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Effects of royal jelly on genotoxicity and nephrotoxicity induced by valproic acid in albino mice

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ABSTRACT

Epilepsy is one of the most common neurological diseases affecting at least 50 million people worldwide. Valproic acid (VPA) is a widely used antiepileptic medication for both generalized and partial seizures of epilepsy. The objective of the study was to investigate the anti-mutagenic and anti-histopathologic effects of royal jelly (RJ) on VPA-induced genotoxicity and nephrotoxicity in male albino mice (*Mus musculus*). 80 Mice were used for 21 days; they were divided into eight groups, (G1) served as normal control group, G2 received VPA (100 mg/kg) and (G3–G5) received RJ at doses 50, 100 and 200 mg/kg respectively. While (G6–G8) were administrated RJ simultaneously with VPA. In RJ treated mice at doses of 50 and 100 mg/kg, the kidney sections showed normal histological structure with non significant changes in chromosomal aberrations (CA) and mitotic index (MI), while RJ at dose of 200 mg/kg showed mild inflammatory cells infiltration and hyperemic glomeruli but not highly significant changes in CA and MI. The cortex of VPA treated mice revealed congested glomeruli with inflammatory cells infiltration, and marked degeneration of almost structures of the glomeruli including some vacuoles in mesangial cells with dark mesangial substances on the ultrastructure level. Some proximal tubules showed degeneration of microvilli on the apical parts of some cells. Cells of the distal tubules attained obliterated lumen and vacuolated lining epithelium. The results also revealed that valproic acid induced a high frequency of CA in bone marrow cells of mice and MI was significantly decreased indicating bone marrow cytotoxicity. The treatment of mice with RJ at doses 50, 100 and 200 mg/kg for 21 days simultaneously with VPA resulted in abating the histological alterations in renal tissues with significant reduction in chromosomal aberrations, for doses of 50 and 100 mg/kg, and elevation in mitotic index ($P < 0.05$). RJ at doses 50 and 100 mg/kg appeared more potent in exerting the ameliorative effect.

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1. Introduction

Valproic acid (VPA), the common name of 2-propylpentanoic acid or dipropylacetic acid (Aktaş et al., 2010), is commonly prescribed worldwide as a broad spectrum antiepileptic drug with specific indications for many forms of epilepsy and many types of seizures affecting both children and adults (Silva et al., 2008). VPA is usually well tolerated. Nevertheless, along with the desired effects, VPA therapy can induce side effects such as dyspepsia, obesity, hematological toxicity, teratogenicity, idiosyncratic hepatotoxicity and important endocrine dysfunctions (Dutheil et al., 2008; Verrotti et al., 2005; Zhang and Wang, 2009).

Verrotti et al. (2000) indicated that patients treated with VPA had an impairment of renal tubular functions. As well, Altunbaşak et al. (2001) reported that epileptic children who were ambulatory and depend on VPA monotherapy developed clinically insignificant proximal renal tubular dysfunction. Recently, the experiment of Mazaheri et al. (2011) concerning children on anti-epileptic treatment with valproic acid recorded signs of renal tubular dysfunction, reflected by N-acetyl beta glucosaminidase/creatinine (NAG/Cr) activity index.

Kortenhorst et al. (2009) reported that VPA treatment caused significant nuclear alterations in normal drug-filtering organs (liver and kidney tissues). VPA further induced a depletion of several members of the structural maintenance of chromatin (SMC) proteins, SMC-associated proteins, DNA methyltransferase and heterochromatin proteins (Marchion et al., 2005).

Long-term mono-therapy or poly-therapy with anti-epileptic drugs leads to the formation of toxic metabolites of these drugs, reactive oxygen species and free radicals (Witczak et al., 2008). In particular, reactive oxygen species and free radicals show genotoxic activity (Mitchell et al., 2004). In order to overcome the potential harmful effect of free radicals and to reduce the damage by oxidants, many natural substances have been tried as antioxidants.

Royal jelly (RJ) is a honeybee product secreted from the hypopharyngeal and mandibular glands of the worker honeybees (Silici et al., 2009). It is a mixture that contains protein, glucose, lipid, vitamins, minerals (Nakajima et al., 2009), aspartic acid, gelatin, sterols, phosphorous compounds, acetylcholine, nucleic acids, and numerous trace ingredients, which are all important in RJ's documented therapeutic and nutritional properties (Çavuşoğlu et al., 2009). These ingredients are in the form of 65% water, 12% crude protein and 10% monosaccharides. The remainder of the royal jelly is composed of an ether-soluble fraction of fatty acids (Spannhoff et al., 2011).

Previous studies have shown that RJ has number of physiological effects, such as anti-inflammatory, anti-tumor, antimetastatic effect (Kimura et al., 2003) anti-allergic, antioxidant activities (Guo et al., 2008), antibacterial, vasodilative and hypotensive activities, disinfectant action and anti-hypercholesterolemic activity (Ramadan and Al-Ghamdi, 2012).

RJ has received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity (Cemek et al., 2010) and used for decreasing the toxic effects of chemical agents (El-Nekeety et al., 2007). Several studies revealed biological evidence supports the use of RJ in the treatment of chemical induced genotoxicity (Türkmen et al., 2009). It has a DNA-protective effect (Inoue et al., 2003) and also stimulates bone marrow formation (Narita et al., 2006). Abd El-Monem (2011) revealed that RJ caused a significant recovery in antioxidant status of reduced glutathione (GSH) and a significant inhibition of malondialdehyde (MDA) production and ameliorated DNA damage and genotoxicity induced by malathion in rat cells.

