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GINGER AMELIORATES THE NEPHROTOXICITY INDUCED BY GABAPENTIN IN RAT FETUSES

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[1]Abstract

Treatment with the antiepileptic drugs is usually associated with adverse side effects, especially when used during pregnancy. The current work aimed to study the possible ameliorative effect of ginger extract on developmental defects of kidney of rat fetuses maternally injected with the antiepileptic drug gabapentin (GBP) during the organogenesis phase. At the histological level, the kidney of fetuses of the GBP group displayed dilatation and vacuolar degeneration in the convoluted tubules epithelium along with hemorrhage between the tubules. In addition, the glomeruli were atrophied and edematous. Similarly, at the immuno-histochemical level, the expression of Bcl-2 in the cytoplasm of convoluted tubule epithelium was remarkably decreased, while the expression of Caspase-3 was increased. At the ultrastructural level, there was obvious thickening of the glomerular basement membranes. Most of the foot processes of the podocytes appeared irregular, short and fused with each other. Cells lining the proximal convoluted tubules showed marked thickening of their basal lamina with partial destruction of the microvilli of the apical brush border. Oral injection of ginger after GBP resulted in evident amelioration at all the investigated levels. In conclusion, the use of GBP as antiepileptic drug should be treated with highly caution during pregnancy and ginger is recommended to be taken in parallel for its ameliorative role in this regard.

Keywords: Gabapentin, ginger, fetal development, kidney, organogenesis, Ultrastructure, immuno-histochemical

1. Introduction

Epilepsy is a commonly encountered serious chronic neurological problem faced by obstetricians, gynecologists and primary health care physicians [1]. It is associated with an increased risk of having malformed babies [2]. One-third of women with epilepsy have an increased risk of seizures during gestational period [3], therefore, most of the epileptic women urgently need to continue taking medication during pregnancy [4, 5]. Currently, management of epilepsy mainly depends on Antiepileptic drugs (AEDs), which are notoriously known for their adverse side effects and this explains the reason for their several generations [6]. Infants of mothers treated with AEDs during pregnancy had a

[1] Antiepileptic drugs, AEDs; Av, apical vacuole; Bb, brush border; Cm, cell membrane; Er, erythrocytes; Gabapentin, GBP; h, hemorrhage; J, juxtamedullary zone; L, lumen; M, mitochondria; MR, medullary ray; Mv, microvilli; Nu, nucleus; P, peroxisomes; Po, podocyte; RC, renal corpuscle; Sc, subcapsular zone; T, renal tubules; V, vacuole.

greater incidence of congenital malformations than those of either normal controls or non-treated epileptic women [7]. Therefore, it is believed that AED therapy rather than the maternal disease or convulsions are the cause of malformations identified at birth [7].

In the recent years, numerous drugs have been introduced to the treatment of epilepsy. AEDs generally may be divided to the older, classic AEDs and the newer, next generation, AEDs [8]. The adverse effects of old drugs are well known, whereas those of the newer drugs are much less recognized [9, 10].

As one of the new AEDs, Gabapentin (GBP) has achieved greater popularity as an adjunctive therapy for chronic pain [11]. Clinically, GBP is indicated as an add-on medication for the treatment of partial seizures [12] and neuropathic pain [13] as well as prophylaxis of migraine [14]. It was also claimed to be beneficial in several other clinical disorders such as anxiety, bipolar disorder and hot flashes [15]. GBP has been labelled category C on the basis of its hazards produced in rodent fetuses [16]. Despite expanding data on the usage of GBP, there is little information, so far, on its teratogenic effects [14, 17].

Kidneys are among the most important organs in the body serving many vital processes [18]. Although the detrimental effects of GBP on kidney, especially in the fetal tissues, are not yet clear, there have been few reports studying the side effects of this drug [19]. Chronic administration of GBP, in a case study with renal impairment, increased serum creatinine level [20]. Afshar et al. [14] reported that consumption of GBP during pregnancy can cause hydronephrosis and hydroureter in rat fetuses. Embryos of valproic treated pregnant rats were found to have renal degeneration [21].

The value of medicinal plants in curing most diseases has been realized since the beginning of life. In fact, the treatment of most human ailments and discomforts can be found in the nature, on which humans are dependent [22]. Ginger is the rhizome of the plant *Zingiber officinale* Roscoe, member of the family of Zingiberaceae. It is a common kitchen spice widely used worldwide as powder or as the whole fresh root [22-24]. Ginger contains several compounds such as gingerol, shogaol, zingiberene, paradol, resin, starch, volatile oil and vitamins C and A [25].

