Effects of exposure to gibberellic acid during pregnancy and lactation on the postnatal development of the renal cortex in the albino rat

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Background
Gibberellic acid (GA3) is one of the plant growth regulators that are widely used in Egypt, to increase the growth of fruits and vegetables. Little is known about the effects of GA3 on the mammalian tissues.
Aim of the work
This work was aimed to reveal the effects of exposure to GA3 during pregnancy and lactation on the postnatal development of the renal cortex in the albino rat.
Materials and methods
Forty pregnant females, weighing 200–300 g, were divided into two equal groups: group A (control) and group B (experimental). The pregnant rats of group B were given GA3 in a dose of 0.2 g/l of drinking water (equivalent to 55 mg/kg of body weight) from the 14th day of pregnancy until day 21 after delivery. The same dose of GA3 was given to the offsprings after weaning (day 21) till adulthood (3 months). The offsprings were sacrificed at the following ages: newborn, 10 days, and adult (3 months). Kidney specimens were processed for light microscopic examination, electron microscopic examination, and immunohistochemical study. In addition, morphometrical and statistical analysis of the renal cortical thickness was done.
Results
The renal glomeruli and the convoluted tubules showed degenerative changes in the three treated studied ages with delayed development of the glomeruli in addition to extravasation of blood and inflammatory cellular infiltration in the interstitial tissue.
Conclusion and recommendation
GA3 has harmful effects on the histological development of the renal cortex. So, it should be used cautionary.

Keywords:
gibberellic acid; postnatal development; renal cortex

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Introduction

Gibberellic acid (GA3) is one of the plant hormones that used worldwide in agriculture to increase the growth of fruits and vegetables [1–3].

People are exposed to GA3 by consumption of fresh fruits and vegetables [4]. Occupational exposure occurs through inhalation of the powder and dermal contact giving the picture of an acute toxicity [5].

Studies indicated that these plant growth regulators induce oxidative stress, leading to cell damage in many organs [6]. Of these free radicals, malondialdehyde is the most important [7].

Materials and methods

Drug

GA3 was obtained from Oxford Company, Biological Company (Oxford Laboratories, Mumbai, India) in a powder form that is soluble in water.

Experimental animals

A total number of 40 adult female and 10 adult male albino rats (3-month old), weighing 200–300 g, were obtained from the Animal House, Faculty of Medicine, Assiut University. They were housed at room temperature and supplied with a standard pellet food with the tap water *ad libitum*. Every four females were housed in a cage with one male and mating was allowed between the female and male rats. Every morning the females were examined for the presence of the vaginal plug and vaginal smears were examined to detect the commence of pregnancy.

Experimental protocol

The pregnant rats were divided into two equal groups:

Group A (control): composed of 20 pregnant female rats. They received no treatment.

Group B (experimental): composed of 20 pregnant female rats which were given GA3 in a dose of 0.2 g/l of drinking water (equivalent to 55 mg/kg of body weight) from the 14^{th} day of pregnancy until day 21 after delivery [8]. The same dose of GA3 was given to the offsprings after weaning (21 day) till adulthood (3 months). Offsprings included in the present study were divided into two groups (control and treated). For each group, the offsprings were sacrificed at the following ages: newborn, 10 days, and adult (3 months).

Histological study

The animals were anesthetized by ether inhalation then subjected to intracardiac perfusion of normal saline 0.9% NaCl. At each of the studied ages the kidney was extracted, fixed in the 10% buffered formalin for light microscopic study, and immunohistochemical staining and 2.5% glutaraldehyde for electron microscopy. The kidneys of the studied rats were prepared for paraffin embedding. Sections (8–10 μm) stained with hematoxylin and eosin stain and periodic acid Schiff (PAS) stain and semithin sections (1 μm) stained with toluidine blue to be examined under the light microscope.

Ultrathin sections (0.1 μm) were prepared for transmission electron microscopic examination using uranyl-acetate and lead citrate.

Immunohistochemical staining was performed using avidin–biotin peroxidase for localization of Bcl-2 protein (B-cell lymphoma 2). The primary antibody used was a rabbit polyclonal antibody (Biological Company, USA).

Morphometrical study of the renal cortical thickness was done by using Image Analyzer Computer System. Statistical differences between the groups were tested by paired *t* test.

Results

Hematoxylin and eosin stain

Newborn rats

The newborn control albino rat renal cortex showed few immature forms of renal glomeruli in the outer cortex with more mature renal glomeruli at the deeper parts. Proximal convoluted tubules showed an acidophilic granular cytoplasm and basal nuclei. Distal convoluted tubules showed a dilated lumen (Fig. 1a and b).

The outer cortex of newborn-treated albino rat renal cortex revealed crowded immature renal glomeruli while deeper cortex showed degenerative glomerular changes. Some renal tubules showed loss of normal architecture of cells, degenerative changes, and mitotic figures. Undifferentiated solid masses of cells were seen. Acidophilic exudates and intertubular extravasation of red blood cells were observed (Fig. 1c and d).

10 days old rats

The renal cortex of a control 10 days old albino rat showed uniform appearance of the cortex with disappearance of all immature forms. Smaller renal corpuscles with compact capillary tuft were seen in the superficial cortex and larger lobulated glomeruli in the deeper cortex (Fig. 2a).

The renal cortex of a treated 10 days old albino rat demonstrated some subcapsular immature forms of the renal glomeruli. The deeper cortex showed degenerative changes in the glomeruli. The epithelial lining of the renal tubules showed degenerative changes. Undifferentiated masses of cells were detected (Fig. 2b).