Therefore, the present study was undertaken to evaluate the possible protective effects of royal jelly against valproic acid induced chromosomal abnormalities in bone marrow cells and histological alterations in kidney tissue of male mice (*Mus musculus*).

2. Materials and methods

2.1. Chemicals

Sodium valproate (salt of Valproic acid) was purchased from pharmacy in the form of tablets with trade name Depakine (Sanofi Synthelabo, France), each containing 200 mg of sodium valproate dissolved in distilled water according to the used dose. Royal Jelly was purchased from pharmacy in the form of capsules (Techno Pharma Egypt for Pharco Pharmaceuticals, Alexandria, Egypt) each containing 340 mg of natural royal jelly dissolved in distilled water according to the used dose. All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Experimental animals

The experimental animals used in this work were 80 random bred adult males of laboratory mice *M. musculus* (20–30 g in weight). All animals were housed in plastic cages with wired covers and kept under normal laboratory conditions. Experiments were performed as per internationally followed ethical standards and according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Institute of Laboratory Animal Resources, 1996). The animals were not treated with antibiotics or insecticides and fed a standard commercial diet (ATMID Company, Egypt) and tap water *ad libitum*.

2.3. Experimental design

A single dose (100 mg/kg) of VPA was selected with reference to the dose range of the cytotoxicity and genotoxicity of VPA (Lee et al., 2007). However, RJ concentrations used in the study were 50, 100 and 200 mg/kg. These concentrations were selected according to (Cemek et al., 2010).

Experimental groups were organized into eight groups including 10 animals per each. Because colchicine is toxic, five animals were used for cytogenetic analysis while the other five animals were used for histopathological studies. The animals of group one (G1) served as normal control receiving 0.9% of NaCl solution by intraperitoneal injection (i.p.) daily

for three weeks. The animals of G2 received intraperitoneal (i.p.) injection of VPA (100 mg/kg) daily for three weeks. In groups 3, 4 and 5, the RJ doses 50, 100 and 200 mg/kg respectively, were given to the animals through oral intubation once/day for three weeks. The animals of the groups G6, G7 and G8 received oral administration of RJ (50, 100 and 200 mg/kg respectively) once/day for three weeks simultaneously with i.p. injection of VPA.

2.4. Cytogenetic assay

Bone marrow cell preparations for the analysis of chromosomal aberrations and mitotic indices were conducted by the colchicine-hypotonic technique. Twenty-four hours after completion of the treatment period, five animals from each group were sacrificed by cervical dislocation. Colchicine was given at the dose of 4 mg/kg intraperitoneally (i.p.) at 2 h prior to sacrifice time. The bone marrow smears of animals in each group were prepared according to Preston et al. (1987). For each group, slides were stained with Giemsa staining method and 50 well spread metaphase plates/animal were analyzed for chromosomal aberrations and the number of mitotic cells in 1000 cells/animal. The percentage of suppressed aberrant cells was calculated according to Shukla and Taneja (2002) as follows:

$$100 - \left(\frac{\%\text{ of aberrant cells in G6 or G7 or G8}}{\%\text{ of aberrant cells in G2}}\right) \times 100$$

2.5. Histopathological assay

2.5.1. Preparation of paraffin section

At the end of the experiment, mice in each group were sacrificed under mild anesthesia by diethyl ether and dissected quickly to remove the kidney. Kidney tissues were fixed in 10% buffered formalin, washed with tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. After routine processing, paraffin wax tissue blocks were prepared for sectioning at 4 microns thickness by rotary microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination (Bancroft and Gamble, 2002).

2.5.2. Ultrastructure preparations

Specimens from kidney were cut into small pieces measuring about 1 mm^3 and kidney biopsies were fixed in 2.5% phosphate buffered glutaraldehyde. Specimens were post-fixed with 1% osmic acid and dehydrated with acetone, the semithin sections of tissue samples, embedded in araldite, were stained with toluidine blue. Then, the thin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Joel CX100 transmission electron microscope.

2.5.3. Statistical analysis

Statistical analysis for the difference in the mean number of chromosomal aberrations and mitotic index between groups was carried out using the student t-test ($P < 0.05$ was considered significant).

3. Results

3.1. Cytogenetic assay

According to the illustrated cytogenetic results, ten structural and numerical chromosomal aberrations were determined in the control and the experimental groups (Fig. 1). The results obtained in the first phase of cell cycle (24 h after sampling time), revealed that VPA given at a dose of 100 mg/kg daily for three weeks (G2), induced a high frequency of chromosomal aberrations in bone marrow cells of mice when compared with the control group (G1). The chromatid breaks and deletions were the most frequent chromosomal aberrations. Other structural and numerical aberrations increased significantly at ($P < 0.05$) over the control group (G1) (Table 1 and Fig. 2), while the mitotic index was significantly decreased to 59.76 ± 3.771, compared to control value of 80.84 ± 1.035 ($P < 0.05$), indicating bone marrow cytotoxicity (Table 2).