Ginger has been used as a traditional medicine for several conditions [24]. Ginger is known for its antioxidant, anti-cancer and anti-inflammatory properties as well as anti-nausea/vomiting properties [26-28]. Some studies have dealt with the effect of ginger on renal damage. Sabraz et al. [29] concluded that the aqueous extract of ginger showed an ameliorative effect against cadmium bromide induced nephrotoxicity. El-Hummadi [30] found that a mixture of honey, black seed and ginger had a curial effect on the pathological changes of anticoagulant Enoxaparin Sodium of the kidney of albino rats.

The aim of the present study is to focus on the possible ameliorative role of ginger against GBP induced nephrotoxicity in rat fetuses maternally injected with GBP during the organogenesis phase of development at histological, histo-morphometric, immuno-histochemical and ultrastructural levels.

2. Materials and Methods

2.1. Animals and treatment

Principles of animal care and use were carefully followed during conducting the present study according to the guide for the care and use of laboratory animals approved by Faculty of Science, Menoufiya University, Egypt (Approval No. MNSE2175) and according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Thirty six healthy mature fertile males (12) and virgin females (24) of Westar 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 ± 2^0C 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 two females with one male 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. The pregnant rats were divided into four groups, six rats each, as follows:

1. Control group, administrated distilled water.
2. Ginger administrated group given oral dose of ginger (200 mg/kg).
3. Experimental GBP group given intraperitoneal injection of GBP (162 mg/kg).
4. Combined GBP and ginger injected group, received intraperitoneal injection of GBP first followed by oral administration of ginger one hour later.

This study was based on 36 fetuses.

2.2. Gabapentin

GBP, with the trade name Gaptin, (Delta Pharma $_{S.A.E}$) was employed for the study. The treatment started on GD 6 and ending on GD 15. The applied dose was 162 mg/kg [16].

2.3. Ginger extract

Fresh rhizomes of *Zingiber officinale* were purchased from a local market at Shebeen El-Koom, Menoufiya, Egypt. They were shade dried at room temperature and then crushed to powder. 125 g of the powder were macerated in 1000 ml of distilled water for 12 h at room temperature and filtered through a 5 µm filter paper to obtain the final aqueous extract. Accordingly, concentration of the obtained extract was 24 mg/ml and equal to 120 mg/kg [31]. Ginger extract was daily administrated orally, one hour after GBP injection, by gavage tube at a dose of 200 mg/kg body weight [32] during the organogenesis phase of gestation.

2.4. Investigated parameters:

2.4.1. Histological examination

For light microscopical examination, the fetal kidney of different groups were fixed by immersion in 10% neutral formalin for 24 hours at room temperature followed by washing under running tap water for 12 hours. All specimens were transferred to 70 % ethanol and then dehydrated in an ascending series of ethanol, cleared in xylol and embedded in molten paraffin.

Five µm thick sections were produced using a rotary microtome (Leica, Model Rm 2125, Germany). Sections were mounted on albumen-coated slides and stored until staining. Histological staining was performed with Ehrlich's hematoxylin and counterstained with aqueous eosin. Histological sections were subjected to microscopical examination and when necessarily photographing using Olympus microscope.

2.4.2. Histomorphometric parameters:

An estimation of fetal nephron numbers was performed by counting representative glomerular numbers in microscopic sections of different groups after H & E staining under X400 magnification (n=6 fetuses per group).

2.4.3. Immuno-histochemical investigation

Avidin-biotin peroxidase method was used for the immuno-histochemical demonstration of the anti-apoptotic mediator Bcl-2 and proapoptotic antigen Caspase-3.

Samples of the fetal kidney were fixed in 10 % formalin for 24 hours and processed as described in our previous work [17].

2.4.4. Ultrastructural investigation

For ultrastructural investigation, which has been done using transmission electron microscope, specimens of 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) and processed as described in our previous work [17].

2.5. Data evaluation and statistical analysis

All data sets were expressed as mean ± standard error of the mean (SEM). The statistical data were based on at least 6 kidneys in each group. The data were analyzed statistically for normal distribution (student's T test) and homogeneity of variances (Levene test) using statistical package of social sciences (SPSS) software for windows, version 22. Differences were considered insignificant whenever $P>0.05$. the significances of the obtained data were classified into two categories, i.e. $P<0.0001$ and $P<0.05$ according to the obtained P values.

