OnLine Journal of Biological Sciences 2(5): 320-324, 2002
ISSN 1608-4127

Methylprednisolone Induced Histological and Histochemical Changes in the Adrenal Cortex of Rat

S.A. Sakr, [1]N. I. El-Desouky and [2]S.M. Hanafy
Zoology Department, Faculty of Science, Menoufia Universiy, Shebin El-Kom, Egypt
[1]Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
[2]Zoology Department, Faculty of Science, South Valley University, Aswan, Egypt

Abstract: In this experiment the effect of the synthetic glucocorticoid, methyl prednisolone on the adrenal cortex of albino rat was investigated by applying histological and histochemical techniques. Animals were orally given methyl prednisolone at a dose level of 1.8 mg/kg/body weight dissolved in sterile saline, 3 times/week for 3 weeks. Histological results revealed atrophy of zona fasciculata, thickness of adrenal capsule and hypertrophy of zona reticularis. Total carbohydrate content of the zona glomerulosa, zona fasiculata and zona reticularis cells showed an obvious decrease after treatment which was prominent after 3 weeks. Total protein content showed a marked decrease in zona glomerulosa and zona fasiculata. On the other hand, the cells of zona reticularis appeared with an increase amount of total protein.

Key words: Methylprednisolone, adrenal cortex, histology, histochemistry

Introduction

Glucocorticoids are commonly used as therapeutic agents for many diseases (Crossland, 1980). Methylprednisolone is widely used as anti-inflammatory drug (Heiman *et al*., 1994). On the other hand, the use of glucocorticoids and their synthetic analogues were accompanied by deleterious effects. Prednisolone leads to change in diurnal periodicity of the hypothalamo-hypophysial-drenocortical system in adult rats (Zlobina *et al*., 1990). Oral administration of prednisolone affected endogenous adrenocortico-trophic hormone (ACTH) concentration and adrenocortical response to exogenous ACTH in dogs (Brockus *et al*., 1999). Methylprednisolone produced diabetes mellitus in dog (Jeffers *et al*., 1991)

Glucocorticoids were found to induce histopathological alterations in the liver (Bhagwat and Deodhar, 1968; Bhagwat and Ross, 1971; Wassef and Demian, 1992). Kidney (Lange and Doorenbos, 1974) and adrenal gland (Challis *et al*., 1974; Wassaf and Demian, 1992). The use of glucocorticoids and their synthetic analogues during pregnancy increased foetal mortality (Seifter *et al*., 1951) and induced cleft Palate in the offspring (Fraser and Fainstat, 1951). So, this study was carried out in order to demonstrate the histological and histochemical effects of the synthetic glucocorticoid. methylprednisolone on the adrenal gland of the albino rats.

Materials and Methods

Adult male albino rats, *Rattus norvegicus* weighing 100 to 120g were used in the present investigation. They were maintained under the standard laboratory conditions of temperature and were fed on standard rodent chow with water provided ad libitum. After one week of acclimatization to the laboratory environment, the animals were divided into two groups:

Group 1: Animals of this group (25 rats) were orally given methylpredenisolone by gastric tube (Urbason, Hoechst Marion Roussel, Germany) at a dose level of 1.8mg/kg/body weight dissolved in sterile saline, 3 times/week for 3 weeks.

Group 2: Animals of this group (15 rats) were given saline and were served as control.

The animals were killed by decapitation after two and three weeks. Their adrenal glands were removed and were immediately fixed. For histological examination, the tissues were fixed in Bouin's fluid, while for histochemical study, they were fixed in Carnoy's fluid. Fixed materials were embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained by haematoxylin and eosin for histopathological examination. Total carbohydrates were demonstrated using Periodic acid Schiff's technique (PAS) (Hotchkiss, 1948). Total proteins were detected using the mercury bromophenol blue method (Mazia *et al*., 1953).