The data illustrated in Table 1 indicated that, when RJ was given alone at doses 50, 100 and 200 mg/kg once daily for three weeks (G3, G4 and G5, respectively), it did not induce or increase the number of chromosomal aberrations over control group (G1) like ring chromosomes, end to end association, centric fusion, centric attenuation and endomitosis. Nevertheless, the number of chromatid breaks, chromatid deletions, value of total structural aberrations, value of total numerical chromosomal aberrations, number of cells with one aberration, percentage of incidence of aberrant cells and number of aberration/cell increased gradually with increasing the dose of RJ over the control group (G1) (Tables 1 and Fig. 2). The RJ was also found to be not cytotoxic at the given doses of 50 and 100 mg/kg as there was no significant changes at ($P < 0.05$) in mitotic index over G1, while RJ at dose of 200 mg/kg showed slight cytotoxicity (Table 2).

Administration of RJ with VPA (G6, G7 and G8) indicated a significant decrease in rates of clastogenetic changes compared with the VPA-treated group (Tables 1 and Fig. 2). All types of chromosomal aberrations induced by VPA including breaks, deletions, fragments, end to end association, centric fusion, and other multiple damages were found to be reduced by RJ but still significantly higher than the normal control group (G1) (Table 1). The mitotic index was found to be increased significantly ($P < 0.05$) over G2 for 50 and 100 mg/kg doses, indicating their anti-cytotoxicity towards VPA (Table 2). The percentages of aberrant cells which were found to be 30.40 ± 1.460 in VPA treated animals, were reduced to 18.80 ± 0.320, 17.60 ± 1.070 and 24.40 ± 1.360 ($P < 0.05$) by RJ at doses 50, 100 and 200 mg/kg respectively (Table 2). Also a significant decrease in the number of aberrations per cell was observed in G6 and G7 over the VPA-treated group (G2). The suppressive effects of RJ, at doses 50, 100 and 200 mg/kg were 38.150%, 42.100% and 19.737%, respectively (Table 2).

3.2. Histopathological assay

3.2.1. Light microscope analysis

Microscopic examination of the kidney of male mice was carried out in all experimental groups after 3 weeks of treatment

