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Hepatorenal Toxicity Induced by Maternal Exposure to Topical Betamethasone Prior to Fertilization in Newborn Rabbits

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ABSTRACT

Background: Synthetic glucocorticoids, such as Betamethasone, are used for treatment of skin disease including Psoriasis. No enough evidences are present about the side effect of maternal exposure to betamethasone, before fertilization, on new-born. So, the goal of the present study was to determine these side effects on liver and kidney.

Methods: In the present study, two doses of betamethasone (0.02 and 0.2 mg/kg b.w) were used. At the end of the experimental period (after 3 weeks of delivery), six new-born rabbits of both sexes (1 newborn rabbit per mother) were dissected under light anesthesia and blood, liver and kidney samples were collected. All the measurements were performed in one assay.

Result: Maternal exposure to betamethasone, for 2 months before fertilization, induced toxicity in new-born rabbits and decrease the relative organ weight of liver and kidney in new-born rabbits. The biochemical changes involved induction of oxidative, alterations in hepatic and renal parameters. Moreover, structural changes in liver and kidney new-born rabbits were observed. So, this study suggested that females must take care in using treatment with betamethasone before fertilization to avoid toxicity of their offspring.

Key words: Betamethasone, Kidney, Liver, Maternal exposure, Psoriasis.

INTRODUCTION

Glucocorticoids (GCs) are a class of steroid hormones that function in virtually all vertebrate cells and tissues (Okajima *et al.* 2001). They regulate numerous physiological processes and are mainstay in the treatment of inflammation, autoimmune diseases and cancer (Masuzaki *et al.* 2001).

Since 1948, synthetic GCs have been used to treat various immune-related disorders such as psoriasis (Dogra and Mahajan, 2016). Although synthetic GCs, including betamethasone, are an essential therapy for a range of conditions, they can exert influences on developments and subsequent offspring physiology and pathophysiology (Cain and Cidlowski, 2017).

It was reported that antenatal steroid administration is associated with alterations in fetal kidney development and hypertension (Hattori *et al.*, 2013) and liver function. Pathological changes in liver demonstrated as lesions in liver, fatty cytoplasmic vacuolation and necrosis of the hepatocytes (Amar *et al.*, 2013).

In this regard, some animal and human-based studies could confirm the beneficial effects of corticosteroids, but some others did not recommend such regimens due to their related adverse consequences (Arimi *et al.,* 2021). No enough evidences are present about the side effect of maternal exposure to betamethasone prior fertilization, on new-born. In addition, previous studies paid attention to intramuscular injection during pregnancy neglecting the long-term impact of the use of topical solution. So, the goal of the present study was to determine these adverse side effects of topical administration of betamethasone solution on liver and kidney tissues.

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MATERIALS AND METHODS

Experimental animals

Twenty-four adult healthy sexual mature, White New Zeeland rabbits *Oryctolagus cuniculus* of both sexes (6 males and 18 females) of average weight of 2.5±0.5 kg, 6-8 months obtained from research station in king Faisal University. The animals were housed in steel cages under controlled condition like temperature 25±2°C and 12 hrs. light /dark cycle with free access to water, labium and pellet food diets. They were left for two weeks for acclimatization before starting the experiment. All experimental procedures were reviewed and approved by the research ethics committee at King Faisal University (Ref. No. KFU-REC/2021-03-05). They were housed in the animal house of College of Science, Building 9, King Faisal University.

Experimental design

The experiment involved three experimental groups as follows:

G I (Control group)

In this group, female rabbits (n=6) were topically exposed – on the shaved area of back skin -to the saline solution for 2 months and allowed to mate with males and then kept until delivery.

G II

In this group, female rabbits (n=6) were topically treated with betamethasone solution at a dose of 0.02 mg/kg body weight on the shaved area of back skin for 2 months, allowed to mate with males and then kept until delivery.

