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AMELIORATIVE EFFECTS OF CURCUMIN ON BETAMETHAZONE –INDUCED MATERNAL AND FETAL NEPHROTOXICITY IN RATS

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ABSTRACT

This study aimed to evaluate the possible adverse effects of the corticosteroid drug betamethazone on the kidney of both mother rats and their fetuses during gestation. Another aim was to ameliorate these possible adverse effects using one of the natural antioxidant namely, curcumin. Four integrated approaches namely, histological, histo-morphometric, ultrastructural and molecular were adopted. Histological examination of the kidney of the drug administered mothers showed atrophic glomeruli with widened capsular spaces of the renal corpuscles. Many proximal and distal convoluted tubules had dilated lumen with debris of hyaline casts and the lining epithelium had vacuolated cytoplasm. The renal cortex of maternally treated fetuses showed severe degeneration of glomeruli along with disrupted proximal and distal convoluted tubules. The epithelial cells lining the convoluted tubules showed cytoplasmic vacuolation and hemorrhage. Electron microscopic examination of the kidney of the mothers and their fetuses injected with betamethazone revealed conspicuous alterations, represented by thickening of the capillary basement membrane. Most of the foot process of podocytes appeared irregular with complete disappearance of their membranes. The lining cells of the convoluted tubules had degenerative changes and displayed partial destruction of the microvilli of the apical brush borders. Administration of betamethazone induced variations in the expressed protein and DNA fragmentation in the kidney tissue of both mothers and their fetuses. Administration of curcumin after betamethazone caused improvement in many adverse changes in the kidney of both mothers and their fetuses.

KEWORDS: Betamethazone, Kidney, Histological, Ultrastructural, Molecular, Fetuses, Curcumin.

INTRODUCTION

Corticosteroids are an important class of naturally occurring and synthetic steroid hormones that affect virtually every aspect of human physiology. The former are produced by the cortex of the adrenal gland and are classified as either glucocorticoids (GCs) or mineralocorticoids.[1] GCs are commonly used during pregnancy to reduce the immune response in allergic or inflammatory or skin diseases. Other indications include promoting fetal lung maturity, treating infertility and maintaining pregnancy, or as replacement hormone therapy.[2] All corticosteroids cross the placenta, but some corticosteroids are more readily inactivated than others. Prednisone and cortisone are the preferred medication for treating maternal disease, since they have shorter half-lives, while dexamethasone and betamethazone are used with fetus requires specific treatment, such as for respiratory distress.[3]

GCs most notably act during late gestation to stimulate surfactant production by the lung. In addition, GCs play an essential role in regulation of metabolism, immune system reaction and more significantly they are essential for normal fetal development and are important for the development and maturation of various fetal tissues including the liver, gut, skeletal muscle and adipose tissue in preparation for extrauterine life.[4] However, there is evidence that prenatal administration of synthetic GCs may have adverse effects on the developing fetus with consequences in later life.[5,6] Therefore, exposure of the developing fetus to inappropriate patterns or concentrations of GCs has been proposed as a mechanism for fetal programming.[7,8,9] Moreover, their chronic administration is associated with numerous systemic side effects, such as bone mass loss[10,11] skeletal muscle atrophy, adipose tissue mass redistribution, glucose intolerance, decrease in insulin sensitivity, which causes or aggravates diabetes and susceptibility to infection.[12]

Short periods of high maternal GCs given during early to mid gestation tend to impact on organ development rather than birth weight for example, nephron number in the kidney is reduced.[13,14] GCs bind to the glucocorticoid receptor, which is present in almost all cells[15] and have anti-inflammatory, immunosuppressive, anti-proliferative, and vasoconstrictive effects.[16] The mechanism of GCs actions during the prenatal life is

poorly understood. Renal damage has been reported as one adverse consequence of early-life GC overexposure.[17] Data suggest that maternal GC administration, e.g., dexamethazone or betamethazone, when the kidney is at a preglomerular stage of development, impairs nephrogenesis and reduces glomeruli numbers, which may contribute to hypertension in late life. The mechanisms by which these effects occur are still largely unknown.[13,18] Among the GCs, betamethazone has become very popular, being more commonly used for a number of diseases including rheumatic disorders such as rheumatoid arthritis and systemic lupus erythematosus, skin diseases such as dermatitis and psoriasis, allergic conditions such as asthma and angioedema, preterm labor to speed the development of the baby, Crohn disease, cancers such as leukemia, and along with fludrocortisone for adrenocortical insufficiency, among others. It can be taken by mouth, injected into a muscle, or applied as a cream.[19]

There is an increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage. Consequently, current research is now directed towards finding naturally occurring antioxidants of plant origin.[20] Recently, turmeric is picking up a great deal of consideration for its potential medicinal properties. Curcumin is a well-known biologically active natural phytochemical phenolic compound found as a major component in turmeric and considered as its most active constituent.[21] Curcumin has been shown to exhibit a variety of biological activities in both *in vitro* and *in vivo* animal studies. It has been shown to exhibit anti-inflammatory[22], antioxidant[23], anti-bacterial[24], immunomodulatory[25], antimicrobial[26,27], hypoglycemic[28] and hypocholestrolemic activities.[29,30] Curcumin injection has been reported to prevent renal damage in streptotocin diabetic rats[31], and to protect against oxidative stress in renal cell lines.[32] Curcumin also prevents or attenuates nephrotoxicity caused by cisplatin[33], adriamycine[34] arsenic[35,36] and diabetes.[37]

Turmeric has been used during pregnancy as a part of traditional medical systems for thousands of years, and no traditional or Western authority recommends against its use during pregnancy.[38] The present study therefore, aimed to investigate the possible adverse effects of betamethazone administration on both mother rats and their fetuses during gestation. Another aim was to ameliorate these possible adverse effects using one of the natural antioxidant namely, curcumin.