Adult rats

The renal cortex of a control adult albino rat showed numerous renal corpuscles. The proximal convoluted tubules appeared lined by pyramidal cells with narrow lumina. The epithelial cells showed indistinct cell boundaries, a rounded vesicular basal nucleus, and an apical brush border with acidophilic granular cytoplasm. The distal convoluted tubules had wide lumina and appeared lined by low cuboidal epithelium with rounded nuclei and less acidophilic cytoplasm with no brush border. Peritubular capillaries were observed (Fig. 2c).

Renal cortex of a treated adult albino rat revealed degenerative changes with interstitial extravasation of blood (Fig. 2d).

Toluidine blue stain

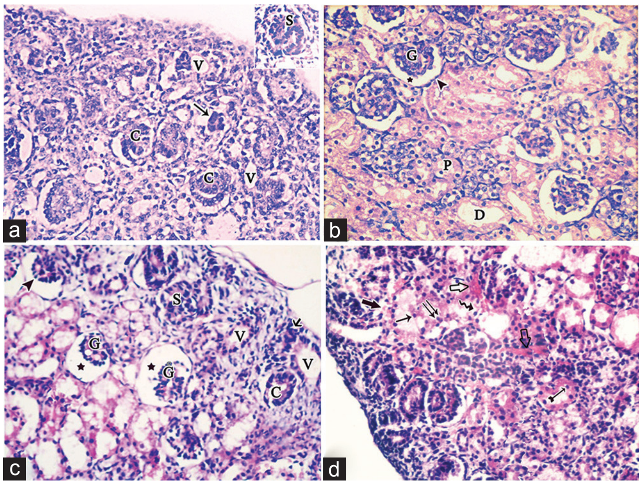
The control rats' renal cortices showed the normal renal cortical structure (Fig. 3a, c, and e).

The renal cortex of the treated rats showed disturbed architecture of glomeruli. The proximal and distal convoluted tubules showed degenerative changes with an interstitial inflammatory cellular infiltration and extravasation of blood cells (Fig. 3b, d, and f).

Periodic acid Schiff stain

The control rats' renal cortices, revealed positive reaction in the mesangium of the glomeruli, the basement

Figure 1



(a) A photomicrograph of a section in the superficial cortex of a control newborn rat showing some immature forms of renal glomeruli such as cell condensate (arrow), comma-shaped (C) bodies, and renal vesicles (V). The inset shows S-shaped bodies (S) (H and E, ×400). (b) A photomicrograph of a section in the deeper cortex of a control newborn rat showing mature renal glomeruli (G) and Bowman's capsule with its parietal layer (arrow head). Note the distinct Bowman's space (asterisk). Proximal (P) and distal (D) convoluted tubules are seen (H and E, ×400). (c) A photomicrograph of a section of the renal cortex of a treated newborn rat showing a cell condensate (short arrow), renal vesicles (V), comma-shaped (C), and S-shaped bodies (S). Note some glomeruli with densely stained pyknotic nuclei (arrow head) and others show shrunken vacuolated glomerular capillary tuft (G) and dilated Bowman's space (asterisk) (H and E, ×400). (d) A photomicrograph of a section in the renal cortex of a treated newborn rat showing the epithelial lining of some tubules with mitotic figures (double arrow) and sloughing of some cells into the tubular lumen (thin arrow). Other cells reveal vacuolated cytoplasm (wavy arrow) and pyknotic nuclei (tailed arrow). Note acidophilic exudates (open arrow) and extravasation of red blood cells (thick arrow) in between the renal tubules (H and E, ×400). H and E, hematoxylin and eosin.

membrane of the parietal layer of the Bowman's capsule and the renal tubules and in the apical microvilli of the proximal convoluted tubules. The positivity of the PAS reaction was increasing from the newborn stage to the adult age (Fig. 4a, c, and e).

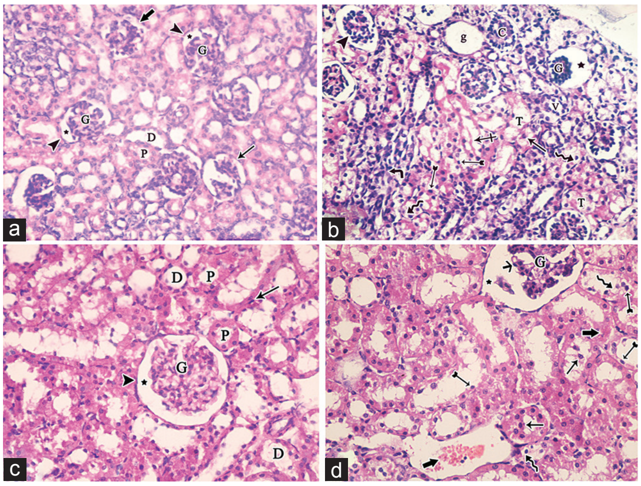
A decreased PAS-positive reactivity was detected in the mesangium of most of the glomeruli in the treated rats' renal cortices. The proximal tubules showed a weak and disrupted reaction along the apical microvilli in comparison with the control rats (Fig. 4b, d, and f).

Electron microscopic examination

The renal cortex of the control newborn rats showed double glomerular basement membranes. The glomeruli showed podocytes with primary and secondary (foot) processes (Fig. 5a).

