoid-induced polycystic kidney disease--a threshold trait. Kidney Int 1990; 37(3): 901-8.
10. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 2007; 18(10):1319-28.
11. Bouhenni RA, Dunmire J, Sewell A, Edward DP. Animal models of glaucoma. J Biomed Biotechnol 2012; 1-11.
12. Mahmood AA, Sidik K, Salmah I, Suzainur KAR. Effect of dexamethasone on rats infected with pasteurella multocida. J Animal & veterinary advances 2004; 3 (1): 39-44
13. Chaweeborisuit P, Rungruang T, Kettawan A, Boonmars T. Long term dexamethasone administration affects morphological and histological changes of plasmodium yoelii-infected ICR-Mice liver. Siriraj Med J 2009; 61(1): 1-4.
14. Matsunaga T, Toba M, Teramoto T, Mizuya M, Aikawa K, Ohmori S. Formation of large vacuoles induced by cooperative effects of oncostatin M and dexamethasone in human fetal liver cells. Med Mol Morphol 2008; 41(1): 53-8.
15. Giudetti AM, Gnoni GV. Short-term effect of dexamethasone on fatty acid and cholesterol synthesis in isolated rat hepatocytes. Biochem Mol Biol Int 1998; 44(3): 515-21.
16. Burt AD, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. Semin Diagn Pathol 1998; 15(4): 246-58.
17. Yu AS, Keeffe EB. Non-alcoholic fatty liver disease. Rev Gastroenterol Disord 2002; 29(1): 11-19.
18. Kashireddy PR, Rao MS. Sex differences in choline-deficeint diet induced steatohepatitis in mice. Experimental biology and medicine 2004; 229: 158-162.
19. Kumar V, Abbas AK, Fausto N. Cell injury, cell death, and adaptation, Robins Basic Pathology. 8th ed. Philadelphia: Saunders; 2007.p.23-24.
20. Turecky L, Kupcova V, Szantova M, Ulikova E, Viktorinova A, Czirfusz A. Serum magnesium levels in patients with alcoholic and non-alcoholic fatty liver. Bratisl Lek Listy 2006; 107(3): 58-61.
21. Corica F, Corsonello A, Ientile R, Cucinotta D, Di Benedetto A, Perticone F, et al. Serum ionized

magnesium levels in relation to metabolic syndrome in type 2 diabetic patients. J Am Coll Nutr 2006; 25(3): 210-5.
22. Bao QD, Yang LL, Wang L, Cui DL. Protective effect of magnesium isoglycyrrhizinate against carbon tetrachloride-induced acute liver injury in mice. Shijie Huaren Xiaohua Zazhi 2008; 16(9): 1004-1007.
23. Ding PP, HE G, Wu XL, Hu CZ, Zhao MC. Efficacy of magnesium isoglycyrrhizinate on the prevention of liver injury in HBV carriers with tuberculosis. J Tropical Medicine 2011; 7: 786-7.
24. Wang ZN, Tang Z, Zhou HC, Li SH, Yu ZH. Clinical observation of liver-protecting effect of Magnesium Isoglycyrrhizinate in FOLFOX on colorectal cancer. J Clinical Med in Practice 2011; 17: 107-9.
25. Martin H, Staedtler F, Lamboley C, Adrian M, Schumacher MM, Chibout SD, et al. Effects of long-term dietary intake of magnesium on rat liver transcriptome. Magnes Res 2007; 20(4): 259-65.
26. Heinrichs C, Yanovski JA, Roth AH, Yu YM, Domene HM, Yano K, et al. Dexamethasone increases growth hormone receptor messenger ribonucleic acid levels in liver and growth plate. Endocrinology 1994; 135(3):1113-8.
27. Soldatovic D, Matovic V, Vujanovic D. Prophylactic effect of high magnesium intake in rabbits exposed to prolonged lead intoxication. Magnes Res 1993; 6(2): 145-8.

**Address for Corresponding Author:**
**Asma Siddiqui,**
M.Phil Student,
Anatomy Department,
Basic Medical Sciences Institute,
Jinnah Postgraduate Medical Center, Karachi. Cell No. 03142116275.