MATERIALS AND METHODS

Animals and grouping

Principles of animal care and use were carefully followed during the conducting of all experiments. Healthy mature virgin females and fertile males of Wistar albino rats (*Rattus norvegicus*), weighing 135 ± 15g and aged 17±1 weeks, were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt. Rats were kept in the laboratory for at least one week before initiation of the experiments for acclimatization. They were housed in specially designed plastic rodent cages at Faculty of Science, Menoufiya University and maintained at 25 ± 2ºC in 12h light: 12h dark cycle. Free access of water and standard diet composed of 50% ground barely, 20% ground yellow maize, 20% milk and 10% vegetables was supplied. Mating was achieved by housing females with males at a ratio of one male with two females overnight. Females were checked daily in the morning for the presence of a copulatory plug and the presence of sperms in unstained native vaginal smears. Therefore, vaginal smears were carried out to give a precise determination of the onset of gestation. The day at which vaginal smear was positive has been considered as the day zero of pregnancy. Day 20 was determined as the end point for experimentation. A total of 60 rats were used for the present study.

The pregnant rats were divided into groups according to their weights in order to ensure equal means and standard errors for total body weight in each group at the start of the experiment. The selected pregnant females were divided equally (10 in each group) into four groups as follows:

1. Control group, administrated distilled water.
2. Curcumin injected group given oral dose of curcumin (15.75 mg/kg).
3. Experimental betamethazone injected group given subcutaneous dose of betamethazone (0.3 mg/kg).
4. Combined betamethazone and curcumin injected group, received subcutaneous injection of betamethazone first followed by an oral injection of curcumin one hour later.

Betamethazone administration

Betasone tablets (each tablet contains Betamethazone 0.5 mg) was manufactured in Memphis Company for pharmaceutical and chemical industries, Cairo, Egypt and purchased from pharmacy in Shebeen El-Koom, Monoufyia. Tablets were ground and dissolved in distilled water and subcutaneously administrated daily by insulin syringe during the organogenesis phase of gestation i.e. starting from the sixth day and ending at the 15th day of gestation. The applied dose was 0.3 mg /kg body weight which is equivalent to the human dose[39]

Water extraction of curcumin

Dry turmeric rhizomes of the plant *Curcuma longa* were purchased from a local market at Shebeen El-koom, Menoufiya, Egypt. One kilogram fresh *Curcuma longa* were crushed into powder, dissolved in distilled water, filtered and orally given daily at a dose of 15.75 mg/kg body weight[40] during the organogenesis phase of gestation.

INVESTIGATED PARAMETERS

A- Histological investigation

For light microscopical examination, maternal kidney as well as the fetal kidney from the control and experimental groups were fixed by immersion in 10% neutral formalin for 24 hours at room temperature and washed under running tap water for 12 hours. The specimens were dehydrated in an ascending series of alcohol, cleared in butanol and embedded on molten paraffin.

Sections of 5µm thickness were cut using a rotatory microtome (Leica, Model Rm 2125, Germany). Sections were mounted on albumin-coated slides. Histological staining was performed with Ehrlich's hematoxylin and counter-stained with aqueous eosin. Histological sections were subjected to microscopical examination and when necessarily photographing using Olympus microscope.

B- Histo-morphometric parameters:

B1- Estimation of nephron numbers

An estimation of fetal nephron numbers was performed by counting representative glomerular numbers in microscopic sections after H & E staining under X40 magnification (n=10 fetuses per group).

B2- Estimation of cortex and medulla diameter

An estimation of cortex and medulla diameter of fetal kidney was performed under X10 magnification (n=10 fetuses per group).

C- Ultrastructural investigation

For ultrastructural investigation which has been done using transmission electron microscope, specimens of maternal and fetal kidney of both control and experimental groups were separated and immediately fixed for 4 hours at room temperature in 2.5% Glutaraldehyde and 2% paraformaldehyde in 0.1 cacodylate buffer (PH. 7.4).

After rinsing in cacodylate buffer, samples were post fixed in buffered solution of 1% osmium tetra-oxide for three hours at 4°C. This was followed by dehydration in ascending grades of ethanol and embedded in epoxy-resin. Ultra-thin (50 nm) sections were cut, mounted on formvar-coated grids and stained with uranyl acetate for 10 minutes. Sections were then stained with freshly prepared lead citrate for 10 minutes and washed with distilled water. Examination of grids was done by using JEOL electron microscope, Electron Microscope Unit, Tanta University. Selected sites were digitally photographed and then printed on Kodak sensitive printing paper.

D- Sodium Dodecylsulfate (PolyAcrylamide Gel Electrophoresis) SDS-PAGE

SDS (Sodium dodecylsulfate)-PAGE of denatured proteins of the maternal and fetal kidney was carried out in 15% polycarylamide gels pH8.8, in a discontinous buffer according to Maziel and Jr,[41] Photograph of the gel was taken using Sony digital camera.

E- Determination of DNA fragmentation

As a measure of apoptotic DNA fragmentation, the presence of DNA ladder was determined according to Woldek *et al*.,[42] Extraction of DNA was done according to the method of Aljanabi and Martinez.[43] The DNA was visualized and photographed with illumination under ultraviolet (uv) light using a photo documentation hood (Fisher Scientific, Pittsburg PA, USA) equipped with a polaroid 667 film with an orange filter (Kodak, Rochester, NY, USA). The UV reacts with the ethidium bromide to show the DNA fragments. Apoptotic bands appeared and located at 200 bp and its multiples.

Data evaluation and statistical analysis

All data sets were expressed as mean ± standard error of the mean (SEM). The data were analyzed statistically for normal distribution (student' t test) and homogeneity of variances (Levene test) using statistical program of social sciences (SPSS) software for windows, version 11. Differences were considered insignificant whenever $P>0.05$. The significances of the obtained data were classified into three categories, *i.e.* $P<0.0001$***, $P<0.001$** and $P<0.05$*.

RESULTS

Number of Glomeruli

Fig. (1), illustrates the changes in the number of glomeruli in the 20 days fetuses of different groups. There was insignificant increase in the total number of glomeruli in the curcumin group (19 ± 0.462) compared with the control group (18.7 ± 0.411). The number of glomeruli were highly reduced in fetuses maternally injected with betamethazone (7 ± 0.801). Injection of betamethazone followed by curcumin led to a marked increase in the total number of glomeruli compared with betamethazone group, but was still lower than the control group (12.37 ± 0.679; 7 ± 0.801; 18.7 ± 0.411 for betamethazone + curcumin, betamethazone and control groups respectively).