In the control 10 days albino rats, the renal corpuscle showed glomerular blood capillaries with fenestrated

Figure 2



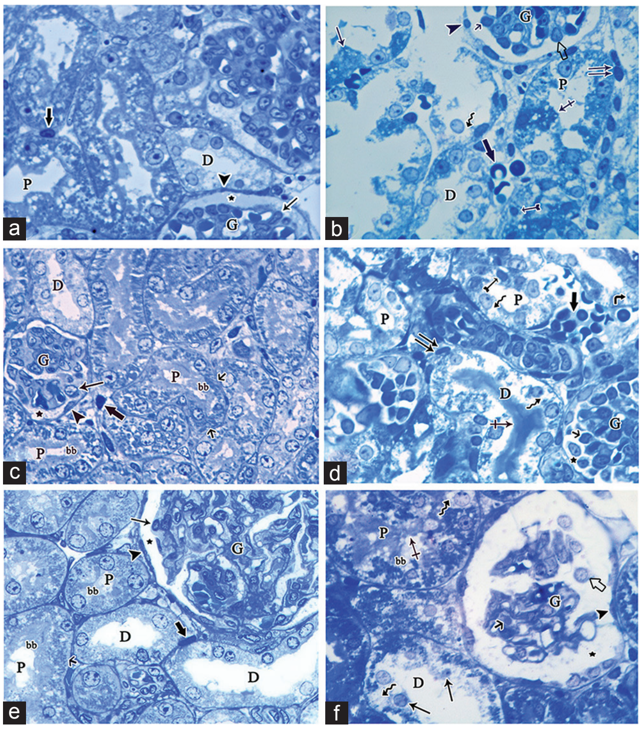
(a) A photomicrograph of a section in the renal cortex of a control 10 days old albino rat showing the renal corpuscles (G) with evident Bowman's spaces (asterisk). Note the parietal layer of the renal corpuscles (arrow head). Note the renal corpuscles in the superficial cortex (thick arrow) and the renal corpuscles in the deeper cortex (thin arrow). Proximal (P) and distal (D) convoluted tubules are seen (H and E, ×400). (b) A photomicrograph of a section of the renal cortex of a treated 10 days old rat showing comma-shaped (C) bodies. Note undifferentiated solid masses of cells (curved arrow). Some glomeruli show hypercellularity (arrow head). Other glomeruli show shrinkage (G) and widening of the Bowman's space (asterisk). Note glomeruli (g) with loss of architecture where only the parietal layer is detected. The epithelial lining of the renal tubules shows pyknotic nuclei (tailed arrows) and cytoplasmic vacuolation (wavy arrows). Note epithelial debris (crossed arrow) and exfoliation of cells into the tubular lumen (thin arrow). Loss of normal architecture of cells in many tubules (T) is noticed (H and E, ×400). (c) A photomicrograph of a section of the renal cortex of a control adult rat showing lobulated glomeruli (G). Bowman's capsule appears with its parietal layer (arrow head). Note the distinct Bowman's space (asterisk). Proximal (P) and distal convoluted tubules (D) are seen. Note peritubular capillaries (arrow) (H and E, ×400). (d) A photomicrograph of a section in the renal cortex of a treated adult rat showing markedly lobulated and fragmented glomeruli (G) with wide Bowman's space (asterisk). The glomeruli (G) also show densely stained nuclei (short arrow). The epithelial lining of some tubules show cytoplasmic vacuolations (wavy arrows) and pyknotic nuclei (tailed arrows). Some tubules show rupture of their lining epithelium and desquamation of the cells into the lumen (thin arrows). Extravasation of blood is seen (thick arrow) (H and E, ×400). H and E, hematoxylin and eosin.

endothelium. The glomerular filtration barriers revealed fused glomerular basement membranes. The podocyte showed a nucleus and processed primary processes and foot processes (Fig. 5c).

The renal cortex of the control adult rats revealed that the glomerular basement membrane consisted of an external and internal laminae rarae and lamina densa in between. Podocytes were seen with primary and secondary foot processes (Fig. 5e).

The renal cortex of the treated rats showed irregular thickness of the glomerular basement membrane with degenerative changes in the endothelial cells and the podocytes (Fig. 5b, d, and f).

Figure 3



(a, c, and e) A photomicrograph of a semithin section in the renal cortex of a control newborn (a), 10 days old (c), and adult (e) albino rats showing a mature glomerulus (G). The parietal layer of the Bowman's capsule (arrow head) and the cells of the visceral layer (thin arrow) are seen. The Bowman's space is also seen (asterisk). Proximal (P) and distal (D) tubules have normal epithelial lining cells. The proximal convoluted tubules of the 10 days old and adult rats (P) show the presence of the brush border (bb) and well-formed basal striations (short arrow). Peritubular capillaries (thick arrow) are also seen (toluidine blue, ×1000). (b, d, and f) A photomicrograph of a semithin section in the renal cortex of a treated newborn (b), 10 days old (d), and adult (f) albino rats showing glomeruli with some nuclei are dense (short arrow) while others appear pale (open arrow). The cells of the parietal layer of the Bowman's capsule show dense nuclei (arrow head). The Bowman's space (asterisk) is obliterated in the 10 days rats and wide in the adult rats. The epithelial lining of the proximal (P) and distal (D) convoluted tubules shows marked cytoplasmic vacuolations (wavy arrow), degenerated cells (thin arrow), apoptotic nuclei (tailed arrow), or rarified nuclei (curved arrow) and cellular debris inside their lumen (crossed arrow). The epithelial lining of the proximal convoluted tubules (P) shows loss of the apical microvilli in the newborn rats and disruption of the brush border (bb) in the adult rats. Note inflammatory cellular infiltration (double arrow) and extravasation of blood (thick arrow) (toluidine blue, ×1000).