3. Results and Discussion

Different animal studies have demonstrated that in utero exposure to teratogenic agents such as drugs during development can produce different developmental alterations due to their interaction [15, 33]. Teratogenicity can be expressed by interference in proliferation, migration or differentiation at the cellular level [34]. Wells et al. [35] stated that the embryonic period and fetal period of embryogenesis are hallmarks of teratological risks as organs are usually more susceptible to abnormal development only if exposed either during early events of their formation, i.e. during organogenesis phase or during their differentiation and functional development. Due to the fact that certain agents have the same metabolic pattern, they are also associated with similar patterns of malformation, resulting in a recognizable syndrome. Some good examples of these effects are the AEDs which are associated with higher risks for both anatomical and behavioral teratogenesis in the embryo [36]. For some AEDs, such as valproate, phenobarbital, phenytoin, and carbamazepine, the risks are widely studied and outcomes for the fetus are more or less clear [36]. However, there is no adequate information concerning the teratogenic effect of more current AEDs, such as GBP, and few investigations have addressed their teratogenic effects [14, 16]. Therefore, there was a need to examine the in utero exposure of GBP, as a

representative of new AEDs generation, on the developing fetal rat kidney, especially that there are few clinical reports which described its effect on kidney [19].

3.1. Histological investigation

The renal cortex of control fetuses showed the presence of two zones, namely, the subcapsular zone and the juxtamedullary zone. The first contained immature forms of the renal developmental stages which included cell condensates, and renal vesicles in addition to nephrogenic mesenchyme. The juxtamedullary zone contained mature renal corpuscles surrounded by proximal and distal convoluted tubules. Medullary rays were seen extending between the two previous zones. Aggregations of mesenchymal cells forming caps were seen in close association with the upper sides of the ureteric buds (Fig. 1A&B). Fetuses maternally administered ginger alone showed normal renal structure, somewhat similar to that of the control group (Fig. 1C).

On the other hand, fetuses maternally injected with GBP exhibited degeneration of renal corpuscles in the form of shrunken or absent glomeruli along with increased periglomerular space (Fig. 1D). In addition, there was dilatation in the tubules lumen as well as degeneration and vacuolation in the epithelium of the convoluted tubules (Fig. 1D&E). Hemorrhage was evident in the renal tubules (Fig. 1E). Co-administration of ginger with GBP resulted in better glomeruli morphology compared with the GBP group. The tubules were more or less normal in their structure with no vacuolation (Fig. 1F).

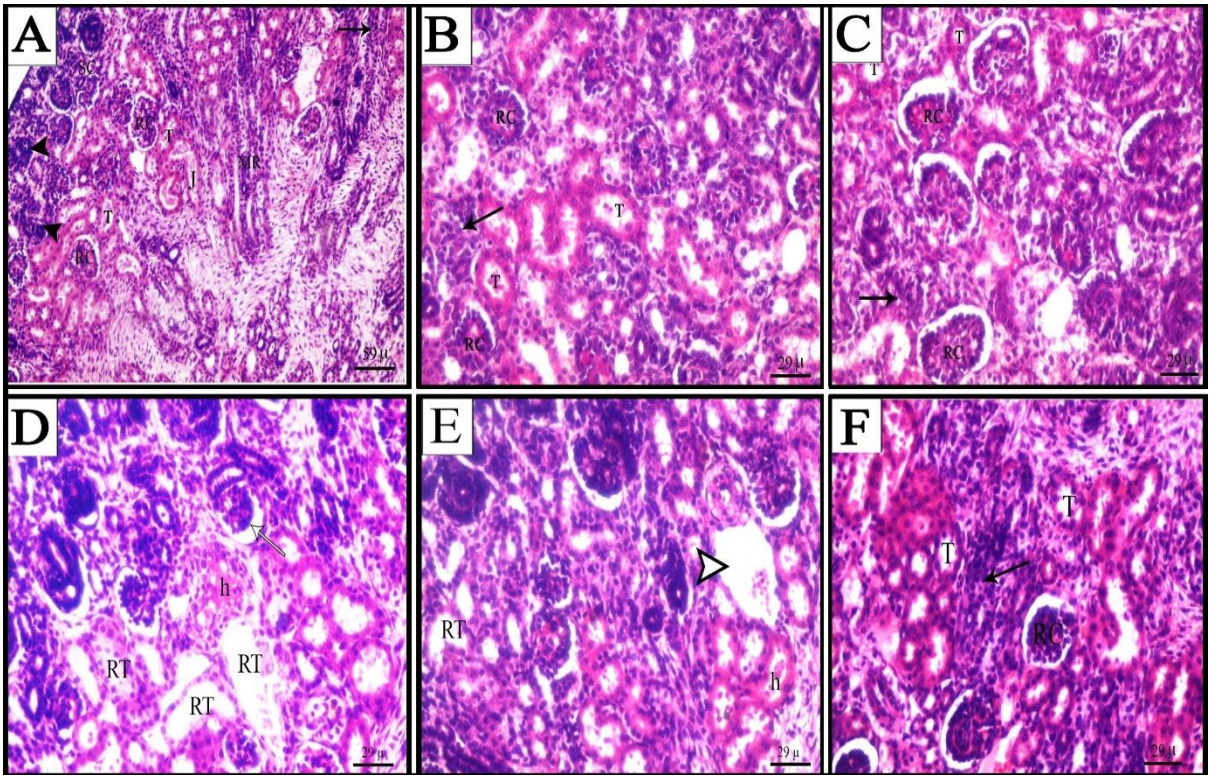

Figure 1: Photomicrographs of transverse sections of fetal cortical region of kidney. (A&B) Control (C) Ginger (D&E) GBP group (F) GBP+ginger group. Immature forms of renal developmental stages