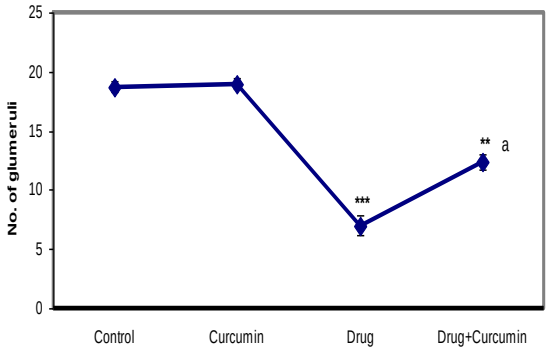

Figure (1): Graph showing changes in the number of glumeruli of experimental fetuses in different groups compared with control. a= highly significant compared with betamethasone group.

Diameter of cortex and medulla

The kidney of fetuses maternally injected with curcumin demonstrated a significant higher diameter of the cortex compared with the control group (Fig. 2). The diameter of the cortex in fetuses maternally injected with betamethazone was highly reduced compared with the control group (2.63 ± 0.204; 3.92 ± 0.083 for betamethazone injected and control groups respectively). There was an evident increase in the diameter of the cortex in the fetuses maternally injected with both betamethazone and curcumin compared with the betamethazone group and this led to a low significant increase compared with control (3.23 ± 0.160; 2.63 ± 0.204; 3.92 ± 0.083 for betamethazone + curcumin, betamethazone, and control groups respectively). In a similar trend, the diameter of the medulla increased insignificantly in the curcumin group, significantly reduced in betamethazone group and evidently increased in the curcumin+betamethazone group compared with the control group (6.15 ± 0.299; 2.23 ± 0.284; 3.98 ± 0.166; 3.16 ± 0.071 for curcumin, betamethazone, betamethazone+curcumin and control groups respectively).

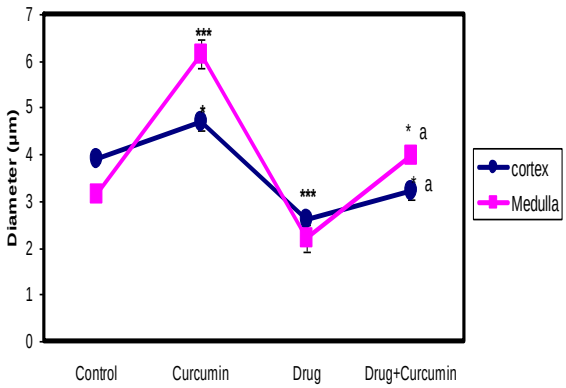

Figure (2): Graph showing changes in the diameter of cortex and medulla of the kidney of experimental fetuses in different groups compared with control. a= highly significant compared with betamethasone group.

Histological observation

A- Maternal Kidney

The renal cortex of the control mothers displayed the presence of the renal corpuscles. Each renal corpuscle was formed of the glomerulus surrounded by Bowman's capsule. The glomerulus composed of a network of anastomotic capillaries. The Bowman's capsule was formed of outer parietal layer of simple squamus epithelium, capsular space and inner visceral layer of modified epithelial cells (podocytes) that completely surround the glomerular capillaries. The renal cortex also showed the presence of many proximal convoluted tubules which lined by cuboidal cells with basal rounded nuclei and apical brush border. The distal convoluted tubules were lined by simple cuboidal cells with rounded and centrally located nuclei (Fig. 3A).

Curcumin injected mothers exhibited normal renal architecture with well developed Bowman's capsule as that of the control group. Normal distal and proximal convoluted tubules can be seen (Fig. 3B).

The kidney of betamethazone-injected mothers showed evident degenerative changes. Examination of the renal cortex revealed an apparent shrinkage of the glomeruli and widening of the capsular space of the renal corpuscles. Few glomeruli were atrophied, while others were clefted or acquired bizarre profile. Moreover, a few glomeruli were markedly congested. The urinary spaces of some Malpighian corpuscles were evidently widened (Figs. 3C). The kidney tubules showed variable degrees of degeneration. Many proximal and distal convoluted tubules had dilated lumen. The lining epithelium of these tubules showed degenerative changes. Some cells had vacuolated cytoplasm and others had damaged luminal borders (Fig. 3D). Lumens of some convoluted tubules were either erased or highly reduced, containing some debris of hyaline casts. Besides, cells lining few kidney tubules were broken down or vanished. In addition, fibrocytes increased in the intertubular tissue (Fig. 3E). Many of the interstitial capillaries were congested, where marked cellular exudates in certain areas are indicative of chronic inflammation. The endothelial cells of some kidney tubules were markedly swollen and these swellings were so obvious that these cells almost blocked the lumina of tubules indicating intertubular haemorrhages brought about by extravasted blood (Fig. 3F).

In the kidney of mothers injected with betametha zone and curcumin injected mothers, despite the presence of mild tubular degeneration and epithelial vacuolization in the proximal tubules, glomeruli maintained a better morphology compared with the betamethazone group. The renal corpuscles showed little shrinkage of the glomeruli with slight widening of the capsular space as compared with betamethasone group. Administration of curcumin to betamethazone-injected mothers resulted in an evident improvement in the structure of proximal tubular cells but little leucocytic infiltration appeared in some areas (Fig. 3G).

B- Fetal kidney

At the light microscope level, the renal cortex of the control fetuses showed the presence of two cortical zones. The subcapsular zone contained immature forms of the renal developmental stages; the juxtamedullary zone contained mature renal corpuscles surrounded by convoluted tubules. The medullary rays were seen extending between these two zones. The subcapsular zone contained nephrogenic mesenchyme in addition to all immature forms of renal developmental stages, which included cell condensates, and renal vesicles. Aggregations of mesenchymal cells forming caps were seen in close association with the upper sides of the ureteric buds (Figs. 3H).

Fetuses maternally injected with curcumin alone displayed normal renal structural similar to that of the control group (Figs. 3I).

Fetuses maternally injected with betamethazone exhibited severe degeneration of glomeruli showing shrinkage along with disrupted proximal and distal convoluted tubules. There was increased periglomerular space associated with hydronephrosis (Figs. 3J). The epithelial cells lining the convoluted tubules showed cytoplasmic vacuolation and hemorrhage (Fig. 3K).

In fetuses maternally injected with both betamethazone and curcumin, despite the presence of mild tubular epithelial vacuolization in the urinary tubules, glomeruli maintained a better morphology compared with the betamethasone group (Fig. 3L).