Results

Histopathological results: The adrenal gland formed of two distinct parts, the adrenal cortex and the adrenal medulla. Section of the adrenal cortex of control rats displayed three zones: zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). The ZG zone lie adjacent to a relatively thick capsule of connective tissue and this zone is a relatively narrowest one. Its cells are arranged in closely packed rounded oval clusters with deeply stained nuclei. The ZF represents the thickest zone of the cortex and consists of straight cords of vacuolated cells in a radical direction toward medulla. The ZR comprises an innermost portion with anastomotic cords of trabeculae of the adrenal cortex and the cells are generally smaller than in ZF and they have deeply stained nuclei (Fig. 1a , b).

Examination of the adrenal of rats treated with methylprednisolone for two weeks showed that ZF zone was

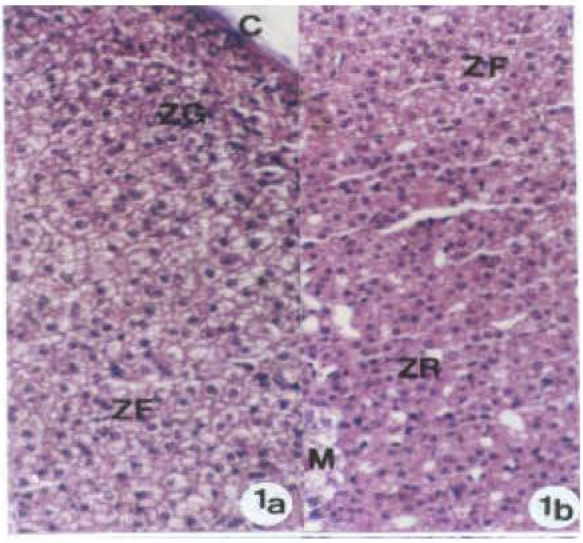

Fig. 1 a,b: Section of adrenal cortex of a control rat showing capsule (c), Zona glomerulosa (ZG), Zona fasciculata (ZF), Zona reticularis (ZR) and medulla (M), (H&E, x400).

Corresponding Author: Saber. A. Sakr, Zoology Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt

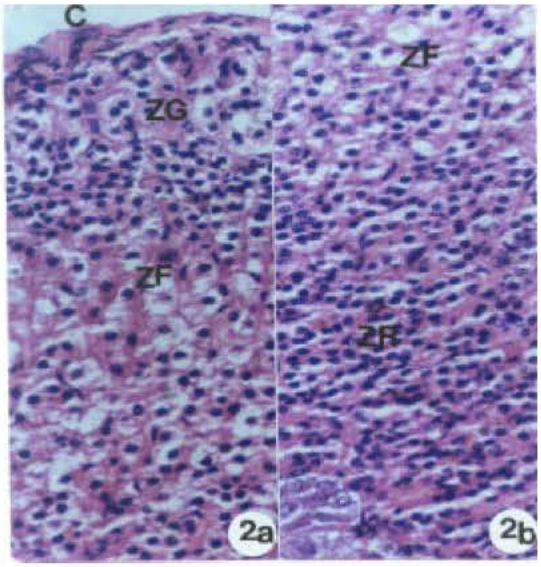

Fig. 2 a,b: Section of adrenal gland of a rat treated with methylprednisolone for 2 weeks showing atrophied ZF and hypertrophy of ZR (H&E, x400).

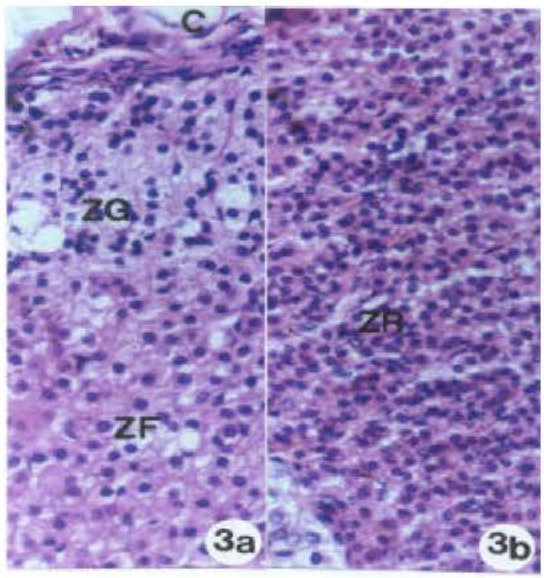

Fig. 3 a,b: Section of adrenal gland of a rat treated with methylprednisolone for 3 weeks showing thick capsule (c), vacuolated ZG, atrophied ZF and hypertrophy of ZR. Note the interference of ZF with either ZG or ZR (H&E, X 400).