(black arrow head), nephrogenic mesenchyme (black arrow), disrupted glomeruli (white arrow), empty renal corpuscle (white arrow head). Scale bar A= 59 µ, B-F= 29µ.

Aktaş et al. [21] reported a very severe damage to the tubules of the kidney in pregnant mice or rats after injection of valproic acid. The histopathologiocal changes of rat kidney tissue treated with valproate revealed that the proximal and the distal convoluted tubules showed hydropic changes and the glomeruli showed hypercellularity [37]. Similar effects were seen when valproate and lamotrigine were injected into epileptic rat mothers were the maternal kidney showed potent nephrotoxicity [38]. Afshar and Golalipour [39] reported that consumption of GBP during pregnancy can cause hydronephrosis and hydroureter in rat fetuses.

3.2. Number of Glomeruli

Table (1) illustrates the changes in the number of glomeruli in the fetuses of different groups. There was insignificant difference in the total number of glomeruli in the ginger group (18.5 ± 0.43) compared with the control group (18.83± 0.31). The number of glomeruli were highly reduced in fetuses maternally injected with GBP (10.83 ± 0.54). Injection of GBP followed by ginger administration led to a marked increase in the total number of glomeruli compared with the GBP group, but was still lower than that of the control group (17.5 ± 0.34; 10.83 ± 0.54; 18.83± 0.31, for the three groups respectively). In humans, nephron numbers were found to be lower in neonates with low birth weight [40, 41].

Table 1: Effect of maternal adminstration of gabapentin and ginger extract, either individually or in combination, on the number of glumeruli.

Groups	Control	Ginger	Gabapentin	Gabapentin + Ginger
Mean±SEM	18.83± 0.31	18.5 ± 0.43	10.83 ± 0.54**	17.5 ± 0.34* c

Data are represented as mean ± SEM.

Asterisks (* - **) refer to the P value compared with the control group.

c= highly significant (P<0.0001) compared with gabapentin group.

**** P< 0.0001**

*** P< 0.05**

3.3. Immuno-histochemical investigation

The majority of proximal and distal tubular epithelial cells of fetuses from control and ginger groups were immuno-reactive to Bcl-2 protein (Fig. 2A&B) and showed insignificant relationship in the mean area percentage expression (43.86%; 36.51%) (Table 2 and Fig. 2). In the glomerulus, Bcl-2 protein was not detected, although few parietal epithelial cells expressed moderate immunostaining intensity. The capillary tuft cells

showed scarce or absent Bcl-2 immunoreactivity. In fetuses maternally injected with GBP, there was a highly significant decrease in the mean area expression with only 5.26% of the cells were immuno-stained. Both the intensity and distribution of Bcl-2 protein expression were decreased and few scattered, mild Bcl-2 positive epithelial cells were observed. In some parietal epithelial cells and podocytes, Bcl-2 was detected (Fig. 2 C&D). Co-administration of ginger after GBP resulted in moderate expression of Bcl-2 antigen, especially in the renal tubules of (Fig. 2E&F) and significant increase when in the mean area percentage when compared with GBP group (16. 91%).Conversely, few cells in the kidney of control and ginger groups showed immunoreaction to Caspase-3 (Fig. 2G&H) (6.91%, 758%). On the other hand, the immune-expression of Caspase-3 was highly significantly increased in the kidney of GBP group as compared with that in normal control fetuses (29.98%). The antigen was located in both the renal tubule cells and Malpighian corpuscle (Fig. 2I-K). The administration of ginger after GBP reduced the elevated Caspase-3 cytoplasmic expression to be more or less to that of the control group (Fig. 2F) (13.57%).

