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

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ABSTRACT

Background: Betamethasone treatment is widely used and recommended worldwide. Although, there are no enough studies to look for the risk or potential alterations due to accumulation of this drug before fertilization. In addition, previous studies payed attention to intramuscular injection during pregnancy neglecting the long-term impact of the use of topical solutions. So, the aim of the present study was to determine this side effect.

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

Result: The results of this study revealed maternal exposure to betamethasone prior to fertilization, induced oxidative stress, alterations in levels of enzymes. Further, these biochemical alterations were supported by histopathological and ultrastructural observations. So, this study suggested that females, desiring to be pregnant, should stop treatment of psoriasis with betamethasone before fertilization to avoid heart toxicity of their offspring.

Key words: Betamethasone, Heart, Newborn, Topical maternal exposure.

INTRODUCTION

Psoriasis is a skin disease with prevalence around 2-3% of the general population. The incidence is highest at the age of 20-39 years in males and 40-59 years in females (Nestle *et al*., 2009).

Although there is a wide range of therapies available for the treatment of psoriasis, either systemic or topical agents, the use of topical therapy remains a key component of the management of almost all psoriasis patients. Topical therapies available for mild-to-moderate psoriasis involve a great number of different agents, including topical corticosteroids (Reich and Bewley, 2011).

Since their introduction to dermatology, more than 50 years ago, topical corticosteroids have become the mainstay of treatment of various dermatoses including psoriasis, mainly due to their immunosuppressive, anti-inflammatory and antiproliferative properties, which makes this class of drugs useful therapy for this immune-mediated disease (Castela *et al*., 2012).

Exposure to glucocorticoids (GCS) hormones exert influences on organ growth, developments and subsequent offspring physiology and pathophysiology (Drake *et al*., 2005). Pharmaceutical industry has developed a number of synthetic analogs of GCS such as dexamethasone, betamethasone and Prednisone (Kis, 2012).

Betamethasone valerate is a mid-potency corticosteroid used to relieve psoriasis, atopic dermatitis and other corticosteroid responsive dermatoses (Shemer *et al*., 2014). However, several studies have demonstrated the disadvantages of using betamethasone during pregnancy. Exposure to the single dose of corticosteroid on the fetus, lead to adverse effects on the heart where it reduced heart rates. The greatest danger lies in the fact that the Doppler cannot detect anything and may give a false reading about fetoplacental vessels (Mariotti, 2004). Administration of glucocorticoids is associated with significant vascular side effects. In healthy sheep and primate fetuses, antenatal glucocorticoid administration induces hypertension (Koenen *et al*., 2002). Corticosterone also increased cardiac oxidative stress and inflammation and induced left ventricle atrophy (Hattori *et al.,* 2013).

Although, there are no enough studies to look for the risk or potential alterations due to accumulation of this drug before fertilization.

Therefore, the goal of the present study was to establish whether maternal topical exposure to betamethasone, prior to fertilization, would induce alterations in newborn rabbits' embryos by studying the induced biochemical, histological and ultrastructural changes.

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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, in building 9 in King Faisal University, in 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:

GI (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.
GII: 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.
GIII: 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.
Mating: Mating process was done according to the method of Wangikar *et al*., (2005).

Application of medication

Betnovate solution - Scalp application 30ml -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).

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 anesthetic exsanguination using the combination of 10 mg/kg xylazine and 100 mg/kg ketamine HCl. The blood samples (5 ml) were collected aseptically through vein puncture from lateral cephalic vein of each rabbit. Heart tissues were collected and kept frozen at -80°C before assays for 24 hours. Collected heart tissue samples were used for biochemical, histological and ultrastructural evaluations. All the measurements were performed in one assay.

Determination of antioxidant markers

Activities of catalase, superoxide dismutase and glutathione peroxidase were determined in chosen tissues using commercial kits.

Determination of inflammatory markers

The presence of TNF-α, IL-4, IL-10 and IL-6 in the heart tissues was measured with a rat standard-ELISA kit.

Determination of creatine kinase activity

It was done according to the International Federation of Clinical Chemistry (1989).

Determination of Lactate dehydrogenase activity

It was done according to Young (1990).