atrophied (Fig. 2a) and their cells appeared highly vacuolated. On the other hand, ZR zone was increased in its thickness and was markedly hypertrophied (Fig. 2b). Such histological changes were increased after three weeks of treatment with methylprednisolone. The adrenal capsule appeared thicker and the cells of ZG were highly vacuolated with pyknotic nuclei. The atrophy of ZF was increased (Fig. 3a) and interfere with either ZG or ZR zones. The ZR zone was obviously hypertrophied (Fig. 3b). The vacuoles were increased in the cells of ZF zone and the blood sinusoids were dilated and congested.

Histochemical results

Total carbohydrates: Sections of control rats stained with PAS

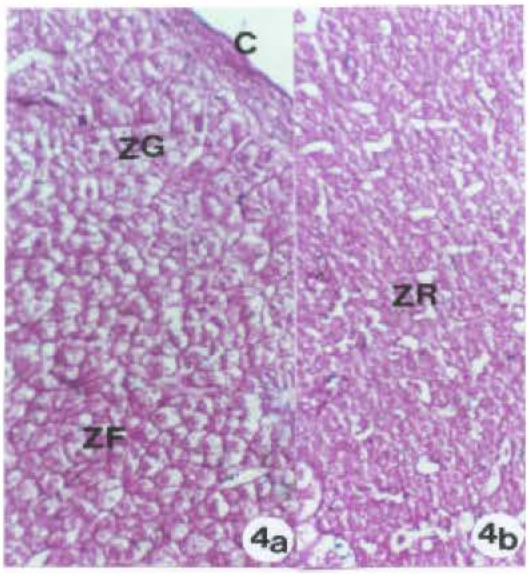

Fig. 4 a,b: Section of adrenal control rat showing PAs - positive inclusions in the cells of the three zone (ZG, ZF, ZR), (PAs, X400).

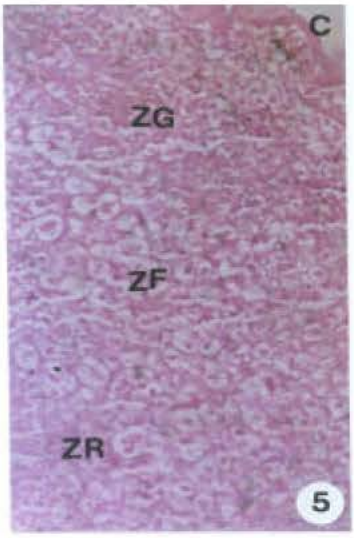

Fig. 5: Section of adrenal cortex of a rat treated with methylprednisolone for 2 weeks showing a moderate amount of total carbohydrates in the cells of the three zone, (PAs, X400).

method showed total carbohydrate content and distribution in the form of deeply stained reddish granules in the cytoplasm of cortical cells. The adrenal capsule exhibited a moderate red colour. The cytoplasm of ZG cells showed a moderate reaction with PAS. The cells of ZF zone showed a relatively moderate PAS-reaction while the cells of ZR zone revealed strong reaction (Figs. 4 a, b) Table 1 showed the change in total carbohydrate content in the adrenal cortex of rats treated with methylprednisolone. Adrenal cortex of rats treated with methyl prednisolone for 2 weeks showed that the adrenal capsule was weakly stained with PAS. The cells of all the zones (ZG, ZF, ZR) appeared with a moderate amount of total carbohydrates (Fig. 5). After 3 weeks of treatment with this drug, the cells of ZG and ZF revealed a marked depletion of their PAS inclusions. In most cells of ZR zone, the PAS inclusions were lacking and in some cells the cytoplasm showed