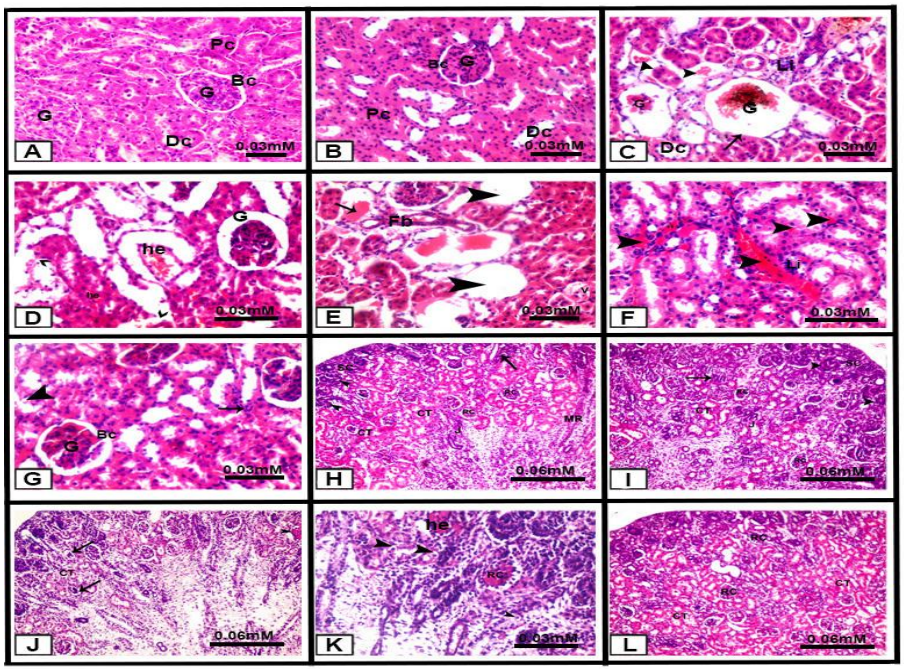

Figure (3): Photomicrographs of transverse sections passing through the cortical region of kidney. (A-G) Maternal kidney (A) Control (B) Curcumin injected (C-F) Betamethazone injected showing shrinkage of the glomeruli, dilated lumen and degenerated epithelium of the distal convoluted tubule (arrow head) and hyaline cast (arrow). (G) kidney of mother injected with betamethazone + curcumin showing somewhat normal structure, arrow indicated leucocytic infiltration while arrow head indicates mild tubular degeneration. (H-L) Fetal kidney (H) Control & (I) Curcumin groups (J&K) Betamethazone group showing disrupted glomeruli (arrow), hemorrhage (arrow head) and vacuolization in the convoluted tubules (arrow head). (L) kidney of betamethazone + curcumin injected group showing almost normal renal corpuscle and convoluted tubules. G. Glomeruli, Pc. Proximal convoluted tubule, Dc. Distal convoluted tubule, Bc. Bowmen's capsule, he. hemorrhages, Fb. fibrocytes, CT. convoluted tubules, RC. renal corpuscle . (H&E)

Ultrastructural observation

A- Maternal kidney

Transmission electron microscope examination of the renal cortex of the control mothers revealed that the podocytes were highly branched and had many primary and secondary processes. Smaller divisions (pedicles) came out of these processes and interdigitates with their counterparts of another podocytes. The narrow spaces between the adjacent pedicles i.e. the filtration silts were evident (Fig. 4A).

Many proximal convoluted tubules which lined with cuboidal cells were evident. Each cell had basally located nucleus, with a clear nucleolus. The nucleus of the cells of the convoluted tubules had an evenly distributed chromatin and intact nuclear envelope. They had well formed microvilli forming the apical brush border. There were many small vesicles at the base of the microvilli. From the basal membrane into the interior of the cell, numerous basal folds emerged, which were inserted with numerous rod-like mitochondria oriented parallel to the cell axis as well as relatively few dense formations (Fig. 4B).

The cells of the distal convoluted tubules of the control rats had thin basement membrane, few microvilli and basal nuclei. Moreover, numerous, elongated and round-shaped mitochondria were seen between the basal enfolding (Fig. 4C).

Like that of the control group, the cells of the proximal convoluted tubule in curcumin injected rats had a brush border of numerous microvilli projecting within the tubular lumen and intense cytoplasm due to high content

of organelles. The nucleus was spherical, euchromatic, surrounded by numerous mitochondria and apical vacuoles (Fig. 4D).

The renal cortex of mothers injected with betamethazone alone possessed changes in the renal corpuscles and the cells of the renal tubules. The glomerular basement membrane showed obvious thickening in most of renal glomeruli. Most of the foot process of podocytes appeared irregular with each other with complete disappearance of their slit membranes (Fig. 4E). The cells lining the proximal convoluted tubules showed thickening of basement membrane and irregularly-shaped nuclei with chromatin margenation and invagination of the nuclear membrane. Some nuclei showed shrunken electron dense appearance. The cytoplasm appeared to be rarified and had many lysosomes and damaged mitochondria (Fig. 4F &G). In addition, vascular congestions with red blood cells clusters occurred both at the level of the capillaries placed at the base of the tubules (Fig. 4H). In some cells of proximal convoluted tubules the microvilli completely disappeared (Fig. 4I). In few tubules the lining cells gave wide blebs into the tubule lumen which became almost erased. The basal enfolding of these cells were poorly developed (Fig. 4J).

Examination of the cells lining the distal convoluted tubules showed variable degrees of damage. Nuclear membrane appeared irregular with chromatin clumps. The cytoplasm appeared to be rarified and had many lysosomes and damaged mitochondria. Vacuolation of the cytoplasm, decrease of the number of mitochondria, damages of the apical pole of some cells and the presence of cellular detritus in the lumens of the tubules resulting from the affected tubules were evident (Fig. 4K).

The proximal tubular cells of the betamethazone and curcumin injected mothers revealed marked amelioration compared with those of betamethazone group. The nucleus appeared spherical with normal pattern of chromatin distribution and nucleolus. The mitochondria appeared healthy with preserved cristae. Moreover, a few apical vacuoles, intact plentiful microvilli and thin basal lamina were observed. The apical cytoplasm of some renal tubules was moderately rarified with some dense bodies (Fig. 4L).