Light microscopic study

Cerebellar specimens were collected from all experimental groups and fixed in 10% neutral buffered formalin and processed routinely to be stained with Haematoxylin and Eosin stain (H&E). Tissue blocks were cut into thin section 5 microns and stained with H&E stain (Bancroft and Gamble, 2002). The stained sections were examined using light microscopy.

Electron microscopic study

The heart from the experimental groups were collected and then prefixed in 3% glutaraldehyde in phosphate buffer (pH 7.4). Following postfixation in 1% phosphate-buffered osmium tetroxide (Sigma Aldrich), the heart samples were dehydrated in an ethanol gradient and embedded in Araldite. Ultrathin sectioning was performed using an ultramicrotome. Staining was performed with 2% uranyl acetate and lead citrate. Inspection and imaging were performed using transmission electron microscope.

Statistical analysis

Results were presented as mean±standard error of the mean (SE). SPSS program was used for the statistical analysis of data with one-way ANOVA to compare the experimental groups. A difference was considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of maternal betamethasone exposure prior to fertilization on oxidative stress and inflammatory markers in heart of newborn rabbits

Oxidative stress occurs when the production of reactive oxygen species (ROS) overcome the antioxidant capacity in the target cell. One of the most crucial determinants of health and disease is an imbalance between pro-oxidant and antioxidant forces (Bebe and Panemangalore, 2005).

Increase free radicals cause oxidative damage to cellular biomolecules (Valko *et al*., 2007). Reactive oxygen species (ROS) such as H_2O_2, superoxide ($O._2$) and hydroxyl radical (OH) are capable to react with biological macromolecules and lead to DNA damage, lipid peroxidation and enzyme inactivation (Bagchi *et al.,* 1995). Human disease developments and cell death were induced in presense of ROS (Maurya *et al*., 2014).

It is well known that the cardiac myocyte is highly susceptible to free radical oxidative damage because of their

Table 1: Effect of maternal betamethasone exposure prior to fertilization on oxidative stress markers in heart of newborn rabbits.

	No.	Heart			
		CAT (U/g tissue)	SOD (U/g tissue)	GPX (U/g tissue)	MDA (nmol/g tissue)
Group I	6	47.7^{a}±0.5	73.0^{a}±0.5	63.4^{a}±0.9	5.4^{c}±0.1
Group II	6	33.4^{b}±50.5	59.5^{b}±0.7	45.8^{b}±0.5	7.3^{b}±0.1
Group III	6	28.3^{c}±0.4	43.2^{c}±0.5	28.9^{c}±0.6	9.6^{a}±0.1
F (p)		0.885 (0.433)	696.618* (<0.001*)	641.532* (<0.001*)	377.966* (<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 2: Effect of maternal betamethasone exposure prior to fertilization on inflammatory markers activities in heart of newborn rabbits.

	Proinflammatory		Anti-inflammatory	
	IL-6 (pg/ gm tissue)	TNF-α (pg/ gm tissue)	IL-4 (pg/gm tissue)	IL10 (pg/gm tissue)
GI	37.3^{d}±1.2	51.4^{d}±1	38.4^{ab}±1.4	52.6^{a}±1.2
GII	164.6^{a}±1.3	193^{a}±1	32.6^{c}±1.1	424.4^{d}±1.2
GIII	334.6^{c}±1.1	315^{c}±1.5	24.6^{b}±0.9	22^{b}±1.2

Data are displayed as mean±SE of n=5 rats/group. Different superscripts within the same column indicate significant differences between the groups (p≤0.05).

Table 3: Effect of maternal betamethasone exposure prior to fertilization on CK and LDH activities in sera of newborn rabbits.

	No.	CK (U/L)	LDH (U/L)
Group I	6	1.6^{c}±0.1	127.2^{c}±0.8
Group II	6	2.1^{b}±0.1	137.8^{b}±0.5
Group III	6	2.7^{a}±0.1	146.7^{a}±0.5
F (p)		47.972* (<0.001*)	260.025* (<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.

oxidative metabolism and relatively poor antioxidant defenses (Greaves, 2011).

In the present study (Table 1), new born rabbits of GII and GIII, showed significant decrease in studied antioxidant enzymes activities and increase in the MDA levels in heart tissues. These alterations were more pronounced in newborn rabbits of GIII than those of GII. These can be considered as indicators of oxidative stress. It was reported that Mother exposure to glucocorticoids (antenatal, neonatal, postnatal or during lactation) can induce oxidative stress in heart (Roy *et al*., 2009).