G III

In this group, female rabbits (n=6) were treated with betamethasone solution at a dose of 0.20 mg/kg body weight on the shaved area of back skin for 2 months, allowed to mate with males and then kept until delivery.

Application of medication

Betnovate solution - Scalp application 30 ml -Betamethasone 0.1% w/w (as Betamethasone Valerate) was topically applied on shaving area of back skin (5×5), for 2 months according to Vose *et al.* (2014).

Mating

The females were moved to the cages of males at evening with observation of mating. Specific grunting sound of male in addition to turning on one side after mating indicates successful mating. Then the females were separated next day morning and consider this is the pregnancy day zero (Wangikar *et al.*, 2005).

Sample collection

At the end of the experimental period (after 3 weeks of delivery), six newborn rabbits of both sexes (1 newborn rabbit per mother) were dissected under light anesthesia. The animals were euthanized via anesthetic exsanguination using the combination of 10 mg/kg xylazine and 100 mg/kg ketamine HCl. The blood samples (5 ml through vein puncture from lateral cephalic vein of each rabbit), liver and kidney tissues were collected. All the measurements were performed in one assay.

Serum preparation

At the end of the experiment, blood sample was collected from each new-born rabbit and whole blood samples collected in clean dry centrifuge tubes, containing no anticoagulation factors were allowed to clot for a minimum of 30 min at 37°C before centrifuged to obtain serum (1500 rpm for 15 min). Then serum was stored at -20°C until the analysis.

Determination of relative organ weight of liver, kidney and heart:

Relative organ weight=

$$\frac{\text{Mean absolute weight of organ gm}}{\text{Final weight of the animal (gm)}} \times 100$$

Determination of oxidative stress markers in liver and kidney tissues:

Preparation of tissue homogenate

Liver and kidney were immediately removed and washed using chilled saline solution. These tissues were minced and separately homogenized (10 % w/v) in ice-cold sodium-potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15 % KCl using a homogenizer (Potter–Elvehjem). The homogenate was centrifuged at 10,000 xg for 20 min at 4°C and the resultant supernatant was used for the assay of the enzyme activities the level of MDA and the protein.

Determination of oxidative stress markers in liver and kidney tissues

Activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) were determined in chosen tissues using commercial kits. The enzymes activities were expressed as U/mg protein.

Determination of hepatic parameters

Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method of Tietz *et al.* (1986), Colorimetric method of Young (1990) and Tietz (1990) respectively. Determination of Bilirubin (TOTAL) was done according to Colorimetric Diazo method of Tietz. (1990). Determination of Albumin level were done according to modified bromocresol green colorimetric method of Tietz (1995). Determination of total protein level was done according to Colorimetric method (Biuret reagent) of Tietz (1990).

Determination of renal parameters

Determination of Urea was done according to Urease-colorimetric method of Tietz (1995). Determination of Creatinine level was performed according to Colorimetric method with deproteinization of Tietz (1990). Uric acid level was determined according to Uricase-POD enzymatic colorimetric method with 4-amino-antipyrine of Tietz (1995).

Microscopical examination

For light microscopy

Liver and kidney specimens were fixed in 10% neutral formalin for 24 hour and routinely processed for being stained with hematoxylin and eosin stain (H&E) (Bancroft and Gamble, 2002). The stained sections were examined using light microscopy.

Statistical analysis

Results were presented as mean± standard error of the mean (SE) obtained from six animals. SPSS program was used for the statistical analysis of data with (one-way ANOVA) to compare the groups. In all the cases, a difference was considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of maternal exposure to topical betamethasone prior to fertilization on relative organ weights of liver and kidney in new-born rabbits

There was a decrease in the relative organ weights of liver and kidney of new-born rabbits (Table 1) which was more pronounced in GIII than in GII. Maternal betamethasone exposure before fertilization induced significant decrease in relative organ weights of liver and kidney in newborn rabbits. This agrees with result of Okajima *et al.*, 2001).