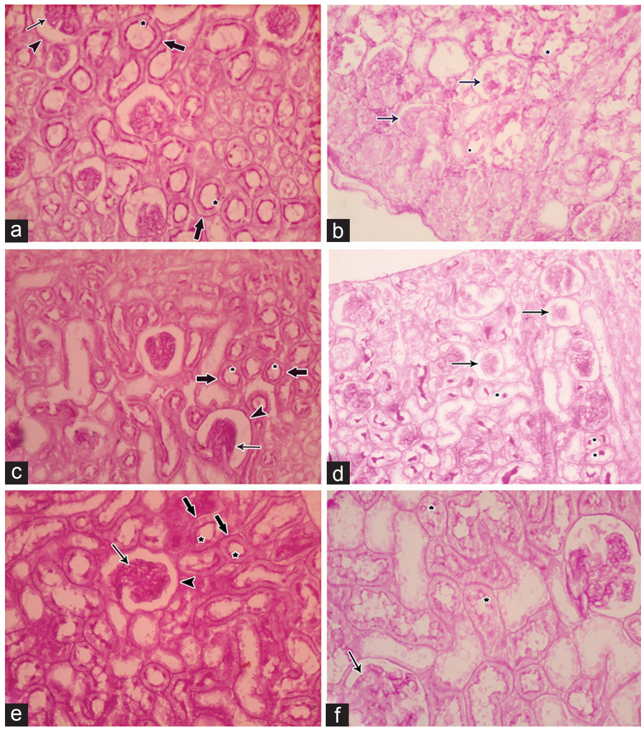
The proximal and distal convoluted tubules showed the normal epithelial lining in the renal cortex of the control rats (Figs. 6 and 7).

The epithelial cell lining proximal and distal convoluted tubule revealed degenerative changes in the renal cortex of the treated rats (Figs. 6 and 7).

Immunohistochemical results (Bcl-2)

The immunostained sections in the control rat's renal cortex revealed an intense brown color of Bcl-2 immunoreactivity within the cytoplasm of the cells lining most of the proximal tubules and weak reaction

Figure 4



(a, c, and e) A photomicrograph of a section in the renal cortex of a control newborn (a), 10 days old (c), and adult (e) albino rats showing positive periodic acid Schiff (PAS) reaction in the mesangium of the glomeruli (thin arrow), the basement membrane of the parietal layer of the Bowman's capsule (arrow head), the basement membrane of the renal tubules (thick arrow), and in the apical microvilli (asterisk) of the proximal convoluted tubules (p) (PAS, ×400). (b, d, and f) A photomicrograph of a section in the renal cortex of a treated newborn (b), 10 days old (d), and adult (f) albino rats showing decrease PAS reaction in the mesangium of most glomeruli (arrow). The proximal convoluted tubules show weak and disrupted PAS reaction along the apical microvilli (asterisk) in comparison with the control rats (PAS, ×400).

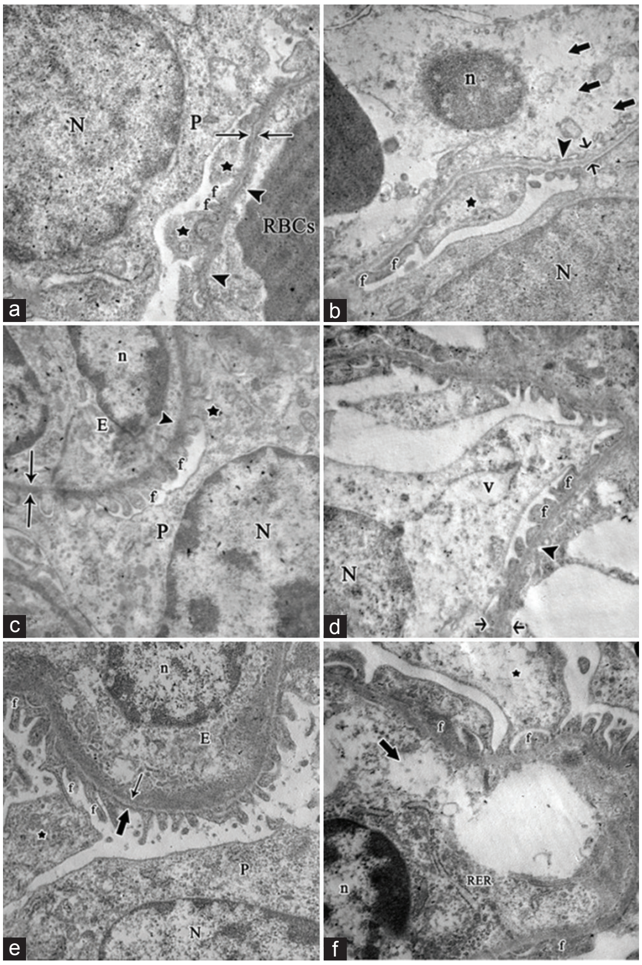
in the distal tubular cells. A very weak staining of the parietal layer of the Bowman's capsule and very weak or absent staining of the capillary unit was detected. The Bcl-2 cytoplasmic site of reaction was stained brown and nuclei stained blue (Fig. 8a, c, and e).

In the treated newborn rats' renal cortex, a weak brown positive Bcl-2 immunoreactivity was detected in the cytoplasm of the cells lining of most of the proximal tubules. Bcl-2 immunostaining was very weak or absent in the cells lining the distal convoluted tubules with a negative Bcl-2 immunoreactivity in the renal glomeruli (Fig. 8b, d, and f).