In the present study (Table 2), new born rabbits of GII and GIII, showed significant decrease in anti-inflammatory markers and an increase in pro-inflammatory markers. Similar result was obtained by Kramer *et al*. (2004).

Effect of maternal betamethasone exposure before fertilization on creatine kinase and lactate dehyd rogenase activities in sera of newborn rabbits

Creatine kinase (KC) is an important clinical marker for muscle damage (Bong *et al*., 2008) and lactate dehydrogenase (LDH) is released into the bloodstream in the presence of damage and toxicity of tissue and cells (Schueren *et al*., 2014).

In this study, there was a significant increase in serum levels of KC and LDH in newborn rabbits from mother exposed to betamethasone before fertilization (Table 3). This may indicate cardiac muscle injury where when cardiac muscle cells are damaged, the membranes permeability increased which results in increasing cardiac enzyme into bloodstream (Upaganlawar *et al*., 2009).

Effect of maternal betamethasone exposure before fertilization on histological structure of heart of new born rabbits

It is worthy to mention that this study is mainly concerned with the myocardial layer of the ventricle. With low magnification the paraffin sections of control tissues fixed in formaldehyde and stained with H&E showed, the myocardium with a network of cardiac muscle fibers have a small amount of faintly stained loose endomysial connective tissue exists between the bundles of muscular fibers (Fig 1a). By the usage of higher magnification, all the muscle fibers are branched, anastomose and are arranged in a linear array with only one elongated centrally located nuclei and a few amount of lipofucsin (Fig 1b).

With low magnification, the low dose of betamethasone caused myopathic alterations as abnormal heart tissue with wide separation between the bundles were illustrated (Fig 1c). The usage of higher magnification showed enlarged space between the bundles of muscle fibers with presence of lytic muscle area. Also, many necrotic cells have pyknotic nuclei were illustrated but still few cells more or less normal. With over view cardiomyocyte width, as a marker of cardiomyocyte hypotrophy was clear when compared to the corresponding controls (Fig 1d).

With low magnification, the high dose of betamethasone -treated group revealed extremely severe and widespread degenerative changes as destruction of muscle fibers with angiogenesis (formation of many capillaries (Fig 1e). With high magnification, completely all the cardiomyocyte are

necrotic cells with pyknotic nuclei and widespread of thick wall blood vessels were illustrated. With over view cardiomyocyte hypotrophy was clearer when compared to the corresponding low dose of betamethasone and controls (Fig 1f).

The present study revealed histological alteration in heart of groups II and III. These results are in accordance with the results of Hattori *et al.* (2013) concerning treatments with corticosterone or glucocorticoids.

Effect of maternal betamethasone exposure before fertilization on ultra structure of heart of new born rabbits

Examination of longitudinal ultrathin section in part of left ventricular muscle in control group showed normal morphological features. Myofibrils, nucleus and mitochondria figures (Fig 2a, 2b). In group II, the nucleus showed abnormal shaped including irregular outline, condensed hetero chromatin and abnormal shaped of nuclear envelope (Fig 2c, 2d). Also, mitochondria showed alteration where they appeared fused or segmented (Fig 2d). Similar alterations were observed in group III (Fig 2e, 2f). In addition, nucleus showed invagination and degenerated area were observed in myofibrils (Fig 2f).

The observed changes in studied tissues in betamethasone treatment groups might be indication of oxidative stress and lipid peroxidation damage of DNA and

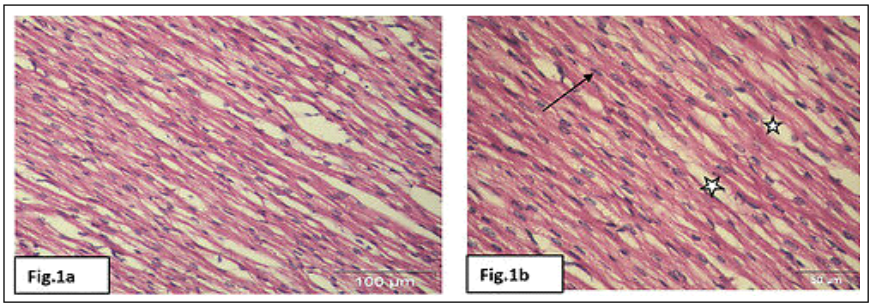


Fig 1a: Light micrograph of control heart section of newborn rabbit stained with H&E, showing: Over view of heart tissue which consists of branched muscle fibers arranged as bundles (100 µm).