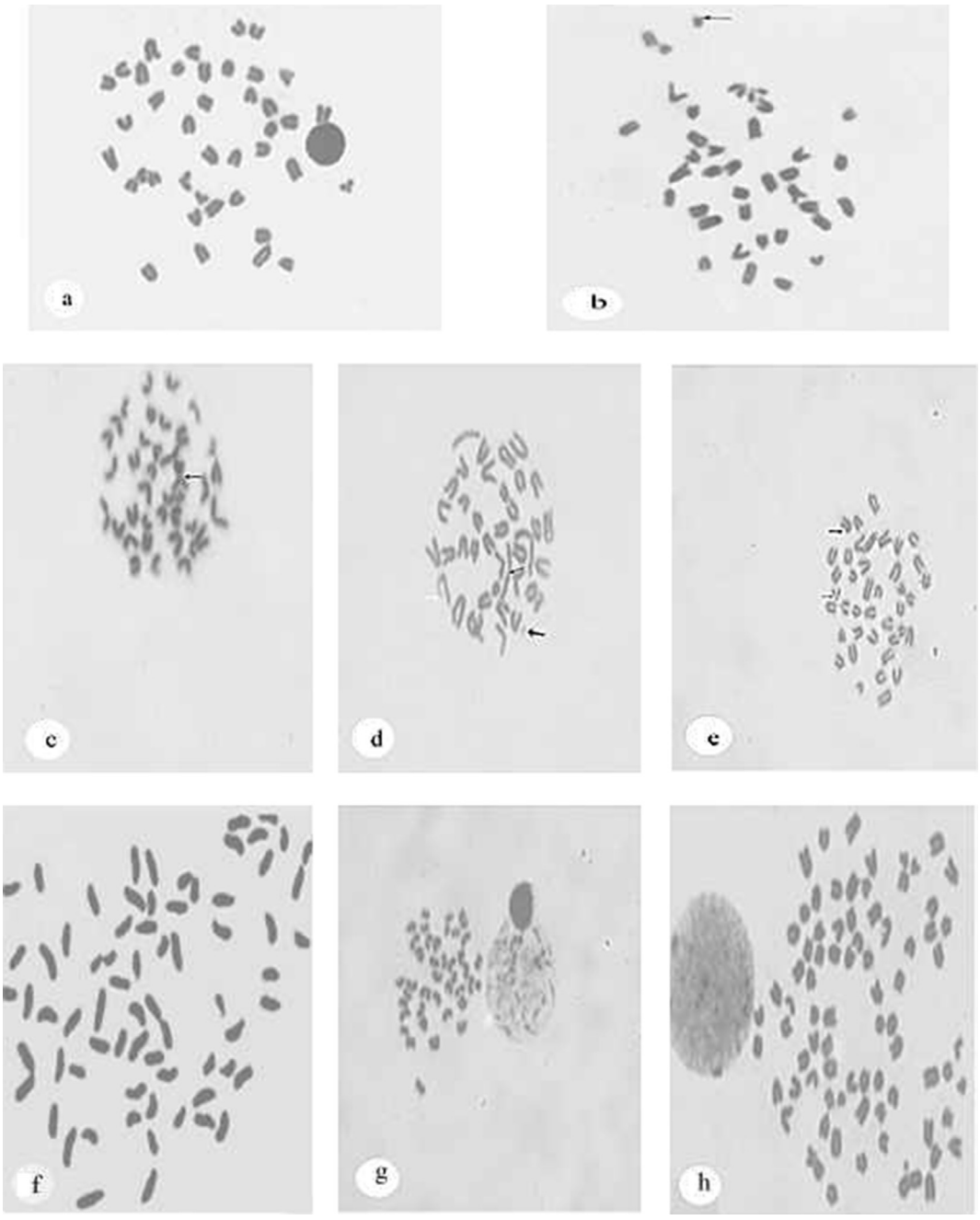

Fig. 1 – (a) Metaphase spread from mouse bone marrow cells showing normal chromosomes spread. (b) Metaphase spread from mouse bone marrow cells showing ring chromosome (↑). (c) Metaphase spread from mouse bone marrow cells showing centric fusion aberration (↑). (d) Metaphase spread from mouse bone marrow cells showing a cell with more than one aberration [deletion (white arrow), fragment (bold black arrow) and end to end association (thin black arrow)]. (e) Metaphase spread from mouse bone marrow cells showing a cell with more than one aberration [chromatid break (thin arrow) and chromatid gap (bold arrow). (f) Metaphase spread from mouse bone marrow cells showing centric attenuation aberration. (g) Metaphase spread from mouse bone marrow cells showing endomitosis. (h) Metaphase spread from mouse bone marrow cells showing polyploidy.

to identify the extent of tissue damage induced by VPA and the ability of RJ for restoring and ameliorating these damaging effects. Light microscopy proved more ameliorative effect at dose 100 mg/kg, so electron microscopy was restricted to this dose. In control mice, the microscopic examination of the kidney sections showed normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle includes the glomerulus that is a small tuft of capillaries covered by Bowman's capsule. The renal tubules consist of proximal convoluted tubules, distal convoluted tubules and collecting

Table 1 – Chromosomal aberrations induced in bone marrow cells of mice after treatment with VPA and 3 different doses of royal jelly (RJ).

Groups	Number of metaphase	Structural chromosomal aberrations									Numerical chromosomal aberrations		
		Chromatid break	Chromatid deletion	Chromatid gap	Ring chromosome.	Fragment	End to end assoc.	Centric fusion	Centric attenuation	TSA[a]	Polyploidy	Endomitosis	TNA[a]
Gr1 (saline)	250	3 (1.20)	4 (2.60)	0	0	0	0	0	1 (0.40)	8 2.20 ± 0.860	0	4 (1.60)	4 0.80 ± 0.200
Gr2 (VPA)	250	18 (7.20)	23 (9.20)	0	1 (0.40)	6 (2.40)	5 (2.00)	4 (1.60)	14 (5.60)	71[b] 14.20 ± 1.529	3 (1.20)	12	15[b] 3.00 ± 0.710
Gr3 (RJ50)	250	3 (1.20)	4 (1.60)	1 (0.40)	0	4 (1.60)	1 (0.40)	0	1 (0.40)	14[c] 2.80 ± 0.860	0	3 (1.20)	3[c] 0.60 ± 0.400
Gr4 (RJ100)	250	7 (2.80)	9 (3.60)	0	0	4 (1.60)	1 (0.40)	1 (0.40)	0	22[b] 4.40 ± 0.510	2 (0.80)	0	2[c] 0.40 ± 0.245
Gr5 (Rj200)	250	9 (3.60)	6 (2.40)	3 (1.20)	2 (0.80)	2 (0.80)	0	0	1 (0.40)	23[b] 4.60 ± 0.245	6 (2.40)	3 (1.20)	9[c] 1.80 ± 0.735
Gr6 (RJ50 + VPA)	250	10 (4.00)	17 (6.80)	0	0	4 (1.60)	3 (1.20)	2 (0.80)	6 (2.40)	42[d] 8.40 ± 0.600	4 (1.60)	6 (2.40)	10[e] 2.00 ± 0.316
Gr7 (RJ100 + VPA)	250	16 (6.40)	12 (4.80)	1 (0.40)	2 (0.80)	2 (0.80)	1 (0.40)	2 (0.80)	6 (2.40)	42[d] 8.40 ± 0.927	1 (0.40)	9 (3.60)	10[e] 2.00 ± 0.707
Gr8 (RJ200 + VPA)	250	15 (6.00)	16 (6.40)	1 (0.40)	0	1 (0.40)	0	0	23 (9.20)	56[e] 11.20 ± 1.463	6 (2.40)	8 (3.20)	14[e] 2.80 ± 0.200

TSA: Total structural aberrations TNA: Total numerical aberrations.
$p < 0.05$ Significant.
[a] Mean ± SE data in brackets %.
[b] Significant increased with −ve control.
[c] Not significant with −ve control.
[d] Significant decreased with +ve group.
[e] Not significant with +ve control.

Table 2 – Clastogenetic changes induced in bone marrow cells of mice in control and treated groups.