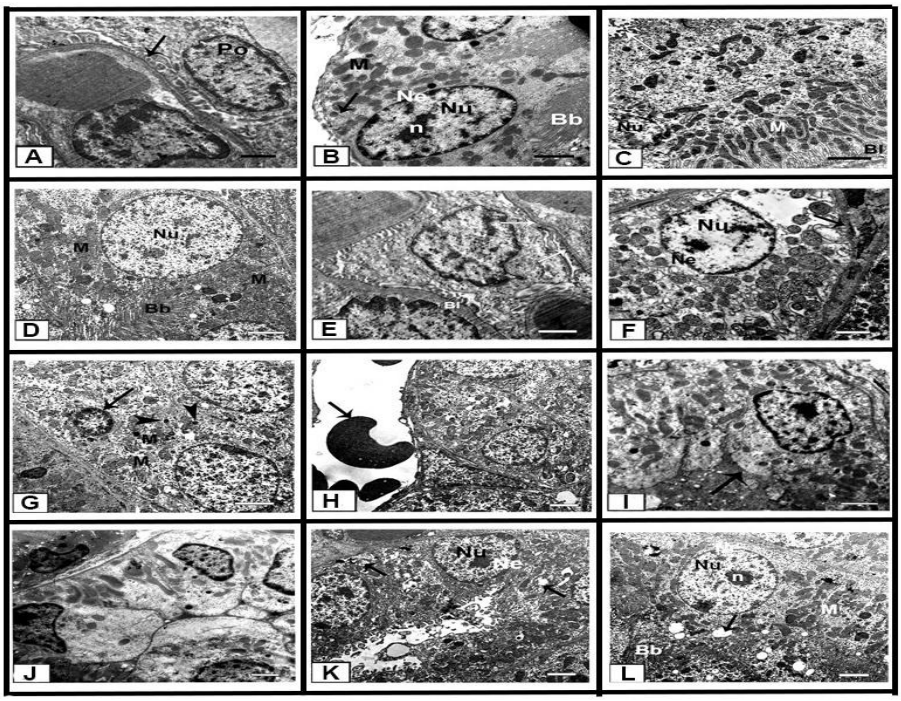

Figure (4): Transmission electron photomicrographs of kidney sections of mothers. (A-C) Control (A) renal corpuscle showing podocytes (po) and the filtration silts (arrow). (B) cells of a proximal convoluted tubule with basal enfolding (arrow) (C) cell of a distal convoluted tubules with few microvilli (arrow). (D) cell of a proximal convoluted tubule of curcumin group (E-K) kidney of betamethazone injected group showing (E) renal corpuscle with irregular foot process of podocytes (arrow). (F-J) cell of a proximal convoluted tubule showing shrunken electron dense nucleus (arrow), many lysosomes (arrow head), vascular congestions (arrow), vacuolated cells with completely appearance of microvilli (arrow) (K) cell of distal convoluted tubule with degenerated mitochondria (arrow). (L) cells of proximal convoluted tubules of betamethazone and curcumin injected mothers showing apical vacuoles (arrow) and intact brush border. Nu. nucleus, Ne. nuclear envelope, n. nucleolus, M. mitochondria, Bb. brush border, Bl. basal lamina. Scale bar = 2µm.

B- Fetal kidney

The renal cortex of the control rat fetuses aged 20 day had glomeruli containing podocytes with flat cytoplasmic sheets and foot processes, in addition to endothelial cells with few fenestrations. The glomerular filtration barriers revealed the presence of double glomerular basement membranes formed of epithelial and endothelial laminae densa. Podocytes possessed flat cytoplasmic sheets and foot processes. These processes rest upon the basement membrane of the glomerulus leaving narrow filtration slits between them (Fig. 5A).

The proximal convoluted tubules of the control group were seen with their lining epithelial cells resting on a basement membrane and had short apical microvilli, oval euchromatic nuclei and electron-dense cytoplasm. Mitochondria were found randomly oriented in the cytoplasm and alongside the lateral cell membrane with few observable basal enfoldings (Fig. 5B). The distal convoluted tubules had a few microvilli at the apical surface, The nuclei were relatively large and the mitochondria were elongated and occupied the cytoplasmic compartment (Fig. 5C).

The renal cortex of 20 day aged rat fetuses maternally injected with curcumin during the organogenesis phase of development had an elaborate shape, well developed microvilli along their lumina and many spherical or elongated mitochondria. The nuclei of such cells were relatively large, mostly euchromatic with prominent nucleoli and always lying the thin basal lamina (Fig.5D).

Investigating the renal cortex of 20-days old fetuses maternally injected with betamethazone revealed evident ultrastructural changes. The foot processes of podocytes were fused and obliterating the filtration slits. The capillary basement membrane was thickened and the urinary space was completely obliterated (Fig. 5E). The proximal convoluted tubules demonstrated marked thickening of their basement membranes. In some parts of the tubules, the microvilli of the brush border were partially degenerated with aggregation of many vesicles and large vacuoles near the basal part of the microvilli. The mitochondria were few and their matrices were condensed so that their fine structures become obscure. The nucleus appeared highly degenerated with irregular nuclear envelope and condensed chromatin. The cytoplasm of most cells became highly rarefied and vacuolated (Fig. 5 F-H). Other cells appeared without nucleus and gave broad blebs into the tubule lumen which became almost obliterated (Fig. 5 I&J).

The distal convoluted tubules appeared vacuolated and freed from organelles except few electron dense mitochondria. The nuclear heterochromatins were aggregated on the inner surface of the irregular nuclear envelope (Fig. 5K).The proximal tubular cells of the 20-day aged fetuses maternally injected with betamethazone and followed by curcumin showed a marked amelioration compared with the previous group. The nucleus appeared euchromatic with prominent nucleolus. The microvilli of these cells appeared intact and nearly unaffected. On the other hand, the nuclei of some cells appeared irregular and somewhat electron dense. The cytoplasm contained many healthy mitochondria (Fig. 5L).