Fig 1b: Enlarged part of Fig (1a) showing: Light -stained spaces separated each bundle from the other (stars), mono-nucleated spindle cells with lipofucsin (arrows) and flat dark fibroblasts nuclei (dashed-arrows) (50 µm).

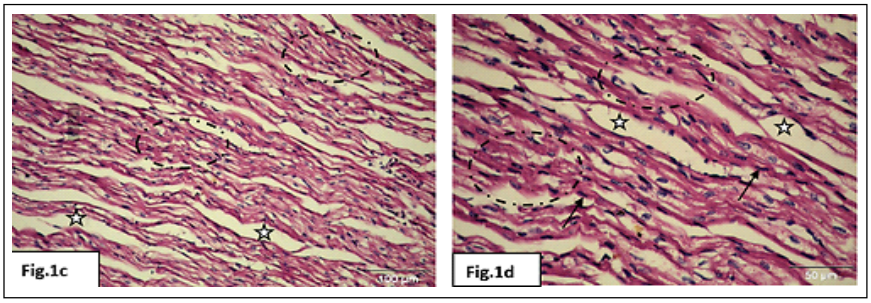


Fig 1c: Light micrograph of low dose of betamethasone -treated heart section of rabbit stained with H&E showing: Abnormal heart tissue (circle) with wide separation (star) between the bundles (100 µm).

Fig 1d: Enlarged part of Fig (1c) showing: Enlarged space between the bundles of muscle fibers (star), lytic muscle area (circle) and necrotic cell with pyknotic nuclei (arrows) (50 µm).

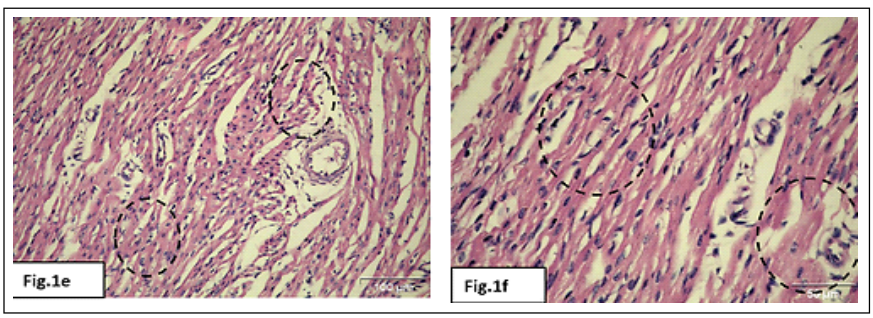


Fig 1e: Light micrograph of high dose of betamethasone -treated heart section of newborn rabbit and stained with H&E showing: Severe degenerative changes as destruction of muscle fibers in some affected areas (dashed-circle) with angiogenesis (arrows) (100 µm).

Fig 1f: Enlarged part of Fig (1e) showing completely necrotic cells with pyknotic nuclei (dashed-circle) and widespread of blood vessels (arrows) (50 µm).

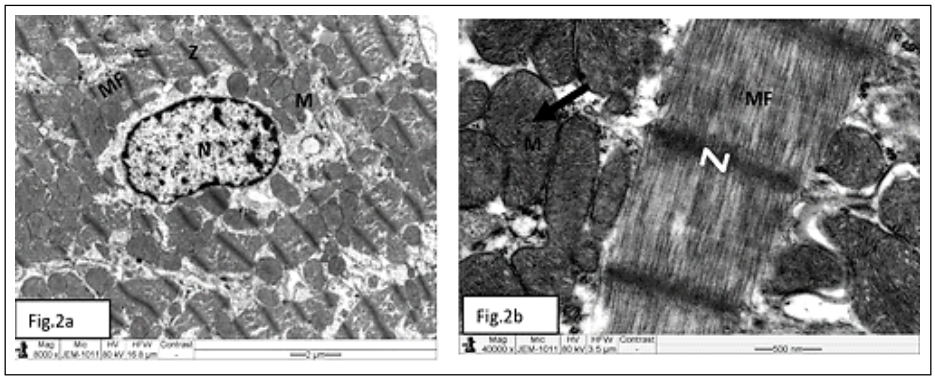


Fig 2a, 2b: Showing cardiac muscle fibers of control group.