Morphometrical

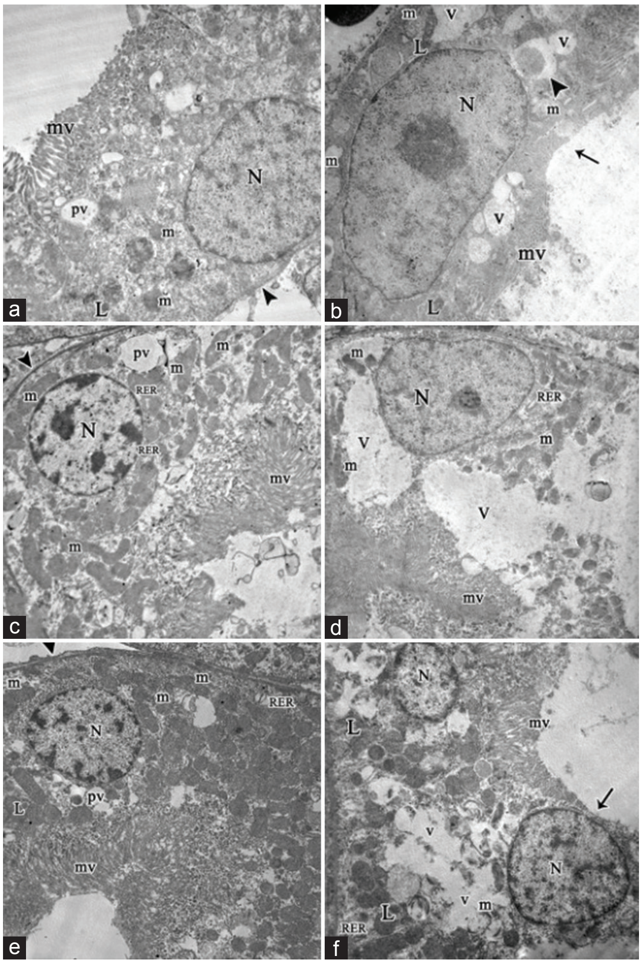
Morphometrical and statistical analysis of the renal cortical thickness showed a decrease in cortical thickness in the treated rats. The decrease was an insignificant ($P > 0.05$) in the treated newborn rats, a highly significant ($P < 0.001$) in the treated 10 days rats and a significant ($P < 0.05$) in the treated adult

Figure 5



(a, c and e) An electron micrograph of a section in the renal cortex of a control newborn (a), 10 days old (c) and adult (e) albino rats showing the glomerular basement membrane consists of endothelial and epithelial laminae densa (arrow) in the newborn rats, appears fused (arrow) in the 10 days old rats and consists of an external (thick arrow) and internal (thin arrow) laminae rarae and lamina densa in between in the adult rats. The glomerular blood capillaries are lined by fenestrated endothelium (arrow head) and contain red blood cells (RBCs). The endothelial cell (E) shows evident nucleus (n). The podocyte (P) contains a nucleus (N) and possesses primary (asterisk) and secondary foot processes (f) (TEM × 14000). (b, d and f) An electron micrograph of a section in the renal cortex of a treated newborn (b), 10 days old (d) and adult (f) albino rats showing an apoptotic nucleus (n) and cytoplasmic vacuolations (thick arrow) in the endothelial cells lining the glomerular capillary. The glomerular basement membrane reveals some areas are thickened (short arrow). Note disruption of the endothelial lining (arrow head). The endothelial cells show large intended nucleus (n) with peripheral chromatin condensation, marked cytoplasmic vacuolations (thick arrow) and dilated rough endoplasmic reticulum (RER). The podocyte shows a nucleus (N), vacuolated cytoplasm (v) and primary processes (asterisk) with broadening and fusion of the foot processes (f) (TEM × 14000).

Figure 6



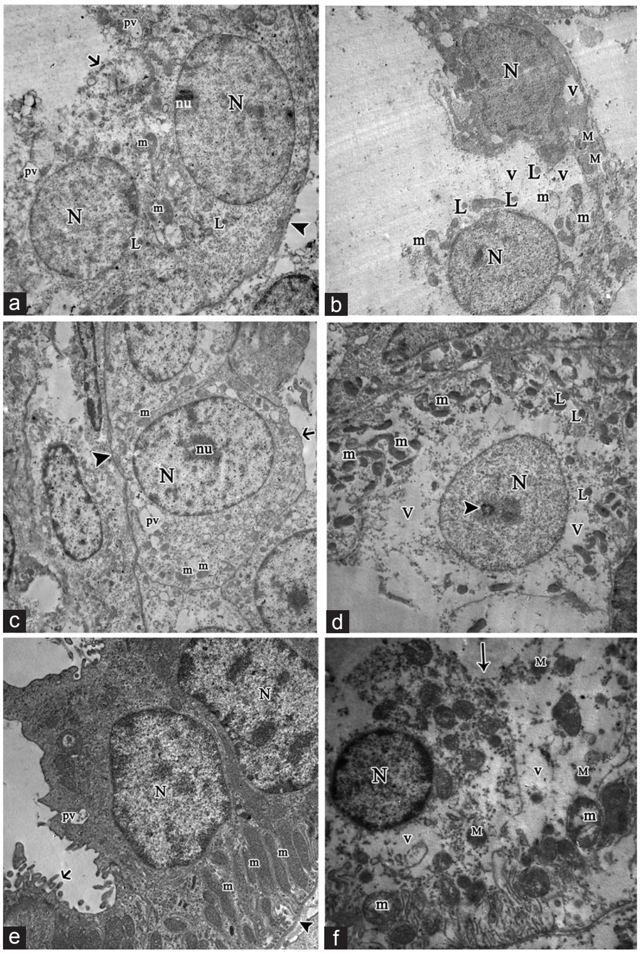
(a, c and e) An electron micrograph of a section in the renal cortex of a control newborn (a), 10 days old (c) and adult (e) albino rats showing part of the proximal convoluted tubule. The lining cells are seen resting on a basement membrane (arrow head) and showing oval euchromatic nuclei (N) with distinct nuclear membrane, few rough endoplasmic reticulum (RER), few short apical microvilli (mv) in the newborn rats and numerous tall tightly packed apical microvilli (mv) forming the brush border in the 10 days old and adult rats. The mitochondria (m) are seen randomly oriented in the newborn rats, elongated within the basal infoldings in the 10 days old rats and apparent healthy within the basal infoldings in the adult rats. Lysosomes (L) and pinocytotic vesicles (pv) are also observed (a (TEM × 7200); c and e (TEM × 4800)). (b, d and f) An electron micrograph of a section in the renal cortices of a treated newborn (b), 10 days old (d) and adult (f) albino rats showing epithelial cell lining proximal convoluted tubules which appears with large irregular electron dense nuclei (N). The cytoplasm shows vacuolations (v), many lysosomes (L) and dilated rough endoplasmic reticulum (RER). The mitochondria appears swollen with disrupted cristae (m) in the newborn rats, degenerated electron dense (m) in the 10 days old rats and degenerated with disrupted cristae (m) in the adult rats. Focal destruction (arrow) of the apical microvilli (mv) is noticed. Note membrane bound the structures with heterogenous content (arrow head) in the newborn rats are seen (b (TEM × 7200); d and f (TEM × 4800)).

rats in comparison with the control rats of the same age (Table 1 and Fig. 9).