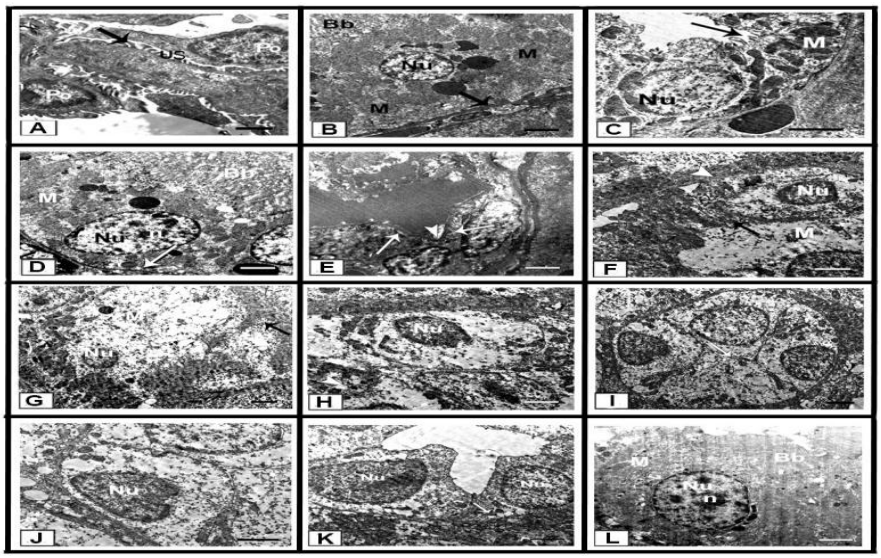

Figure (5): Transmission electron photomicrographs of kidney sections of 20-days old rat fetuses. (A-C) control (D)Curcumin group (E-K) Kidney of rat fetuses maternally injected with betamethazone showing (E) part of the glomerulus with fused foot process (arrow), marked thickening in the basement membrane (arrow head) (F-J) a proximal convoluted tubule cells with marked thickening of their basal lamina (arrow head), partial degeneration of the apical microvilli (arrow) and obliterated lumen (arrow). (K) vacuolated distal convoluted cell with degenerated mitochondria (arrow). (L) a proximal convoluted cell maternally injected with betamethazone and curcumin. Nu. nucleus, n. nucleolus, M. mitochondria, Bb. brush border, Bl. basal lamina. Scale bar = 2µm

Proteomic (SDS- PAGE) analysis

SDS-PAGE for protein of the kidney in curcumin injected mothers showed similar type of expression as control. Administration of betamethazone showed variations in the expressed protein which lacked bands at 250, 25 and 20 KDa. SDS-PAGE of betamethazone and curcumin group exhibited similar expression in protein bands as control except at band 250 KDa which disappeared in this group (Fig. 6).

Comparative protein pattern for molecular weight of betamethazone injected fetal kidney showed that 250, 100, 37, 25, 20 and 10 KDa protein were all down regulated whereas, 50 and 75 KDa were up regulated compared with control and curcumin groups. Administration of curcumin after betamethazone exhibited an evident increase in all protein bands compared with betamethazone alone (Fig. 6).

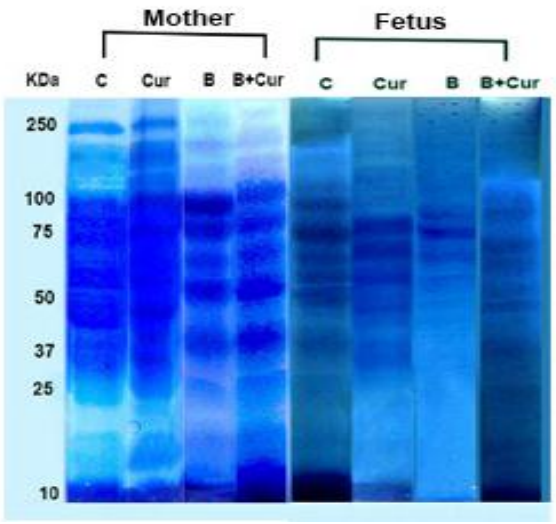

Figure (6): Changes in protein banding patterns of maternal and fetal kidney of different animal groups (control (C), curcumin (Cur), betamethazone (B) and betamethazone+curcumin (B+Cur)) using SDS-PAGE.

DNA fragmentation

A- Maternal Kidney

As can be seen in Figure (7), there was a lack of DNA fragmentation in the kidney cells of the control and curcumin injected mothers. DNA degradation was extensive in the kidney cells of betamethazone injected mothers. The 5th lane which represents the kidney cells of the mothers injected with both betamethazone and curcumin exhibited some DNA fragmentation but less than that injected with betamethazone alone.

DISCUSION

Although many types of corticosteroids are available, few studies have looked at the teratogenic potential of each one independently. Most of the studies analyzed the various drugs together, making it difficult to evaluate the teratogenic risk for a specific drug. In the current study, prenatal betamethazone administration during the

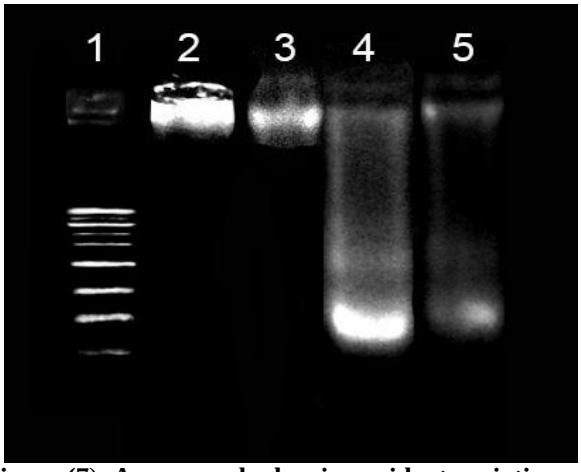

Figure (7): Agarose gels showing evident variations of DNA fragmentation in extracts from the mother' kidney cells, marker (Lane 1), control (Lane 2), curcumin (Lane 3), betamethazone (Lane 4) and betamethazone+curcumin (Lane 5).

B- Fetal Kidney

Cells of the kidney of fetuses aged 20 days of control and curcumin injected mothers possessed intact DNA without any fragmentation. Highest incidence of genomic DNA fragmentation was markedly increased in fetuses of betamethazone injected mothers. On the other hand, Fetuses of mothers received betamethazone followed by curcumin during the organogenesis phase of gestation revealed less genomic DNA fragmentation compared with betamethazone group (Fig.8).