Groups	Number of[a] cells with one aberration	Number of cells[a] with more than one aberration	Incidence of[a] aberrant cells %	Number of[a] aberration/cell	Suppressive effect %	Mitotic index[a]
Gr1 saline	4.80 + 2.039	–	4.80 + 2.039	0.032 + 0.020		80.84 + 1.035
Gr2 VPA	13.20 ± 1.241	2.00 ± 0.316	30.40 ± 1.460[b]	0.344 ± 0.034[b]		59.76 ± 3.771[b]
Gr3 RJ50	3.40 ± 0.678	–	6.80 ± 0.680[c]	0.068 ± 0.013[c]		71.34 ± 0.717[c]
Gr4 RJ100	3.20 ± 0.489	0.80 ± 0.374	8.00 ± 0.450[c]	0.096 ± 0.013[b]		69.73 ± 0.872[c]
Gr5 RJ200	6.40 ± 0.600	–	10.80 ± 0.750[b]	0.128 ± 0.012[b]		62.03 ± 1.456[b]
Gr6 RJ50 + VPA.	8.60 ± 0.510	0.80 ± 0.374	18.80 ± 0.320[d]	0.208 ± 0.010[d]	38.150	62.22 ± 0.610[e]
Gr7 RJ100 + VPA.	6.40 ± 1.288	2.00 ± 0.447	17.60 ± 1.070[d]	0.208 ± 0.018[d]	42.100	64.31 ± 0.855[e]
Gr8 RJ200 + VPA.	11.00 ± 1.673	1.20 ± 0.800	24.40 ± 1.360[e]	0.280 ± 0.027[e]	19.737	58.33 ± 0.521[e]

$p < 0.05$ Significant.
[a] Mean ± SE data.
[b] Significant increased with –ve control.
[c] Not significant with –ve control.
[d] Significant decreased with +ve group.
[e] Not significant with +ve control.

tubules. Proximal convoluted tubules are lined by simple cuboidal epithelium, having prominent brush borders and acidophilic cytoplasm. Distal convoluted tubules are identified on account of simple cuboidal epithelium, clearly defined and wider lumen than those of the proximal convoluted tubules (Fig. 3a). In control RJ-treated mice, the microscopic examination of the kidney sections showed normal histological structure of the renal corpuscles and renal tubules, except for RJ at a dose of 200 mg/kg that showed mild inflammatory cells infiltration and hyperemic glomerulus (Fig. 3b). The microscopic examination of the kidney sections of VPA-administered mice showed periglomerular oedema infiltrated with a number of inflammatory cells (Fig. 3c and d), karyomegally of some epithelial cells lining the renal tubules (Fig. 3c and e) and hyperemic glomerulus (Fig. 3c, e and f). Other corpuscles showed adhesion of the glomerular tufts with Bowman's capsules (Fig. 3e). Dilatation and congestion of blood vessels (Fig. 3d), swelling of renal tubules (Fig. 3f) and degeneration of the epithelial cells lining the renal tubules (Fig. 3g) were detected. In addition, the endothelium of intertubular blood vessels degenerated and the muscle fibers showed hydropic degeneration and perivascular oedema (Fig. 3h).

Photomicrographs of kidney sections of mice treated with VPA and RJ (200 mg/kg) revealed atrophied glomerular tuft and degeneration of epithelial lining of the renal tubules (Fig. 4a). In addition, dilated blood vessels engorged with blood cells and perivascular oedema were noticed (Fig. 4b). Light microscopic examination of the kidney of mice treated with VPA and RJ (50 mg/kg) revealed regeneration of renal tubules and glomeruli but still with slight inflammatory cells infiltration (Fig. 4c and d). While the kidney of mice treated with VPA and RJ (100 mg/kg) revealed nearly normal structure of renal corpuscles and renal tubules (Fig. 4e and f).

3.3. *Transmission electron microscopic analysis*

3.3.1. *Renal corpuscles*

The renal corpuscles of control normal mice appear as dense round tufts of capillaries. The outer layer of

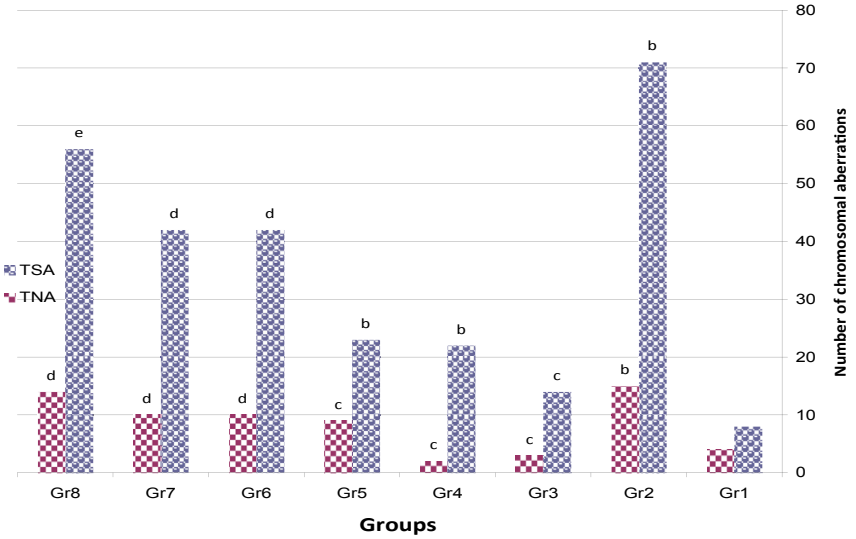

Fig. 2 – Showing the number of total structural and total numerical chromosomal aberrations in the experimental groups.