Effect of maternal exposure to topical betamethasone prior to fertilization on oxidative stress marker in new-born rabbits

GII and GIII new-born rabbits, showed significant decrease in SOD, CAT and GPx activities and an increase in the MDA levels in liver and kidney tissue indicating oxidative stress s. These alterations were more pronounced in new-born rabbits of GIII than those of GII (Table 2). Similar evidences were reported (antenatal, neonatal, postnatal or during lactation) by Jeje and Raji (2015) and Bi *et al.* (2014).

Table 1: Effect of maternal betamethasone exposure before fertilization on relative organ weight of liver and kidney of new-born rabbits.

Experimental group	Relative organ weight (g/kg b.w)			
	Liver		Kidney	
G1	22.4^{a}±0.7		5.5 ^{a}±0.5	
G2	20.6 ^{b}±0.4		4.8 ^{b}±0.5	
G3	19.9 ^{c}±0.4		4.3 ^{c}±0.7	
F (p)	436.547* (<0.001*)	888.065* (<0.001*)	552.959* (<0.001*)	585.189* (<0.001*)

Data are presented as means ± SE. N = Six experimental animals per group. Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Effect of maternal exposure to topical betamethasone prior to fertilization on indicators of liver function

There was a significant increase in serum ALP, ALT and AST activities and bilirubin level and was decrease in the concentrations of total protein and albumin (Table 3) in new-born rabbits (GII and GIII) relative to (GI). Liver enzymes are marker for liver function and integrity (Hasona *et al.*, 2017). Their activities change under several physiological and pathological circumstances (Al-Ghanim, 2014). This effect is similar to the effect of other GCs such as corticosteroid (Kondratjeva and Birgele, 2014) and may be result of oxidative stress.

Also, there was a decrease in total protein and albumin levels and increase in bilirubin level which may be due to induction of oxidative stress where reactive oxygen species are potentially very damaging to cells, leading to oxidation of essential cellular constituents including proteins, lipids and DNA (Paradies *et al.*, 2002). The observed depletion of protein content is in agreement with the fact that glucocorticoids cause protein break down (Wolthers *et al.*, 2000). The observed increase in bilirubin level was previously reported by Pettit *et al.* (2014) and may suggest altered liver function.

Table 2: Effect of maternal betamethasone exposure before fertilization on oxidative stress markers in liver and kidney of new-born rabbits.

	No.	Liver				Kidney			
		CAT (U/g tissue)	SOD (U/g tissue)	GPX (U/g tissue)	MDA (nmol/g tissue)	CAT (U/g tissue)	SOD (U/g tissue)	GPX (U/g tissue)	MDA (nmol/g tissue)
Group I	6	45.7^{a}±1.0	66.6^{a}±0.5	72.3^{a}±0.4	6.3^{c}±0.2	46.0^{a}±0.8	64.2^{a}±0.7	59.3^{a}±0.6	5.3^{c}±0.1
Group II	6	35.3^{b}±0.6	42.7^{b}±0.6	54.4^{b}±0.5	16.3^{b}±0.2	34.3^{b}±0.6	36.7^{b}±0.8	38.8^{b}±0.6	8.8^{b}±0.2
Group III	6	25.1^{c}±0.6	35.8^{c}±0.6	42.4^{c}±0.5	27.1^{a}±0.2	26.3^{c}±0.8	27.3^{c}±0.7	24.9^{c}±0.5	10.2^{a}±0.3
F (p)		189.087* (<0.001*)	875.224* (<0.001*)	1255.397* (<0.001*)	3229.926* (<0.001*)	194.908* (<0.001*)	660.017* (<0.001*)	873.731* (<0.001*)	150.380* (<0.001*)

Data are presented as means ± SE. N = Six experimental animals per group. Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 3: Effect of maternal betamethasone exposure on some hepatic parameters in sera of new-born rabbits.