Table 1: Changes in total carbohydrates and total proteins in adrenal cortex of rats treated with methylprednisolone

Weeks after treatment	Total carbohydrates			Total proteins		
	ZG	ZF	ZR	ZG	ZF	ZR
Control	+ +	+ +	+ + +	+ +	+ +	+ + +
Two weeks	+ +	+ +	+ +	+	+	+ +
Three weeks	+/-	-	+/-	+	+	+ + +

ZG: zona glomerulosa; ZF zona fasciculata; ZR: zona reticularis. (+ + +): strong, (+ +): moderate, (+): slight, (-): depleted

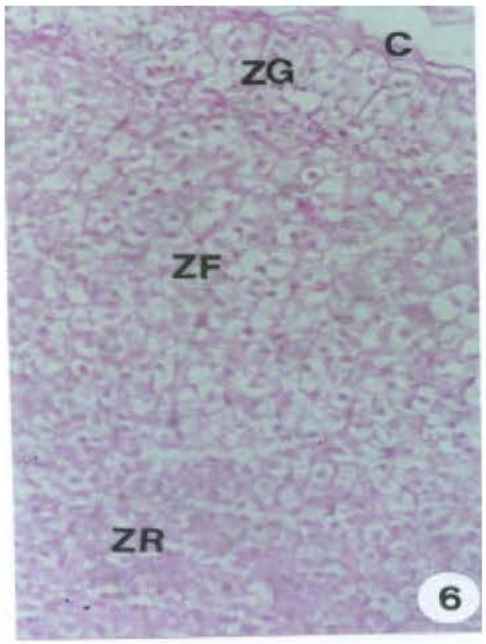

Fig. 6: Section of adrenal cortex of a rat treated with methylprednisolone for 3 weeks showing marked depletion of total carbohydrates in the capsule, ZG and ZF. Cells of ZR showing moderate amount of carbohydrates (PAs, X400).

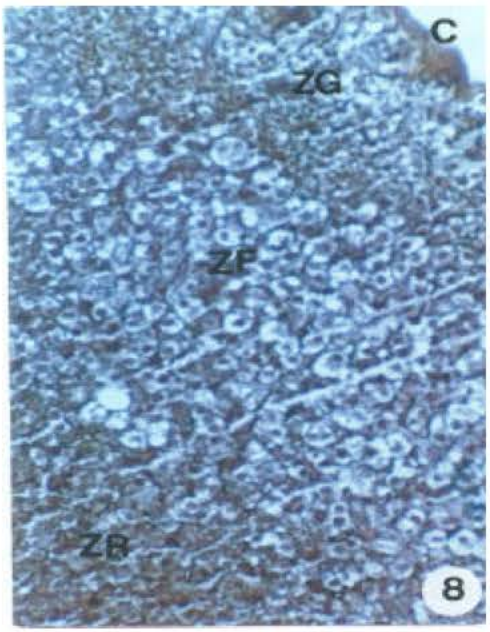

Fig. 8: Section in the adrenal cortex of a rat treated with methylprednisolone for 2 weeks showing slight amount of total proteins in ZG&ZF, and moderate amount of total proteins in the cells of ZR. (Bromophenol blue, X400).

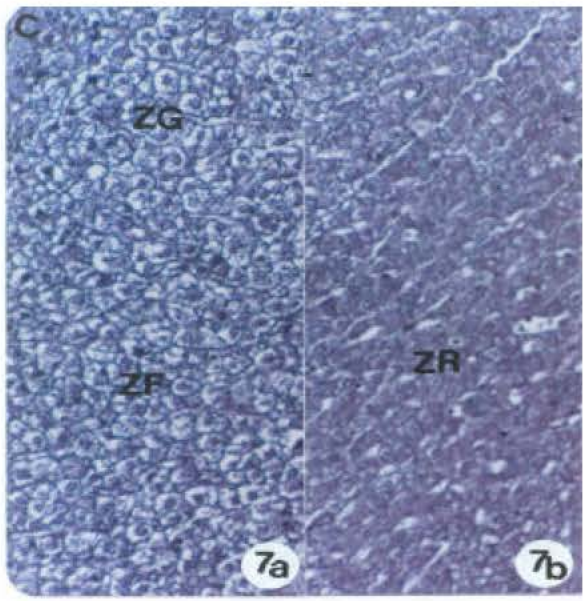

Fig.7a, b: Section of adrenal cortex of a control rat showing total protein, content of the capsule and cells of ZG, ZF and ZR (Bromophenol blue, X400).