Bowman's capsule is the parietal layer and the inner visceral layer applies closely to the glomerular capillaries. Podocytes surround the capillaries. Each capillary loop is lined with endothelial cells. Podocytes give rise to primary processes which in turn give numerous secondary foot processes or pedicels that rest on a thin basal lamina. Each podocyte has a large nucleus and abundant cytoplasm. The pedicels are separated by slit pores. The filtration barrier includes thin basal lamina. The mesangium comprises two components, mesangial cells and mesangial substance and provides support for several capillary loops. The mesangial cell is characterized by large heterochromatic nucleus. The mesangial matrix largely encloses the mesangial cells (Fig. 5a).

Electron micrographs of kidney sections of mice given VPA for three weeks showed renal corpuscles with marked degeneration of almost structures of glomeruli, including some vacuoles in mesangial cells, segmented nuclei and irregular basal lamina (Fig. 5b). The mesangial cell appeared vacuolated with dark mesangial substance. Moreover, the mesangial matrix showed marked expansion (Fig. 5c), marked thickening of the basal lamina and fusion of foot processes were also noticed (Fig. 5d).

After treatment with RJ "at a dose of 100 mg/kg" in concomitant with VPA, the renal corpuscle of mice showed marked amelioration of almost structures including the podocytes, mesangial cells retained their normal structure and the basal lamina appeared thin compared to that of mice treated with valproic acid alone (Fig. 5e).

3.3.2. *Renal tubules*

The normal proximal tubule cells have a brush border of numerous microvilli. The nucleus is spherical in shape. Numerous mitochondria fill the cytoplasm, the basal lamina is thin and the basal infoldings run upwards among the mitochondria (Fig. 6a). The proximal tubules of mice given VPA (100 mg/kg) for three weeks showed dissolution and degeneration of some parts of the cytoplasm, degeneration of microvilli on the apical parts of some cells, in addition to increased lysosomes (Fig. 6b). The most pronounced feature revealed marked thickening of the basal lamina and vacuolation (Fig. 6c and d). Moreover pyknotic nucleus and condensed mitochondria were observed (Fig. 6e). The most damaged organelle, the mitochondria, showed swelling (Fig. 6c). Treatment with RJ "at a dose of 100 mg/kg" in concomitant with VPA showed marked reduction of basal lamina thickness and the apical cell membrane and mitochondria appeared normal but irregular nucleus and some cytoplasmic vacuolation were still observed. However, the cytoplasmic changes were milder than those seen in VPA treated group (Fig. 6f).

3.3.3. *Distal tubules*

The distal tubules of normal control mice showed normal structure, few or no microvilli and deep infoldings embracing elongated, spherical or filamentous mitochondria were observed (Fig. 7a). In VPA treated group, the distal tubule cells attained obliterated lumen and vacuolated lining epithelium. The basal infoldings if appeared were shorter. Some damaged mitochondria were also observed. The nuclei appeared normal (Fig. 7b and c).

After administration of royal jelly (100 mg/kg) with VPA, the basal infoldings of the distal tubules extended deeply in the cytoplasm among the mitochondria. Regular basal lamina and restoration of the number of mitochondria were found (Fig. 7d).

4. Discussion

Epilepsy is one of the most prevalent neurological diseases affecting approximately 50 million people throughout the world (El-Sayyad et al., 2013). Many of the antiepileptic drugs have been found to be mutagenic and teratogenic in laboratory animals (Biswas et al., 2004). Valproic acid (VPA), a frequently used drug for the treatment of epilepsy, has been used worldwide. However, it causes mild to severe side effects and may lead to death (Baran et al., 2004). The present study was designed to investigate the protective role of royal jelly (RJ) against the cytogenotoxicity and histopathological alterations of VPA in mice on light and ultrastructural levels. VPA effects are associated with several biochemical/molecular modes of action that have been put forward to account for VPA action, such as interference with folate–methionine metabolic pathways (Dawson et al., 2006), activity as a histone deacetylase inhibitor (Eikel et al., 2006), alteration of gene expression (Okada et al., 2005), generation of reactive intermediates via lipoxygenases or the prostaglandin synthetase co-oxidation pathway (Miranda et al., 1994), altered antioxidant enzyme activities (Graf et al., 1998) or increase in the formation of toxic VPA metabolites (Baran et al., 2004) such as 2, 4-diene-VPA which can form glutathione conjugate altering glutathione homeostasis (Tabatabaei and Abbott, 1999). In addition, Tabatabaei et al. (1997) indicated that the increased flux of oxygen free radicals during the VPA metabolism leads to nuclear DNA damage, resulting in ensuing cell death. Furthermore, another study showed that free radicals play an important role as regulatory mediator in cellular signaling processes. Excessive amount of free radicals production has been related with an increase in oxidative stress described in series of treatment with VPA in epileptic patients (Martinez-Ballesteros et al., 2004).

The present investigation showed mice treated with VPA had increase in number of chromosomal abnormalities and decrease in mitotic index of bone marrow cells. The chromosomal aberrations induced after treatment with VPA were structural and numerical aberrations. These results were in agreement with previous studies which reported a significant increase in chromosomal aberration rates in adults treated with antiepileptic drugs (Denli et al., 2000) and in epileptic children undergoing long-term antiepileptic drug (Curatolo et al., 1986). Recently, Witczak et al. (2010) indicated that an increase in sister chromatid exchange frequency and in the frequency of chromosome aberrations (including chromosome gaps) was observed in pregnant women when received different antiepileptic drugs in mono- and poly-therapy.