Discussion

The extensive use of plant growth hormones in agriculture, make it an interesting subject to detect its possible harmful effects [7,9]. GA3 is one of the plant growth regulators that are widely used in Egypt, to increase the growth of fruits and vegetables [10]. Although GA3 is extensively used in Egypt, little is known about its potential hazardous effects on the human health [11]. So, the present

Figure 7



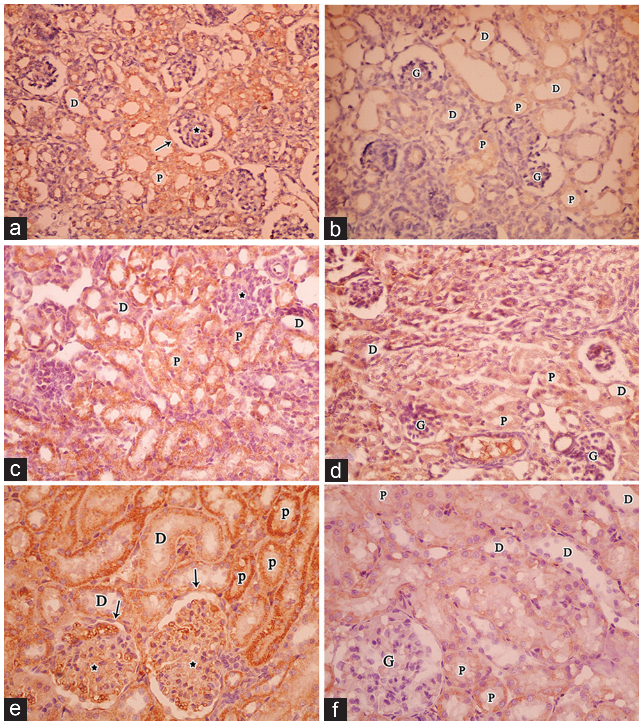
(a, c, and e) An electron micrograph of a section in the renal cortex of a control newborn (a), 10 days old (c), and adult (e) albino rats albino rat showing part of the distal convoluted tubule. The lining cells are seen resting on a basement membrane (arrow head) with round euchromatic nuclei (N) revealing a distinct nuclear membrane and a prominent nucleolus (nu). Mitochondria (m) appear randomly oriented in the cytoplasm in the newborn and 10 days rats and elongated lodged in the basal infoldings perpendicular to the basement membrane in the adult rats. Lysosomes (L) and pinocytotic vesicles (pv) are also observed. Note the presence of few short apical microvilli (short arrow). (a and c, TEM, ×7200; e, TEM, ×4800). (b, d, and f) An electron micrograph of a section in the renal cortex of a treated newborn (b), 10 days old (d), and adult (f) albino rat showing distorted cells of distal convoluted tubule with many vacuoles (v). The mitochondria appear swollen degenerated mitochondria with disrupted cristae (m) or electron dense mitochondria (M). The nuclei (N) appear as an irregular electron dense in the newborn rats, irregular containing dense inclusions (arrow head) in the 10 days old rats and with peripheral condensation of chromatin in the adult rats and irregular in shape with variable sizes in the 10 days old rats. Note many lysosomes (L). Note loss of the apical microvilli (arrow). (a and c, TEM, ×7200; e, TEM, ×4800).

work was designed to detect the toxic effect of GA3 on the developing renal cortex. Light microscopic, electron microscopic, immunohistochemical studies in addition to morphometrical and statistical analysis of the renal cortical thickness were used to achieve this goal.

Three postnatal ages of the rat were used in the present study (newborns, 10 days old, and adults) to detect postnatal changes in the kidney tissue in both the control and treated animals. In the present work, light microscopic examination of newborn control rats revealed immature forms of renal developmental stages in the subcapsular zone with more mature renal corpuscles in the juxtamedullary zone. The same findings were observed by others [12–14].

By light microscopy, the proximal convoluted tubules showed an acidophilic granular cytoplasm and basal

Figure 8



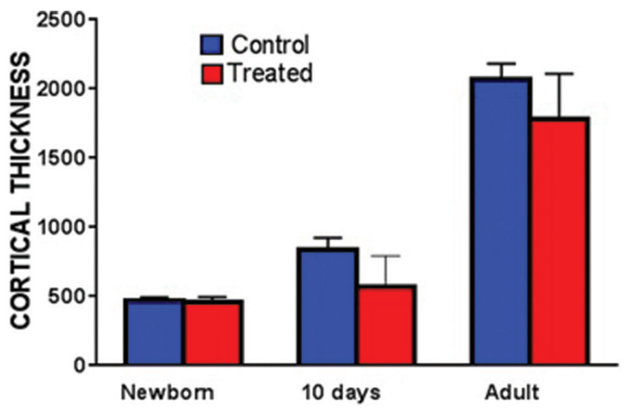
(a, c, and e) A photomicrograph of a section in the renal cortex of a control newborn (a), 10 days old (c), and adult (e) albino rats showing a strong positive Bcl-2 immunoreactivity in the cytoplasm of the cells lining of most of the proximal tubules (P) and weak reaction in the distal convoluted tubules' cells (D). Note very weak staining of the parietal layer of Bowman's capsule (arrow) and very weak or absent staining of the capillary unit (asterisk) (Bcl-2 immunostaining, ×400). (b, d, and e) A photomicrograph of a section in the renal cortex of a treated newborn (b), 10 days (d), and adult (f) albino rats showing weak positive brown Bcl-2 immunoreactivity in the cytoplasm of cells lining most of the proximal convoluted tubules (P). Bcl-2 immunostaining was very weak or absent in the cells lining the distal convoluted tubules (D). The glomeruli (G) reveal negative Bcl-2 immunostaining (Bcl-2 immunostaining, ×400).