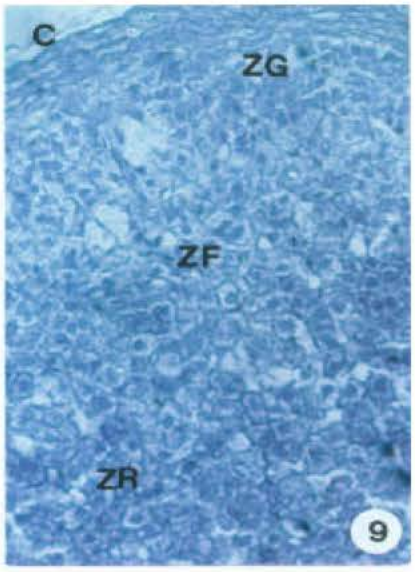

Fig. 9: Section in the adrenal cortex of a rat treated with methylprednisolone for three weeks showing a marked decrease of total proteins in ZG & ZF, while ZR showing an increase amount of total proteins. (Bromophenol blue, X400).

diffuse stainability (Fig. 6).

Total proteins: Total proteins appeared in the adrenal cortical cells of control rats in the form of small bluish granular bodies in the cytoplasm. The cell membrane and the nuclear membrane were intensely stained. The chromatin particles and the nucleoli were also stained. The adrenal capsule showed a high content of total proteins. The cells of ZG and ZF zones showed a moderate amount of total proteins, while ZR zone displayed a strong stainability (Fig. 7 a,b). Animals treated with methylprednisolone for 2 weeks showed that total proteins were decreased in the cells of ZG and ZF zone, while the cells of ZR showed a moderate amount of proteins (Fig. 8). A marked reduction of proteins was observed in the cells of ZG and ZF zones after 3 weeks of treatment. On the other hand, the cells of ZR showed a moderate to strong stainability (Table 1, Fig. 9)

Discussion

These results revealed that methylprednisolone caused histopathological changes in the adrenal cortex of rats. Among these changes was the atrophy of zone fasciculata. Similarly, Okazaki *et al.* (1992) and Nagashima *et al.* (1992) reported that predenisolone farnesylate decreased the weight of adrenal gland and caused atrophy of zone fasciculata in rats and beagle dogs.

Glucocorticoids were found to affect adrenal gland of different animals (Tuchmann–Duplessis, 1970, Challis *et al.*, 1974; Wassef and Demian, 1992). Adrenocortical hypofunction was recorded under the effect of some glucocorticoids (Brockus *et al.*, 1999). Thus, in this experiment, it is speculated that methylprednisolone has a degenerative effect on the adrenal cortex of rats and this effect in secondary to the adrenocortical hypofunction. This is supported by the work of Mopy and Kolanowsk (1986) who found that in adrenocortical insufficiency, the excretion of epinephrine is reduced proportionally to the decrease in adrenocortical activity.

In this study, it has been observed that methylprednisolone induced a decrease of total carbohydrates in the adrenal cortex of the rats and this decrease was marked after 3 weeks. These results are similar to those reported in different tissues under the effect of glucocorticoids. Olejniczak and Lee (1984) reported that treating rats with methylprednisolonate and methyl 17-deoxyprednisolonate caused significant decrease in liver glycogen content, plasma corticosterone level and relative adrenal gland weight. Furll *et al.* (1976) observed that metoprion caused decline of glucose and pyruvate concentrations in plasma and drop of liver glycogen in pigs. Rooney *et al.* (1986) examined the effect of dexamethasone and triiodothyrorine (T3) alone and in combination on glycogen and fatty acids in fetal rat lung. The hormones were administered to the mothers on the 2 days before delivery and on days 17-22 of gestation. Their results showed that there is increase in lung glycogen on days 17-20 with a decrease thereafter and an increase in the rate of fatty acid synthesis between days 20-21.