Our results suggested that valproic acid was capable of being genotoxic and cytotoxic because administration of VPA increased chromosomal abnormalities and decreased

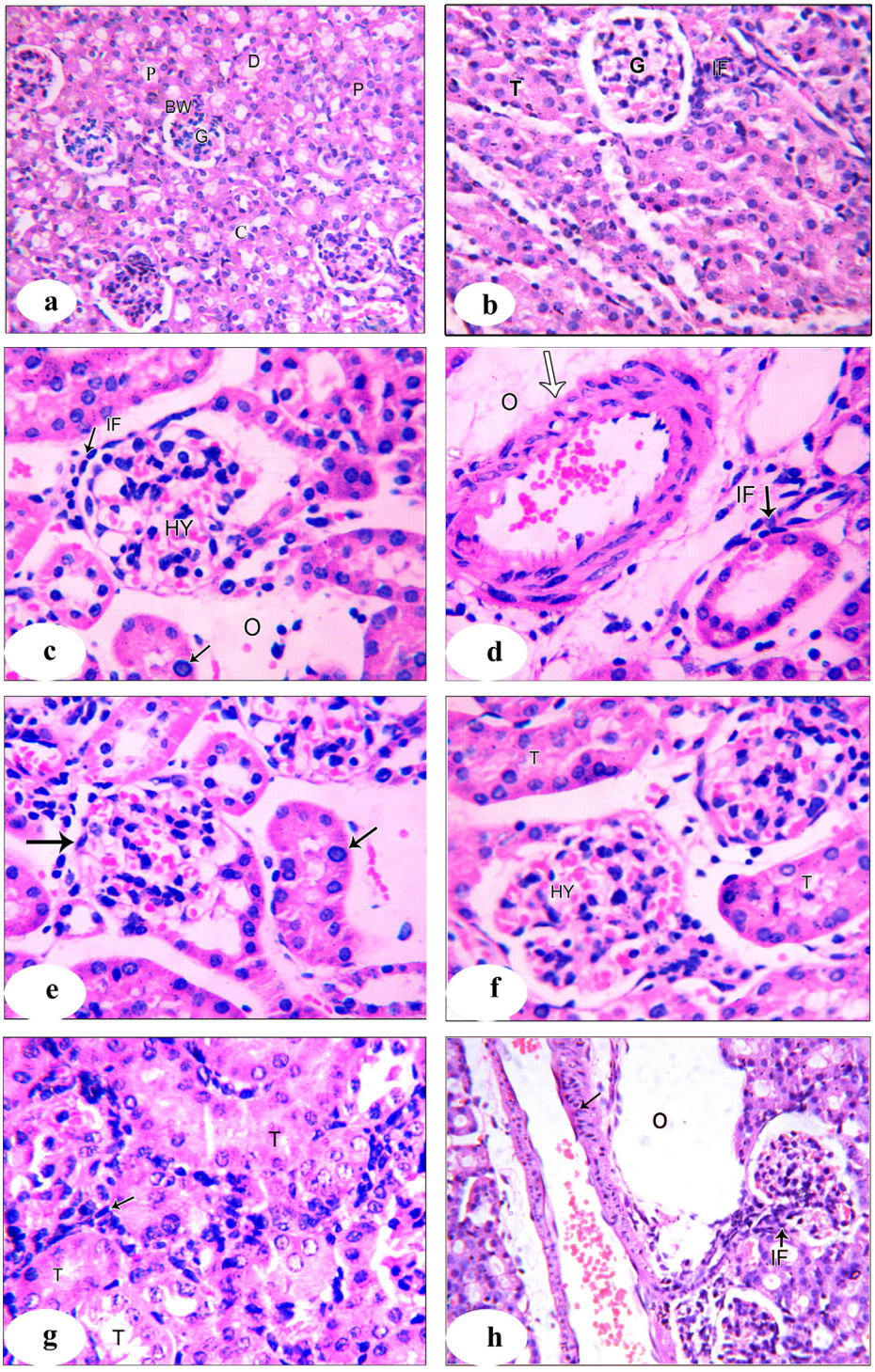

Fig. 3 – (a) A photomicrograph of kidney section of normal control mice showing the glomerulus (G), Bowman's capsule (BW), in addition to proximal tubules (P), distal tubules (D) and collecting tubules (C) (H&E ×100). (b) A photomicrograph of kidney section of normal mice administered royal jelly (200 mg/kg) showing hyperemic glomerulus (G), inflammatory cells infiltration (IF) and dilated tubules (T) (H&E ×400). (c) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing a periglomerular oedema (O) with inflammatory cells infiltration (IF), hyperemic glomerulus (HY) and karyomegally of some epithelial cells lining the renal tubules (↑) (H&E ×400). (d) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing dilated blood vessel congested with blood cells (white arrow) and perivascular oedema (O) with inflammatory cells infiltration (IF) (H&E ×400). (e) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing karyomegaly of some epithelial cells lining the renal tubules (thin arrow) and adhesion of the glomerular tuft with Bowman's capsule (thick arrow) (H&E ×400). (f) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing hyperemic glomerulus (HY)