	No.	Hepatic parameters					
		ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dl)	Albumin (g/dl)	Protein (g/dl)
Group I	6	38.4^{c}±0.7	55.3^{c}±0.9	25.9^{c}±0.6	0.2^{b}±0.1	4.6^{a}±0.1	7.0^{a}±0.1
Group II	6	50.6^{b}±0.8	73.3^{b}±0.8	45.0^{b}±0.5	0.4^{a}±0.1	3.9^{c}±0.1	5.9^{b}±0.1
Group III	6	76.6^{a}±0.7	104.6^{a}±1.2	86.6^{a}±0.9	0.6^{c}±01	2.6^{b}±0.1	4.7^{c}±0.1
F (p)		662.680* (<0.001*)	637.726* (<0.001*)	2116.665* (<0.001*)	103.883* (<0.001*)	54.345* (<0.001*)	3.377 (0.062)

Data are presented as means ± SE. N = Six experimental animals per group. Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Effect of maternal exposure to topical betamethasone prior to fertilization on indicators of kidney function in new born rabbits

Maternal exposure to topical betamethasone prior to fertilization induced significant increase in the values of urea, uric acid and creatinine levels in new born rabbits (GII and GII) relative to (GI) which suggested the induction of renal damage (Table 4). This is in accord with Hasona *et al.* (2017) in case of treatment with dexamethasone or Prednisolone (Wolthers *et al.*, 2000).

Effect of maternal exposure to topical betamethasone prior to fertilization on histological structure of liver of new-born rabbits

Light micrographs of liver tissue of control group (GI)

Table 4: Effect of maternal betamethasone exposure on some renal biochemical parameters in sera of new-born rabbits.

	No.	Renal parameters		
		Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group I	6	$24.8^{c}\pm0.6$	$0.3^{c}\pm0.1$	$2.0^{b}\pm0.1$
Group II	6	$39.2^{b}\pm0.6$	$0.5^{b}\pm0.1$	$2.5^{c}\pm0.1$
Group III	6	$46.0^{a}\pm0.6$	$0.7^{a}\pm0.1$	$3.5^{a}\pm0.1$
F (p)		318.940* (<0.001*)	571.740* (<0.001*)	49.468* (<0.001*)

Data are presented as means ± SE. N = Six experimental animals per group. Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