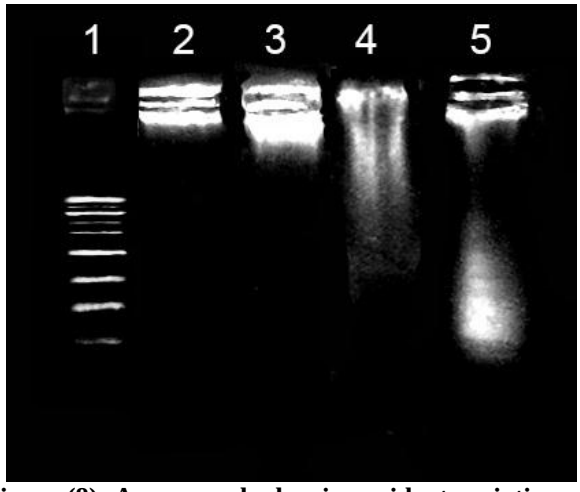

Figure (8): Agarose gels showing evident variations of DNA fragmentation in extracts from the fetuses kidney cells, marker (Lane 1), control (Lane 2), curcumin (Lane 3), betamethazone (Lane 4) and betamethazone+curcumin (Lane 5).

organogenesis phase of development i.e. from the sixth to the 15th day of gestation was shown to induce pronounced changes in both maternal and fetal kidney.

The histological examination of the maternal renal cortex revealed several adverse effects for betamethasone administration. Similar effects were also seen by Pereira

et al.[44], who reported renal adverse effects under the continuous exhibition with corticoids, this event was characterized by the edema and a great infiltration by the excessive number of macrophages and lymphocytes. This in turn, suggests an alteration in the glomerulus filtration caused by the renal toxicity of the corticosteroid. Sekita-Krzak *et al.*[45] reported that the renal corpuscles were highly dilated after administration of dexamethazone in the toxic dose of 120 mg/kg/24 h. Glomerular damage, congestion, hemorrhages, and tubular degeneration cleared after rabbit treated with 5 mg/Kg bwt boldenone (Anabolic-androgenic steroid).[46]

On contrary, the administration of 10 mg/kg dexametazone in rats for single and twice repeated injection as well as for 7 days was verified in the histological and histochemical analysis that both single and twice administration of dexamethazone do not cause the damage of the kidney parenchyma, whereas after 7 days of everyday application of the preparation irreversible changes can be observed.[47] The present histological analysis of the fetal kidneys revealed a severe degeneration of glomeruli in the form of shrinkage along with disrupted proximal and distal convoluted tubules. There was increased periglomerular space in sections of the betamethazone injected group which showed hydronephrosis. The epithelial cells lining the renal tubules showed cytoplasmic vacuolation and hemorrhage. Moreover, there was an evident reduction in the diameter of both cortex and medulla of the fetal kidney. Ortiz *et al.*[13] reported that rats which received prenatal dexamethasone on days 15 and 16 of gestation had an increase in interstitial fibrosis and glomerulosclerosis.

Betamethazone administration resulted in a reduction in glomerular number of the 20 day old fetuses compared with control. This is in agreement with Ortiz *et al.*[13] who demonstrated that short periods (2d) of exposure of the pregnant rat to dexamathazone at specific times during gestation had varying effects on kidney structure. Maternal dexamethazone (0.2mg/kg) administration on 15-16d or 17-18d caused a reduction in glomerular number in 2 month old male and female offspring. A similar period of maternal dexamathazone administration in mid-gestation to the spiny mouse caused a reduction in nephron number in the offspring.[14] Celsi *et al.*[48] found that injection of dexamathazone (0.1mg/kg/d) from day 1 to parturition in the rats resulted in a significant reduction in glomerular number in the 20 day old fetuses. In the sheep, short-term mid-gestation betamethazone administration (0.17mg/kg, 80-81d) decreased nephron number in the 135d fetuses.[49] The administration of dexamethazone to pregnant rats (0.1 mg/kg/day) throughout gestation resulted in 50% reduction in the number of glomeruli when assessed at 20 days of age.[50]

Singh *et al.*[51] revealed that a 2-day exposure to dexamethazone inhibits ureteric branching morphogenesis and nephrogenesis and alters the expression of several genes known to regulate ureteric branching morphogenesis. These findings suggest that the inhibition of ureteric branching morphogenesis may be one of the major mechanisms through which GC exposure results in a reduced nephron endowment. In addition, there appears to be a specific time during renal development when the kidney is susceptible to the adverse effects of GCs. Nephrogenesis commences in the rat at 12 to 13 days of gestation and continues until approximately one week after birth.[52]

Many anti-inflammatory drugs have been reported to cause multiple kidney damage. Our results showed that various ultrastructure alterations were observed in the renal cells of mothers and their fetuses in the betamethazone group. It has been reported that kidneys of rats given the therapeutic dose of the anti-inflammatory drug ketoprofen daily for eight weeks showed disorganized glomerular capillaries and their lining endothelium showed degeneration. The podocytes were deteriorated with rarefied cytoplasm and fragmented primary processes. Also, the foot processes appeared occasionally broad. In some cells of proximal convoluted tubules the microvilli completely disappeared. Occasionally, these cells gave broad blebs into the tubule lumen which became almost obliterated. The cytoplasm of few cells lining the distal convoluted tubules became moderately rarefied and vacuolated.[53] Likewise, Abdel- Gawad.[54] and D'Agati[55] observed necrotized glomeruli and glomerulosclerosis in albino rats after long-term administration of piprofen and asprin respectively. Also, Abdel-Kader *et al.*[56] noticed atrophied glomeruli and ruptured parietal layer of Bowman's capsules in rats treated with indomethacin. Fong and Cohen[57] reported that the anti-inflammatory drug, ibuprofen, induced acute necrosis of the cells of proximal tubules, loss of the brush border of these cells and poorly developed basal infoldings. Moreover, Abdel-Kader[56] found that indomethacin induced degeneration of the epilthelial layer lining the proximal convoluted tubules in kidney of rats accompanied with appearance of hyaline casts in some tubules. In addition, injection of Diethylstilbestrol, synthetic estrogen to male rats showed dramatic changes of the components of the cortex. The podocytes were hypertrophied that erase urinary spaces and compressed the capillary tuft. The capillaries were congested and mitochondria were swollen with loss of cristae and numerous vacuoles.[58] The observed nuclear and cytoplasmic changes in nephrocytes in betamethazone injected group might be attributed to the induction of oxidative stress and lipid peroxidative damage of DNA and other cytoplasmic macromolecules which may induce damage in the membranes and cause degeneration of the cells.