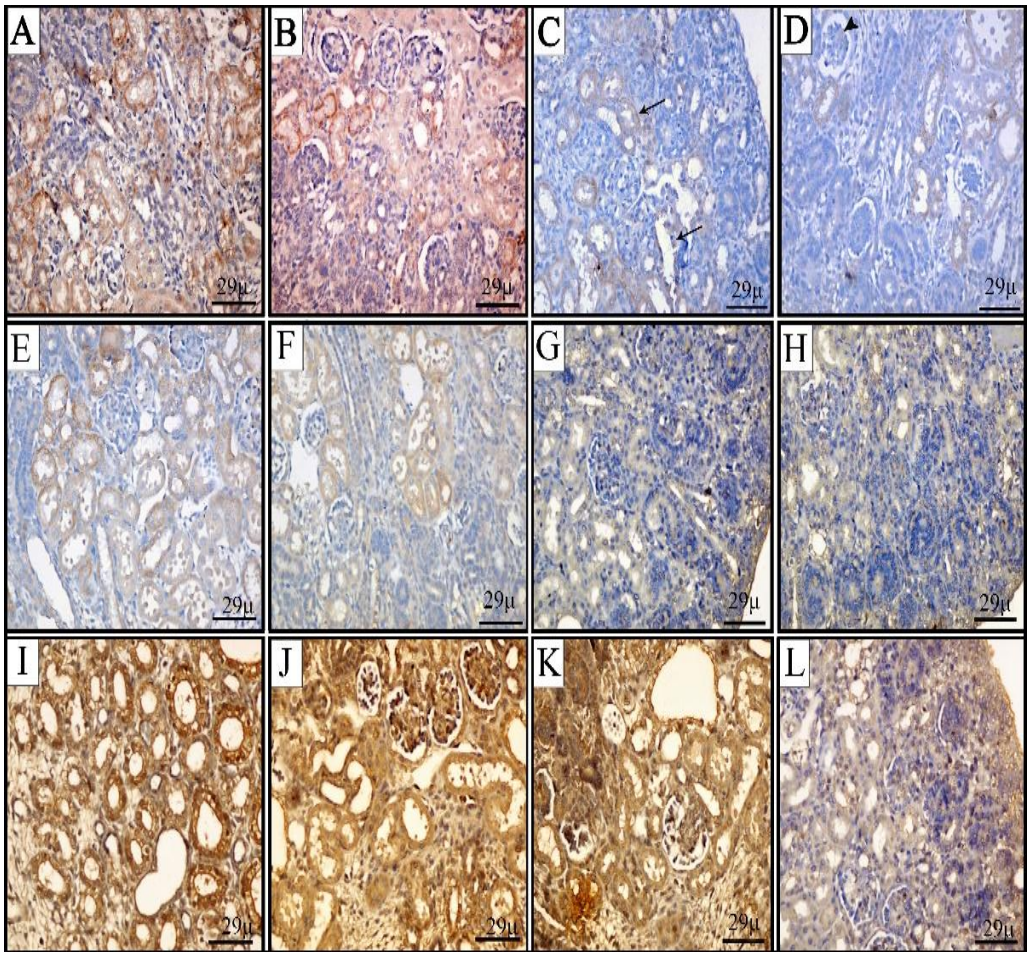

Figure 2: Photomicrographs showing immuno-histochemical localization of Bcl-2 (A-F) and Caspase-3 (G-L) antigens in kidney transverse sections of 20-day old rat fetuses. (A&G) Control group (B&H) Ginger group (C, D, I, J & K) GBP group (E, F& L) GBP+ginger group. Scale bar= 29µ.

Table 2. The mean area % of Bcl-2 and Caspase-3 expression in the fetal kidney of all experimental.

Groups	Control	Ginger	GBP	GBP + Ginger
Bcl-2	43.86 ± 0.88	36.51 ± 1.24	5.26 ± 0.78**	16.91 ± 1.00* a
Caspase-3	6.91 ± 0.24	7.58 ± 0.37	29.98 ± 0.047**	13.57 ± 0.28* a

groups.

Data are represented as mean area % ± SEM.

Asterisks (* - **) refer to the P value compared with the control group.

a= significant (P<0.05) compared with GBP group.

**** P < 0.0001 * P< 0.05**

The immuno-histochemical investigation showed that maternal injection of GBP induced apoptosis in the fetal renal cells. Bcl-2 expression in the kidney of control fetuses was massive in the proximal and distal convoluted tubule cells, while it showed weak expression in the glomerulus or Bowman's capsule. On the contrary, different parts of the renal cortex of GBP group showed rare expression of Bcl-2. Different parts of kidney from the GBP group showed increased expression of Caspase-3. These results were quite similar to the study of Nakopoulou et al. [42] who found that Bcl-2 was detected in a few parietal epithelial cells in the glomerulus with a moderate staining intensity, but it was not expressed at all in the capillary tuft and it was detected in proximal, distal and collecting tubule epithelial cells. Chen et al. [43] made similar observations in renal tubular epithelial cells of Adriamycin-treated rats. They reported that Adriamycin in these cells induced apoptosis and stimulated activities of Caspase-3. Similarly, increased expression of Caspase-3 marker and hence apoptosis of renal tubular epithelial cells was noticed in fetuses, whose mothers were administered Adriamycin before their planned pregnancy [44]. The effect of aflatoxin B1 on the renal tubules was supported with a significant decrease of the antiapoptotic Bcl-2 immunostaining in the tubular cells [45].