Total proteins showed noticeable decrease in the zone glomerulosa and zone fasciculata of methyl prednisolone– treated rats Similarly, Bezdrobny and Germaniuk (1976) reported that treating rats with the glucocorticoid hydrocortisone induced inhibition of protein synthesis in skeletal muscles. Baxter *et al.* (1972) mentioned that the catabolic actions of glucocorticoids result in decreased synthesis and increased degradation of protein and RNA in the adrenal gland, skin, muscle and connective tissue. Clark *et al.* (1986) investigated the effect of dexamethasone on cardiac protein metabolism of rats. They found that body weight was significantly lowered in the treated animals in comparison with controls and a 13% increase in protein degradation was occurred. England and Jurkovits (1992) observed that protein degradation in skeletal muscle is accelerated in rats with chronic renal failure. Exogenous glucocorticoids do not alter protein degradation, but inhibit protein synthesis in BC 3HI myocytes. Silbermann and Maor (1979) examined the influence of triamcinolone hexacetonide, a long-acting synthetic analogue of cortisol, on nucleic acid and protein synthesis in condylar cartilage of neonatal mice. Following a single injection of the hormone the DNA, RNA and protein contents were significantly reduced. Significant changes became apparent by 24 hours and persisted for 72 hours after administering the hormone. Correlative relationship was noted between the inhibitory effects on the uptake and the subsequent incorporation of the above precursors into their respective nucleic acid. This study clearly indicates that corticosteroid hormones possess a significant inhibitory effect on the proliferative activity of neonatal chrondrocytes and upon the latter's protein synthetic pathways, thereby affecting the normal process of endochonral bone growth.

Total proteins increased in the cells of zona reticularis of methylprednisolone - treated animals. This is due to the hypertrophy of this zone observed in the present work. Welsh *et al.* (1982) found that administration of the synthetic glucocorticoid, dexamethasone inhibited testosterone production and this inhibition was accompanied by marked decreases in androstenedione and 17-alphahydroxyprogestrone with reduction of gonadal function. Thus, the hypertrophy of zona reticularis, recorded in the present work, may be due to the involvement of the cells of this zone in secretion of androgens

From these results, it is speculated that one or more metabolites of methylprednisolone are responsible for induction of adrenal hypofunction in rats and resulted in the observed histological and histochemical changes.

References

Baxter, J.D., H. Peter and M.A. Forsham, 1972. Tissue effects of glucocorticoids. Am. J. Med., 53: 573-589.

Bezdrobny, I.U.V. and I.A.L. Germaniuk, 1976. Effect of glucocorticoid hormones on nuclear RNA-polymerase activity and RNA metabolizability in rat skeletal muscles. UKr Biokhim. Zh., 48: 650-652.

Bhagwat, A.G. and R.C. Ross, 1971. Prednisolone-induced hepatic injury, ultrastructure and biochemical changes in rabbits. Arch. Pathol., 91: 483-492.

Bhagwat, A.G. and S.D. Deodhar, 1968. Experimental hepatic injury produced in the rabbit by glucocorticods. Arch. Pathol., 85: 346-356.

Brockus, C.W., A.R. Dillon and R.J. Kemppaien, 1999. Effect of alternate-day prednisolone administration on hypophyseal-adrenocortical activity in dogs. An. J. Vet. Res., 60: 698-702.

Challis, J.R., I.J. Davis, K. Benirschke, A. Hendricks and K.J. Ryan, 1974. The effect of dexamethasone on plasma steroid levels and fetal adrenal histology in the pregnant rhesus monkey. Endocrinol., 95: 1300-1305.

Clark, A.F., G.N. DeMartino and K. Wildenthal, 1986. Effects of glucocorticoid treatment on cardiac protein synthesis and degradation. Am. J. Physiol., 250: 821-827.

England, B.K. and C. Jurkovitz, 1992. Effect of glucocorticoids and extracellular pH on protein metabolism in cultured cells. Miner Electrolyte Metab., 18: 316-319.