GC hormones are known to have a wide variety of molecular effects by induction or repression of proteins at the transcriptional level via a receptor system. GCs, depending on the nature of the target tissue, can not only regulate carbonhydrate, protein and nucleic acid

metabolism but can also accelerate or inhibit cellular growth or differentiation.[59] The results of the present study demonstrate that betamethazone is capable of inducing extensive effect on DNA and total protein in the tissues under investigation in both mothers and their fetuses. The cells of betamethazone injected mothers showed extensive DNA fragmentation compared with control group. However, SDS-PAGE for protein of the renal tissues in betamethazone injected group exhibited a marked lose in most protein bands compared with the control group. Similar to the outcome of the present results, an earlier study by Slotkin *et al*.[60] revealed that administration of 0.2 or 0.8 mg/kg of dexamethasone to pregnant rats on gestational days 17, 18, and 19, the DNA and protein content of the kidney became markedly subnormal during the first postnatal week, the ontogenetic period of rapid cell division. Distelhorst[61] showed that GCs induce extensive DNA fragmentation in acute lymphoblastic leukemia cells. It can be speculated that the lethal event in GC-induced cell death is a destruction of the regular chromatin structure.[62] Furthermore, changes in protein expression in response to GC overexposure in albino Wistar rats were identified during late fetal development.[63]

The present study ascertained that, there is no difference between control and curcumin extract groups. These results are in agreement with observations of previous workers who reported that curcumin is safe and well tolerated.[64] Indeed, the present study showed that curcumin caused marked improvements against the adverse effects caused after betamethazone administration. It is highly possible that the evident improvements in all changes caused by betamethazone administration is owing to the fact that curcumin has antioxidant and anti-inflammatory properties.[65] Many studies confirmed the ameliorative effect of curcumin on the induced nephrotoxicity. Curcumin co-treatment ameliorated the acetaminophen-induced histopathological renal changes in rats.[66] In another study by Venkatanarayana *et al*.[67] found that supplementation of vitamin E and curcumin to CCl4-injected rats was found to ameliorate the renal toxicity. Regenerative changes in glomerulus and convoluted tubules observed in curcumin and vitamin E supplemented rats. In support of our results earlier findings also have suggested that the administration the turmeric powder in food of diabetic rats resulted in a considerable inhabitation of renal pathological injuries. It decreased dilation of urinary space and hyaline cast in the tubules.[37] Other experiments also observed that in mice[68] and rats[69] which were pre-treated with curcumin and subsequently treated with cadmium chloride showed improvement of glomeruli and proximal tubular cell damage observed with cadmium chloride alone. Kumar *et al*.[70] clarified that chlorpyrifos administered mice followed by administration of curcumin showed regeneration of glomerulus, Bowmen's capsule and renal tubules.

Recent study by Soliman *et al*.[71] showed that prior treatment with curcumin could significantly attenuate the lead-induced nephrotoxicity. The kidney of lead acetate-intoxicated group showed hyaline casts in the lumen of renal-convoluted tubules with vacuolar degeneration of tubular epithelium and congestion of renal blood vessels, perivascular fibrosis, oedema around congested blood vessels, and degenerated tubular epithelium. The kidney in curcumin and lead acetate injected rats showed restoration in normal nephron structure. Moreover, El-Mahalaway[72] demonstrated that curcumin reduced the harmful effects of aflatoxin on the histological structure of the rat's renal cortex. The tubules of aflatoxin injected rats showed degenerative and necrotic changes with disruption of basal lamina, but the glomeruli showed an enlargement with dilation of their capillaries lumina in some areas, while the other areas showed glomerular atrophy with obliteration of their capillaries lumina. Administration of curcumin led to a marked improvement of various changes. These results alongside the outcome of the present study are in agreement with the findings of some investigators who reported the protective effect of curcumin against nephrotoxicity.[73]

Curcumin had ameliorative effects on DNA fragmentation and total protein in all studied maternal and fetal tissues. Recent study by Elsayed[74], found that curcumin and green tea had ameliorative effects on DNA of the liver of mice as a result of gasoline inhalation, and these results were in agreement with the studies of Li *et al*.,[75] in their study of green tea, curcumin, grapestone and resveratrol caused protective potential effects on DNA against nitrobenzene-induced DNA adductions. Furthermore, Aziza *et al*.[76] found that curcumin has ameliorative effect on DNA fragmentation in colon cancer induced by 1,2 dimethylhydrazine in rats. Mathuria and Verma[77] showed that curcumin injection along with aflatoxin significantly ameliorates aflatoxin-induced changes in DNA, RNA and protein contents in the liver and kidney of mice. Cheng *et al*.[78] investigated the inhibitory effects of curcumin on nicotine-DNA adduction *in vivo*. They suggested that curcumin is beneficial to prevent the harmful adduct formation and thus to block the potential carcinogenesis induced by nicotine. Another study by Madkour[79] showed that curcumin administration to lambda cyhalothrin-treated animals showed a marked decrease in liver DNA laddering when compared with the lambda cyhalothrin - treated group. Siddique *et al*.[80] stated that, curcumin inhibits the generation of ROS that are responsible for DNA damage. This action of curcumin was explained by Piwocka *et al*.[81] who declared that curcumin leads to attenuated DNA fragmentation due to the elevation of GSH.

Similar to our result, Sarvalkar *et al*.[82] demonstrated that the electrophoretic changes in protein content in mice was significantly decreased during aging, after curcumin treatment in protective and curative groups, it was again increased significantly. Thus, curcumin is able

to ameliorate the stress induced changes in protein profile during aging. Ameliorative effect of curcumin in mice might be due to its antioxidative property. Also, Sharma *et al*.[83] showed that *Curcuma longa* injection along with aflatoxin ameliorates aflatoxin-induced changes in protein contents in the kidney of mice. Changes in total protein could be due to increased necrosis in the kidney. Thus reduction in protein biosynthesis as well as increased necrosis could be responsible for this defect. Many other investigators have also reported a decrease in protein content in kidney of aflatoxin-fed animals.[84]

In the light of histological, ultrastructural results and molecular findings, the present data confirmed that betamethazone had adverse effects on the kidney of both mothers and their fetuses. Furthermore, curcumin might be a potential candidate agent against experimentally induced betamethazone maternal and fetal nephrotoxicity via its antioxidant and free radical-scavenging properties. However, further investigations are needed to demonstrate the exact mechanism of curcunin on betamethazone induced nephrotoxicity in mother rats and their fetuses.

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