Fig 2a: Showing muscle fibers (MF) with Z line (Z), mitochondria (M) and nucleus (N), X8000.

Fig 2b: Showing muscle fibers (MF) with Z line (Z), mitochondria (M) with clearly visible mitochondrial cristae (arrow), X40000.

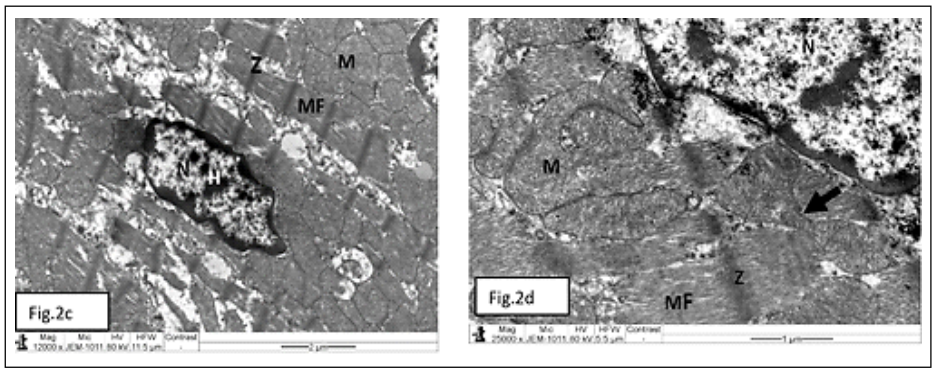


Fig 2c, 2d: Showing cardiac muscle fibers group II.

Fig 2c: Showing nucleus (N) with irregular outline condensed heterochromatin (H). MF: Myofibril, Z: Z line, M: Mitochondria, X12000.

Fig 2d: Showing fused mitochondria (M) and segmented mitochondria (arrow). N: Nucleus, MF: Myofibril, Z: Z line, X25000.

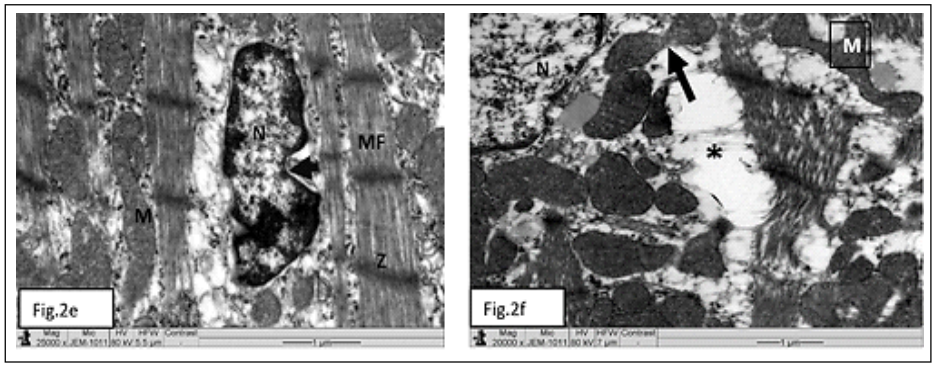


Fig 2e, 2f: Showing cardiac muscle fibers of group III.

Fig 2e: Showing nucleus (N) with invagination (arrow) and condensed heterochromatin (H). MF: Myofibril, Z: Z line, M: Mitochondria, X25000.

Fig 2f: Showing fused mitochondria (M) and segmented mitochondria (arrow), X20000.

Fig 2a-2f: Transmission electron micrograph of longitudinal ultrathin section in part of left ventricular muscle.

other cytoplasmic macromolecules which may induce damage in membranes and causes degeneration of cells (Badawy *et al*., 2016).

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.

CONCLUSION

Accordingly, it was concluded that the treatment of mother with betamethasone prior to fertilization, adversely affect the heart of newborn rabbits by biochemical, histological and ultrastructural changes. So, according to the above mentioned, it is recommended to stop betamethasone treatment by mother before fertilization as it results to serious alterations in the heart of the off spring.

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Conflict of interest: None.

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