3.4. Ultrastructure investigation

Transmission electron microscope investigation revealed that the renal cortex of the control fetuses had glomeruli containing podocytes, a modified epithelial cell, situated between the glomerular capillaries as well as a wide urinary space. These cells were branched and had many foot processes which were extended and rested on a double glomerular basement membrane and interdigitated with their counterparts of other podocytes. The narrow spaces between the adjacent processes, i.e. the filtration slits were

evident (Fig. 3A). Proximal convoluted tubules of control fetuses were seen with their lining epithelial cells resting on a thin basal lamina and lined with cuboidal cells with a basally located euchromatic nucleus. The nucleus of these cells had an evenly distributed chromatin, a prominent nucleolus and intact nuclear envelope. They had well-formed microvilli forming the apical brush border. The highly electron dense cytoplasm contained numerous mitochondria of various sizes with clear cristae profiles. From the basal membrane into the interior of the cell, basal folds emerged which were inserted with numerous rod-like mitochondria oriented parallel to the cell axis (Fig. 3B). The distal convoluted tubules of the control fetuses revealed a thin basal lamina and few microvilli at the apical surface. Moreover, numerous, elongated and round-shaped mitochondria were seen between the basal enfolding occupying most of the cytoplasmic compartment. These cells showed a relatively large basal euchromatic nuclei (Fig. 3C).

The glomerular podocytes of the renal cortex of fetuses maternally administered with ginger as well as proximal and distal tubule cells had a more or less similar ultrastructure compared with those of the control group (Fig. 3D, E&F, respectively).

The renal cortex of fetuses maternally injected with GBP revealed evident ultrastructural changes in both the renal corpuscle and renal tubule cells. There was narrowing of the urinary spaces accompanied by an obvious thickening of the glomerular basement membranes. Most of the foot processes of the podocytes appeared irregular, short and fused with each other obliterating most of their slit membranes. The nuclei of the podocytes were electron dense with irregular nuclear envelope (Fig. 3G). The proximal convoluted tubules showed marked thickening of their basal lamina. The nuclei of most proximal tubule cells were electron dense and shrunken. In some parts of the tubules, the microvilli of the apical brush border of the lining cells were partially degenerated. Swollen mitochondria with vacuolation of the inner compartment were conspicuous. The cytoplasm of some cells showed increase in the number of peroxisomes as well as vascular congestions with red cell clusters both in-between and inside the tubular cells. There was obvious demolishing of their basal enfolding (Fig. 3H). Examination of the cells lining the distal convoluted tubules showed variable degrees of damage. Nuclear membrane appeared irregular and ruptured in some places with chromatin clumps. The cytoplasm had many vacuoles with dilated basal enfolding (Fig. 3I).

Co-administration of ginger after GBP resulted in better ultrastructure of the fetal kidney. The glomerular capillaries and the podocytes of renal corpuscles appeared, somewhat, similar to the control group. The podocytes retained the extended shape of foot processes with obvious slit membranes, however, the urinary space was still narrow compared with the control group (Fig. 3J). The cells of the proximal convoluted tubule

exhibited normal cellular organelles and intact microvillus border. The cells rested on a thin basal lamina with euchromatic, rounded nucleus. The mitochondria appeared rounded with preserved crista (Fig. 3K). Cells lining the distal convoluted tubules were cuboidal and rested on a thin basal lamina. Basally located euchromatic rounded nucleus was evident. Elongated mitochondria were observable in the slightly rarefied cytoplasm. Red blood cells were seen in the intercellular space between the renal tubule cells (Fig. 3L).