Fraser, F.C. and T.D. Fainstat, 1951. The production of congenital defects in offspring of pregnant mice treated with cortisone. Pediatrics, 8: 527-533.

Furll, M., H. Seidel and B. Furll, 1976. Behavior of various metabolites of carbohydrate metabolism in the liver of young pigs following inhibition of C-11 hydroxylase and adrenalectomy. Arch. Exp. Vet. Med., 30: 491-496.

Grassland, J., 1980. Lewis's Pharmacology, Churchill Livingstone Press, London, 5th ed., pp: 347-768.

Heiman, A.S., D. Hong and H.J. Lee, 1994. Receptor binding affinity and antiproliferative activity of new anti-inflammatory antidrugs: 6-methoxycarbonyl prednisolone and its derivatives. Steroids, 59: 324-329.

Hotchkiss, R.D., 1948. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., 16: 131

Jeffers, J.G., K.J. Shanley and R.O. Schick, 1991. Diabetes mellitus induced in a dog after administration of corticosteroids and methylprednisolone pulse therapy. J. Am. Vet. Med. Assoc., 199: 77-80.

Lange, W.E. and H. Doorenbos, 1975. Meyler's side effects of drugs, vol. VIII. American Elsevier Publishing Co. Inc., New York. 7th ed., pp: 812-833.

Mazia, M., P.A. Brewer and M. Affert, 1953. The cytochemical staining and measurements of protein with mercuric bromophenol blue. Biol. Bull., 1: 57-67.

Mopy, M. and J. Kolanowski, 1986. Urinary catecholaminc excretion in patients with secondary adrenocortical insufficiency. J. Endocrinol. Invest., 9: 255-253.

Nagashima, Y., F. Hisaoka, M. Ide, K. Tamura, K. Shimura, G. Tanaka and H. Tanaka, 1992. A 13-week percutaneous toxicity study of prednisolone famesylate (PNF) gel in beagle dogs with a recovery period of 5 weeks. J. Toxicol. Sci., 17: 123-60.

Okazaki, S., S. Nishimura, K. Tamura, T. Aikawa, K. Hatayama, H. TANAKA and G. Tanaka, 1992. A 52-week dermal toxicity study of prednisolone famesylate (PNF) gel in rats with a recovery period of 8 weeks. J. Toxicol. Sci., 17: 91-122.

Olejniczak, E. and H.J. Lee, 1984. Systemic effects of chronically administered methylprednisolonate and methyl 17-deoxyprednisolonate steroids, 43: 657-662.

Rooney, S.A., L.I. Gobran and A.J. Cliu, 1986. Thyroid hormone opposes some glucocorticoid effects on glycogen content and lipid synthesis in developing fatal rat lung. Pediatr. Res., 20: 545-550.

Seifter. J., J. Christian and W.W. Ehrich, 1951. Effect of cortisone and other steroids on the hibernating gland of the pregnant white rat. Fed. Proc., 10: 334-338.

Silbermann, M. and G. Maor, 1979. Effect of glucocorticoid hormone on the content and synthesis of nucleic acids in cartilage of growing mice. Growth, 43: 273-28.

Tuchmann-Duplessis, H., 1970. Influence of certain drug on prenatal development. J. Gynec. Obstet., 8: 777-797.

Wassef, N.W. and M.W. Demian, 1992. Hislopathological effects of prednisolone treatment on the kidney, adrenals and liver of pregnant hamsters and their offspring. J. Egypt. Germ. Soc. Zool., 8: 267-277.

Welsh, T.R., J.R. Bambino and T. H. Hsush, 1982. Mechanism of glucocorticoid-induced suppression of testicular androgen biosynthesis *In vitro*. Biol. Reprod., 27: 1138-1146.

Zlobina, N. A., S.B. Lure and O.A. Donilove, 1990. The effect of prednisolone administration in early postnatal on circadian activity of the hypothalamo-hypophyscal adrenal cortical system in adult rats. Probl. Endocrinol., 36: 58-62.