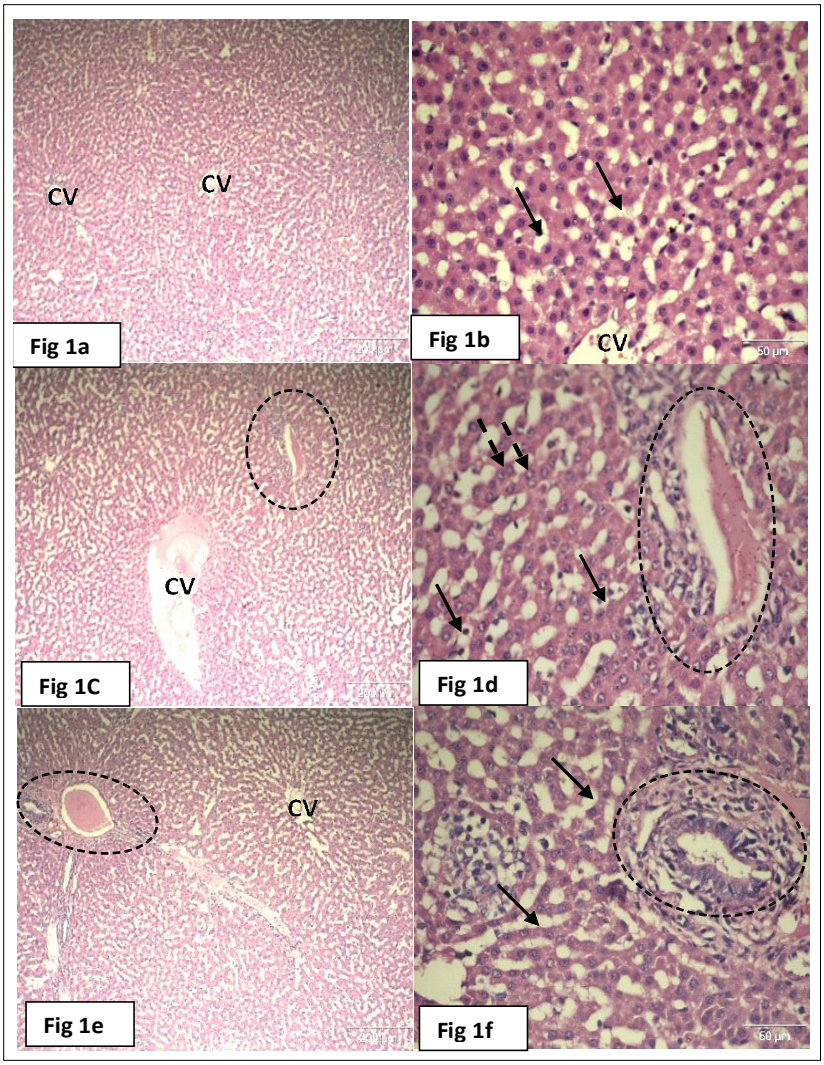

Fig (1a-1f): Light micrographs of liver sections stained with H&E. Fig 1a,1b: Hepatic tissue of control –group showing typical architecture. Fig 1c,1d: Hepatic tissue of group II, showing congested dilated central vein, portal triads with cellular infiltration, binucleated hepatocytes. Fig 1e,1f: Hepatic tissue of III –group showing necrotic hepatocytes, wide blood sinusoid, portal triads with cellular infiltration. sinusoid (arrows), central vein (CV), portal triads (dashed-circle), binucleated hepatocytes (dashed arrows). Figs.1a,1c, 1e, X100, Figs.1b,1d,1f X400.

revealed normal hepatic architecture formed of anastomising and branching polygonal hepatocytes plates arranged radially around central vein (Fig 1a). Hepatocytes are separated by vascular blood sinusoids (Fig 1b).

Chronic exposure to low dose of betamethasone (0.02 mg/kg/B. W) (group II) induced alterations in the hepatic architecture (Fig 1c). These alterations involve the presence of hepatocytes with binucleation, wide sinusoids, Kupffer cells hyperplasia, portal triads with chronic perivascular inflammation and congestion (Fig 1d). Chronic exposure to relatively high dose of (0.20 mg/kg/B.W) (group III) demonstrated more pathological alterations (Fig 1e) in which liver appeared with necrotic hepatocytes strands, portal area having major cellular infiltration and disorganized bile ductile with wide blood sinusoids (Fig 1f). Similar results were obtained in experimental animals treated with betamethasone (Badawy, 2017).

Effect of maternal exposure to topical betamethasone prior to fertilization on histological structure of kidney of new-born rabbits

Control group showed normal glomerulus surrounded by the Bowman's capsule, distal tubules with wide lumen and lined by a single layer of low cuboidal cells, proximal tubules with narrow lumen and a regular basal lamina lined by a single layer of high cuboidal cells (Fig 2a,2b). On the other hand, renal cortex of the animals in group II appeared with degenerative renal tubules, atrophied glomeruli and narrow urinary space (Fig 2c,2d). In group III, the typical architecture of cortical tissue was disappeared (Fig 2e). Atrophied glomerulus with no urinary space, cytoplasmic vacuolation, desquamated distal tubules and proximal tubules were recorded (Fig 2f). Similar changes were reported by Badawy *et al.* (2016). The presence of this vacuolation may be due to the fact that glucocorticoids influence the sodium-potassium pump, disrupting electrolyte balance and