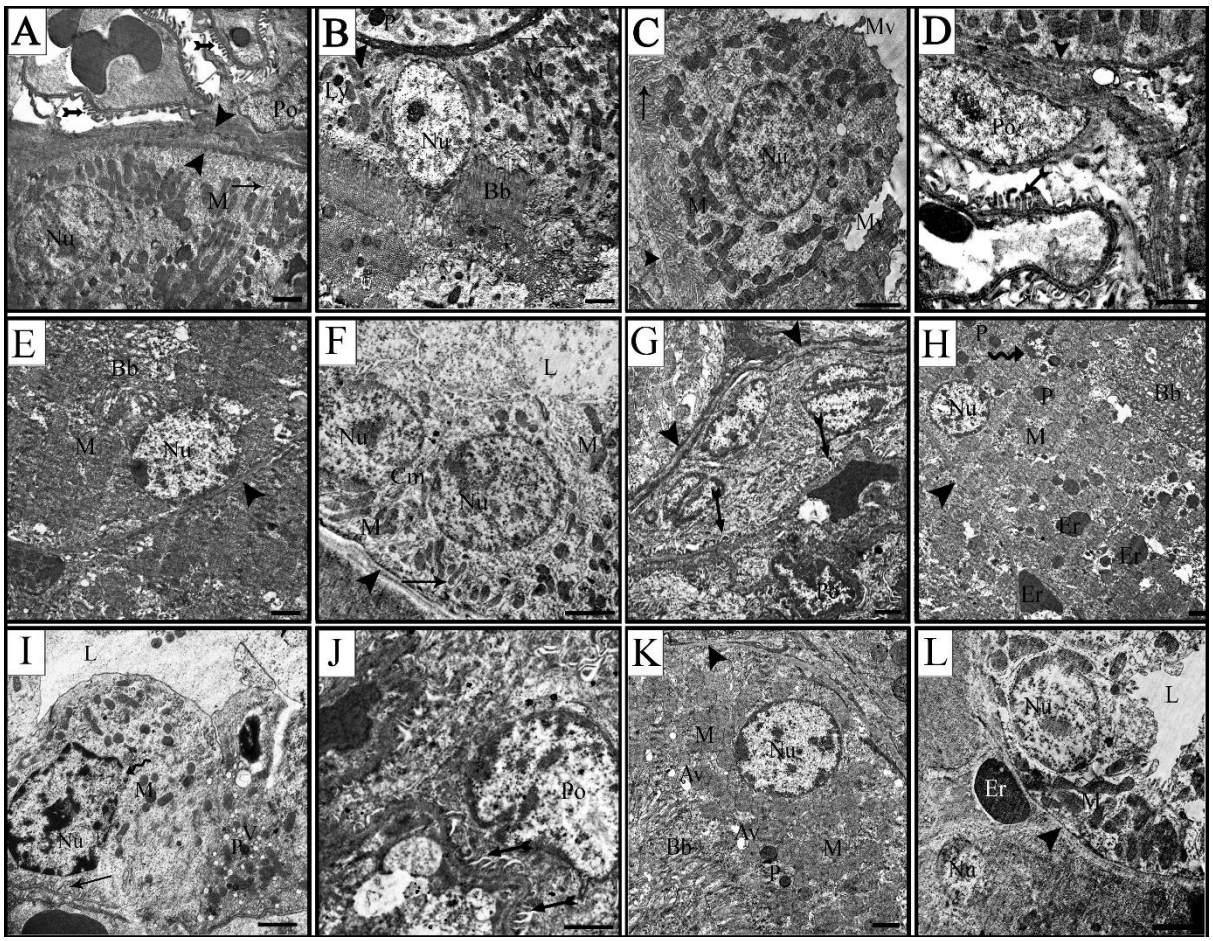

Figure 3: Transmission electron photomicrographs of kidney sections of rat fetuses. (A-C) control group (D-F) ginger group (G-I) GBP group (J-L) GBP+ginger group. Podocytes (Po) with foot processes (tailed arrow), basal folds (arrow), basal lamina (arrow head), degenerated nucleus (Nu) with abnormal nuclear envelope (wavy arrow). Scale bar = 2µm.

Ultrastructurally level, GBP was found to induce deleterious effects on the fetal kidney. According to the present study, the podocytes showed diminished foot processes as well as pyknotic nuclei, they were hypertrophied so that they erased the urinary spaces and compressed the capillary tuft which were congested. Thickened basement membrane was evident in both proximal and distal tubule cells accompanied by shrunken nuclei and altered basal enfolding as well as partial destruction of brush border. The mitochondria were swollen and vacuolated with loss of cristae. These results were in line with El-Sayyad et al. [38] and Aktaş et al. [21] who found that valproate and lamotrigine induced

similar results in epileptic rat mothers. The observed findings are supported by the work of many authors in patients treated with lamotrigine [46] or sodium valproate, carbamazepine and phenobarbital [47].

Fragmentation of foot processes of the podocytes was explained by Pavenstädt et al. [48] who reported that foot processes of podocytes with filtration slits in-between play a major rule in selective permeability of glomeruli, injury to podocytes resulting in retraction of foot processes leads to proteinuria. Damage to the brush border of the proximal tubules could have been as a result of toxin binding to the brush border [49].