Table 1 Renal cortical thickness of the control and treated rats in the three studied age groups

Age group	Group	Mean±SE	*P*
Newborn	Control	467.0±7.951	0.7233 NS
	Treated	461.2±10.05	
10 days	Control	839.5±24.08	<0.0001***
	Treated	504.8±14.04	
Adult	Control	2068±35.82	0.0187*
	Treated	1786±104.0	

NS, no significant difference (*P*>0.05). *Significant (*P*<0.05). ***Highly significant (*P*<0.001).

Figure 9



The mean renal cortical thickness of the control and treated rats in the three studied age groups.

nuclei. The distal convoluted tubules showed a dilated lumen. These results support the findings of the studies done by other researchers [13,14].

The observed double glomerular basement membranes in the newborn control rats' renal cortices was concomitant with those of other researchers who revealed the presence of double glomerular basement membranes in the newborn rats' renal corpuscles [12].

The podocytes of the control newborn rats' renal cortices appeared mature with primary and secondary (foot) processes in harmony with other investigators [15].

In the present study, the ultrastructure of the proximal convoluted tubules of the newborn control rats' renal cortices showed that their lining epithelial cells had few short apical microvilli. These findings were in accordance with the study of other investigators [12,13].

In this study, the proximal and distal convoluted tubules of the control newborn rats' renal cortices showed mitochondria which were randomly oriented in the cytoplasm. These findings were similar to those of other researchers [12]. In the undifferentiated stages of nephron, the mitochondria are scattered throughout the cytoplasm with no particular orientation. As the nephron matures, the mitochondria become parallel to the lateral cell membranes [16].

In the present study, the proximal and distal convoluted tubules of the control newborn rats' renal cortices showed lysosomes and pinocytotic vesicles in accordance with other researchers who attributed it to onset of tubular absorption in the newborn rats [17].

In the present work, light microscopic examination of 10 days old control albino rats' renal cortices were in line with those of other researchers who observed the disappearance of the primitive subcapsular zone between postnatal days 9 and 12 [12,15]. The superficial cortex revealed smaller renal corpuscles with compact capillary tuft while, the renal corpuscles in the deeper cortex appeared large lobulated. In rats as well as other mammals, nephrogenesis in the metanephros proceeds centrifugally. Thus a higher degree of nephrological maturation was observed in the juxtamedullary zone, where nephrons were more differentiated than the subcapsular ones [18].

Ultrastructural observations of the renal cortex of the 10 days old control rats showed that the glomerular filtration barriers revealed fused glomerular basement membranes. These findings were in agreement with those of others [12]. Interactions between some macromolecules contained within the dual membrane unite the two structures together. This fusion provides a denser, more effective barrier to the passage of plasma proteins [19].

The electron microscopic examination of the proximal convoluted tubules of 10 days old control rats showed numerous tall tightly packed apical microvilli forming the brush border. These results were concomitant with those of other investigators[12] and confirmed the earlier study of others who demonstrated that at postnatal day 10, microvilli of the proximal convoluted tubules are taller and are of a more consistent height [20].

The proximal convoluted tubules showed elongated basal mitochondria while randomly oriented mitochondria appeared in the distal convoluted tubules. However, other researchers detected that the proximal and distal convoluted tubules of the 10 days' old rats showed mitochondria which appeared elongated, lodged in the basal infoldings perpendicular to the basement membrane [12].

Light microscopic examination of the renal cortex of the control adult rats in this work revealed the normal histological structure. The current findings were similar to those of other authors [9].

In the present study, electron microscopic examination of the renal cortex of adult control rats was in agreement with other researchers who detected trilaminar appearance of the glomerular basement membrane in the adult rat's renal cortex [21].

The lining cells of the proximal convoluted tubules of the adult control rats' renal cortices in this study appeared with numerous tightly packed apical microvilli forming the brush border. These findings were in agreement with those of Abdel-Aziz and Mohamed [13].

In the present work, the lining cells of the proximal and distal convoluted tubules of the adult control rats' renal cortices showed elongated mitochondria lodged in the basal infoldings perpendicular to the basement membrane. These findings were similar to those noticed by other investigators [22].

The proximal and distal convoluted tubules of the three postnatal ages in the current work showed pinocytotic vesicles and lysosomes which are involved in reabsorption and degradation of small amounts of protein that have leaked through the glomerular filter [23].

The results obtained from the present study revealed an obvious nephrotoxic effect of GA3.

In the present work, the effect of GA3 administration on the structure of the developing renal cortex showed a more frequent presence of immature developmental forms of renal glomeruli in the treated newborn and 10 days old rats which indicate a delayed development of the cortical glomeruli. In addition, the presence of undifferentiated solid masses of cells is considered another evidence for delayed development of kidneys treated with GA3. The present findings were in line with those of other investigators who detected delayed development of the renal cortex of the rats whose mothers were treated with monosodium glutamate [13].

The observed damage that occurred in the renal corpuscles in the studied age groups of the treated rats was in agreement with that seen by other workers [9]. Some investigators referred this lesion as 'cystic glomerular atrophy,' based on the small size of some glomeruli within the dilated Bowman's space [24]. Other authors attributed the pathogenesis of this lesion to periglomerular fibrosis and stated that thickening of glomerular basement membrane resulted in disturbance of glomerular outflow that led to cystic changes in Bowman's space [25].