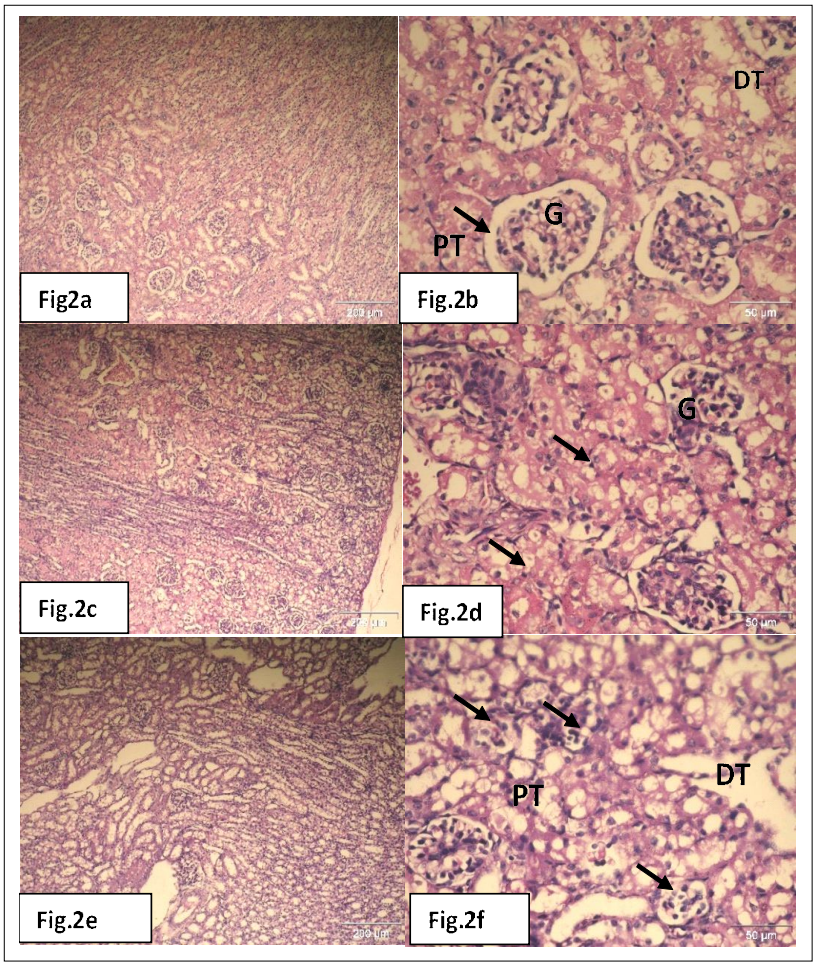

Fig 2a-2f: Light micrographs of renal cortex stained with H&E. Fig 2a,2b: Cortical tissue of the control group with no tissue damage. Fig 2b: normal well define glomerular tuft (G) with urinary space (arrow), normal proximal tubule (PT) and distal tubule (DT). Fig 2c, 2d: congested cortical tissue of group II. Fig 2d: degenerative renal tubules (arrows), atrophied glomerulus (G). Fig 2e,2f: Highly affected cortical tissue of group III. Fig 2f: atrophied glomerulus with no urinary space (arrow), desquamated distal tubules (DT) and proximal tubules (PT). Fig 2a, 2c, 2e X100. Figs.2b, 2d, 2f X400.

increasing the osmotic pressure in the cell, which leads to cell blebbing and vacuolation (Abdelhalim and Jarrar, 2011).

CONCLUSION

In summary, the results of this study suggest that maternal exposure to topical betamethasone prior to fertilization can induces biochemical and structural changes in liver, kidney of new-born rabbits. So, caution should be exercised in the use of Betamethasone therapy before fertilization. They may stop this treatment by enough period (more than 2 months) to avoid toxicity of their offspring.

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Ethical statement

The experimental protocol of this investigation was approved by Institutional Animal Care and Use Committee (IACUC) at the King Faisal University with Research Ethics Committee number: KFU-REC/2021-03-05.

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Conflict of interest: None.

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