The mitochondrial swelling and degeneration of the mitochondria cristae observed accompanied with changes in the nucleus, such as chromatin condensation and irregular nuclear envelope indicate that these organelles are affected in a major way and most probably indicating apoptosis [50]. This was confirmed by the decreased expression of Bcl-2 and increased expression of Caspase-3 antigens the cytoplasm of these cells. It is known that Bcl-2 is localized to intracellular sites of oxygen-free radical generation, including mitochondria, endoplasmic reticula and nuclear membranes [51]. Moreover, it plays a pivotal protective role in preserving mitochondrial structure and function, preventing onset of mitochondrial permeability transition, and finally inhibiting the apoptosis [52]. From these findings, it may be hypothesized that GBP exerts its apoptotic action via the mitochondrial pathway.

Natural antioxidants strengthen the endogenous antioxidant defense mechanism and restore the optimal balance by neutralizing the reactive species and thus the search for crude drugs of plant origin with antioxidant activity has become a central focus of study in recent years [53]. Ginger has been extensively studied for a broad range of biological activities, especially antioxidant activities [54]. It is considered a safe herbal medicine with only few and insignificant adverse side effects [25, 55]. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals [56]. Besides, other researches showed that ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant [57]. The present study confirmed that, there was no difference between control and ginger extract groups. According to the present study, co-administration of ginger extract in dose of 200 mg/Kg with GBP partially ameliorated the histological, immuno-histochemical and ultrastructural changes produced in the kidney due to GBP toxicity.

Several studies have ascertained the protective role of ginger on kidney from different xenobiotics [58]. Ramudu et al. [59] found that ginger effectively attenuated the progression of structural nephropathy in diabetic rats. Similarly, the pathological changes observed in the kidney of STZ-induced diabetic rats were decreased when they were

treated with ginger [60]. Previous studies showed the beneficial effect of ginger to inhibit cisplatin-induced nephrotoxicity [61, 62]. Yon et al. [63] stated that [6]-gingerol and ethanolic ginger extract exhibit protective effects against cisplatin-induced oxidative stress and acute renal failure. Studies have shown that ginger has ameliorative effect against cadmium-induced kidney injury in rat models [29].

The present study revealed that ginger lead to suppression of apoptosis confirmed by increased expression of Bcl-2 and decreased expression of Caspase-3 antigens in the combined ginger and GBP group compared with the GBP only. This was concomitant with the study of Sakr and Badawy [64] who confirmed that ginger reduced apoptosis induced by metiram in the testis of male albino rats. Ali et al. [65] indicated that ginger has a protective effect against cisplatin induced renal damage in rats evidenced by decreased expression of Bax pro-apoptotic protein in the renal cells. In the study of Abd-Allah and Sharaf El-Din [66], rats treated with ginger powder had a decreased expression of protein levels of the pro-apoptotic Bax in the injured intestinal tissues, while those of the antiapoptotic Bcl-2 protein levels increased. Ginger increased the expression of Bcl-2 more than what appeared in the case of cadmium group [67]. Moreover, Baiomy and Mansour [68] showed that cadmium increased the expression of Caspase-3, while ginger reduced its expression in the kidney tubular epithelium of rabbit. Hegazy et al. [69] concluded that 6-gingerol exerts an ameliorative role against gentamicin-induced renal apoptosis via reducing the expression of Caspase-3 antigen.

Transmission electron microscopy investigation in the present study showed that the renal cells retained their normal ultrastructure after combined treatment with ginger and GBP. These results were in agreement with Mahmoud et al. [70] who showed that ginger administration improved the renal ultrastructure in a model of renal failure in albino rats. Similar results were seen in the study of Ali et al. [65] who found that ginger led to an improvement in the ultrastructural renal injury induced by cisplatin.

Although a number of antioxidant compounds have been isolated from ginger [71] the exact mechanism by which ginger exerts antioxidant effect is not yet clear [53]. However, it has been postulated that ginger may attenuate free radical mediated toxic effects by lowering lipid peroxidation and maintaining the activities of antioxidants and this may be attributed to curcumin and zingerone components present in ginger. Moreover, the antioxidant value of ginger is possibly due to its ability to scavenge a number of free radicals and protect cell membrane lipids from oxidation and inhibiting lipid peroxidation, leading to regeneration of damaged tissues and cells in a dose dependent manner [72-74]. In conclusion, the present study demonstrates that ginger exerts significant ameliorative effects against GBP-induced nephrotoxicity in rat fetuses maternally injected with GBP,

possibly via its antioxidant and free radical-scavenging properties. However, further investigations are needed to demonstrate the exact mechanism of ginger on GBP induced nephrotoxicity.

3.5. Conclusion

The present study showed that maternal injection of GBP in pregnant rats during the organogenesis period resulted in adverse effects in the fetal kidneys on the histological, immunohistochemical and ultrastructural levels. On the other hand, co-administration of ginger ameliorated the damage caused by gabapentin and resulted in better renal structure. Therefore, it is advised that ginger should be taken in parallel with GBP during pregnancy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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