However, in the present study, some glomeruli in the renal cortex of the treated rats showed hypercellularity with obliteration of the Bowman's spaces. Some authors attributed that change to compensate for the decrease in mature glomeruli with enlargement of vascular glomeruli tightly filling the Bowman capsule [26]. In addition, GA3 increases the mitotic division so has growth promoting effects in animal tissues [3].

Ultrastructurally, glomerular affection appeared in the present work in the form of irregular thickness of the glomerular basement membrane. These results were in agreement with those of other authors [27]. The large surface area of the glomerular capillaries renders them susceptible to damage from circulating toxins and immune complexes [9].

In the present study, podocytes showed broadening and fusion of the foot processes. Loss of foot process architecture was referred to as effacement which is invariable feature of proteinuric glomerular diseases [19,28].

In the present work, the epithelial lining of the renal tubules in the studied age groups of the treated rats showed vacuolations and pyknotic nuclei. Also, there was desquamated epithelial cells and epithelial debris inside their lumina. These findings were in agreement with those of other investigators [9,27]. The cytoplasmic vacuolization was one of the important primary responses to all forms of cell injury. They implied increased permeability of cell membranes leading to an increase of intracellular water which produce cytoplasmic vacuolization [29].

In the present study, electron microscopic examination of the renal cortex of the treated rats in the studied age groups showed loss of most organelles and destruction of the apical microvilli of the proximal tubular lining cells. Moreover, loss of polarity of polarized epithelia of proximal convoluted tubules due to its contact with toxins resulted in their ischemia, then eventual necrosis [30].

In the present study, the mitochondria of the epithelial lining of the renal tubular cells of the treated animals appeared degenerated. These findings were similar to those of other authors who detected swollen mitochondria in the renal tubules of GA3-treated rats[27] and others who detected abnormal mitochondria in the hepatocytes of the liver tissue in the rats treated with GA3 [31]. Many earlier studies disclosed that mitochondrial dysfunction contributed to apoptosis via the production of reactive oxygen species [32].

In the present study, the epithelial lining of the renal tubular cells of the treated rats revealed many lysosomes which reflected accelerated intracellular degradation of macromolecules [33].

In the present work, the epithelial lining of the renal tubular cells and the endothelial cells of the glomerular capillaries of the treated rats showed dilated rough endoplasmic reticulum. Similar findings were detected that in the pancreatic acinar cells of GA3-treated rats [34]. The dilatation of rough endoplasmic reticulum indicated increased endoplasmic reticulum stress [35].

Glomerular and tubular degeneration and necrosis occurred as a result of oxidative stress and lipid peroxidation which were detected by increased malondialdehyde level in the kidney. This was accompanied by decreased antioxidant enzymes [3].

The interstitial tissue of the renal cortex in the treated groups showed congested blood vessels, extravasation of red blood cells, interstitial hemorrhage, and inflammatory cellular infiltration. These results were similar to those detected by other authors [9,36]. These findings were considered as signs of toxicity and consequent activation of the defensive mechanism of the treated animals [36].

In the present study, PAS positivity was detected in the mesangium of the glomeruli, the basement membrane of the parietal layer of the Bowman's capsule and the renal tubules which contain sulfated proteoglycan and in the brush border of the proximal convoluted tubules since it is formed of microvilli which is coated with a dense glycocalyx in the renal cortex of the studied control age groups. These results agreed with other authors [9,37].

In this study, the positivity of the PAS reaction was increased from the newborn stage to the adult age in the control rats. These findings were in agreement with those of the study of other authors who explained that as being due to an increase in gluconeogenesis with age and accumulation of extracellular matrix protein [38].

GA3-treated groups showed a decrease in PAS activity in the glomeruli and renal tubules. These findings were in agreement with those of other investigators[9] and were attributed to marked reduction in both carbohydrates and proteins after administration of gibberellins to rats [39].

The results of the immunohistochemistry were in agreement with other researchers who detected that the glomeruli showed weak staining of Bcl-2 in the parietal layer of the Bowman's capsule, whereas the capillary units showed negative staining with low expression in the distal tubular epithelial cells [40,41]. The strongest expression of Bcl-2 was found in the mature proximal convoluted tubules [42].

The function of Bcl-2 protein was related to their ability to interfere with mitochondrial apoptosis pathways as it suppresses the initiation of the cell-death process by inhibiting mitochondrial permeability [43].

As the amount of mitochondria is similar in proximal and distal tubules, the difference in expression of Bcl-2 might be attributable to functional differences between proximal and distal tubule mitochondria [44].

A weak brown positive Bcl-2 immunostaining was detected in the cytoplasm of the cells lining of most of the proximal tubules of the treated rats. Similarly, a study was done by other researchers found a significant decrease in Bcl-2 expression in the renal cortex of the rats treated with GA3 for 8 weeks [27]. The reduction in protein content was attributed partially to the decreased level of protein synthesis in the affected renal cells and to the hyperactivity of hydrolytic enzymes [45].

Bcl-2 deficiency resulted in kidney maldevelopment as the Bcl-2 was important for the morphological development of the kidney [42].

In the present work, morphometrical and statistical analysis of the renal cortical thickness showed a decrease in the cortical thickness in the treated rats when compared with the control ones. These findings could be attributed to atrophy of the glomeruli and degeneration of the renal tubules that occurred after GA3 treatment. Thinning of the renal cortex is likely the result of tubular and glomerular atrophy [46].

Conclusion and recommendation

GA3 has harmful effects on the histological development of the renal cortex. So, it should be used cautionary. Also, producers and consumers should be conscious on the toxic effects of these chemicals, which could affect the public health.

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Conflicts of interest

There are no conflicts of interest.

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