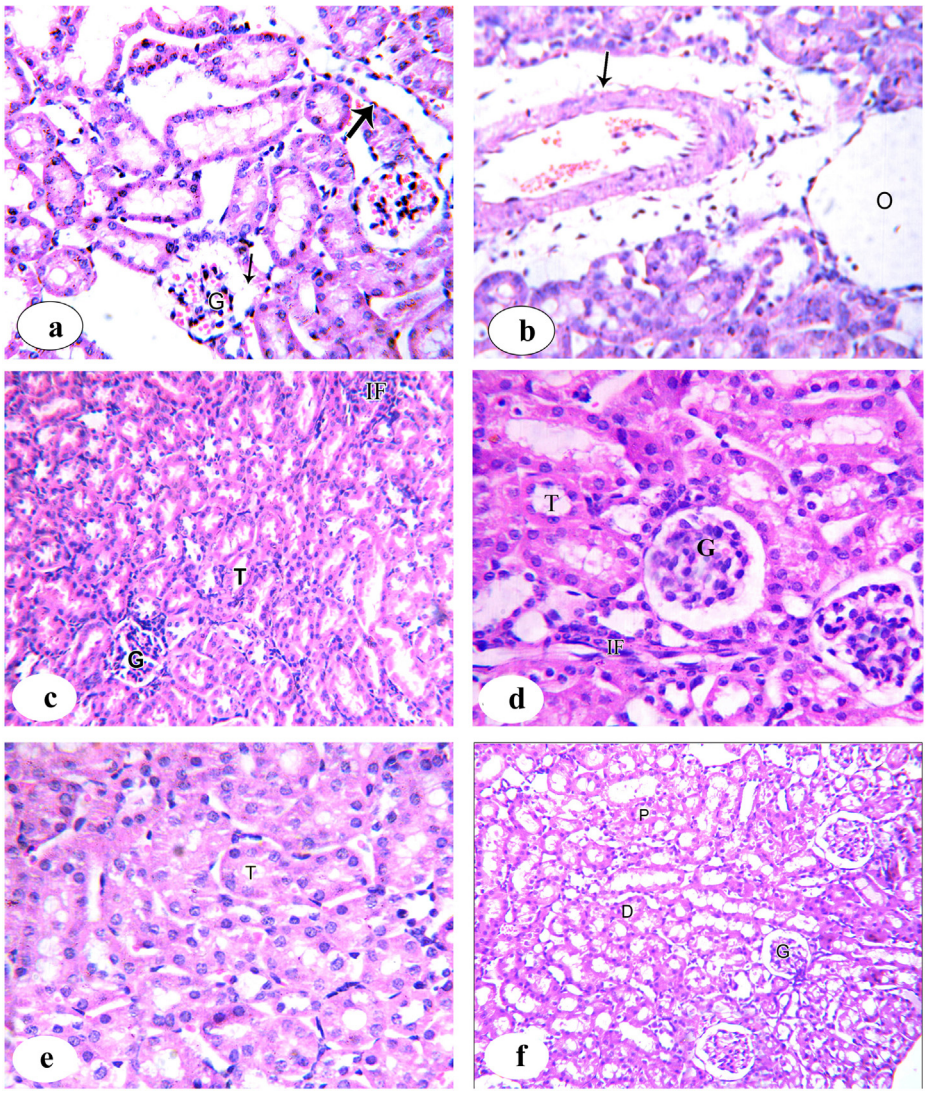

Fig. 4 – (a) A photomicrograph of kidney section of mice treated with valproic acid (100 mg/kg) and royal jelly (200 mg/kg) for 3 weeks showing atrophied glomerular tuft (G), widening of the Bowman's space (thin arrow) and degeneration of the epithelial lining of the renal tubules (thick arrow) (H&E ×400). (b) A photomicrograph of kidney section of mice treated with valproic acid (100 mg/kg) & royal jelly (200 mg/kg) for 3 weeks showing dilated blood vessel (↑) engorged with blood cells and perivascular oedema (O) (H&E ×400). (c & d) Photomicrographs of kidney sections of mice treated with valproic acid (100 mg/kg) & royal jelly (50 mg/kg) for 3 weeks showing nearly normal renal tubules (T) and glomeruli (G) but inflammatory cell infiltration (IF) is still present. c (H&E ×100); d (H&E ×400). (e) A photomicrograph of kidney section of mice treated with valproic acid (100 mg/kg) & royal jelly (100 mg/kg) for 3 weeks showing almost normal renal tubules (T) (H&E ×400). (f) A photomicrograph of kidney section of mice treated with valproic acid (100 mg/kg) & royal jelly (100 mg/kg) for 3 weeks showing almost normal proximal tubules (P), distal tubules (D) and glomeruli (G) (H&E ×100).

mitotic index of mice bone marrow cells. Previous studies that examined the genotoxicity of valproic acid by analyzing sister chromatid exchanges in peripheral lymphocytes from patients treated with valproic acid had shown contradictory results. Sister chromatid exchanges occur via the homologous recombination (HR) repair pathway and are used as an indicator of chromosomal stability after exposure to a potentially mutagenic agent

and swelling of renal tubules (T) (H&E ×400). (g) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing inflammatory cells infiltration in the intertubular space (↓) and damaged dilated tubules (T) (H&E ×400). (h) A photomicrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing that the endothelium of intertubular blood vessels is degenerated and the muscle fibers show hydropic degeneration (↓), perivascular odema (O) and inflammatory cells infiltration (IF) (H&E ×400).

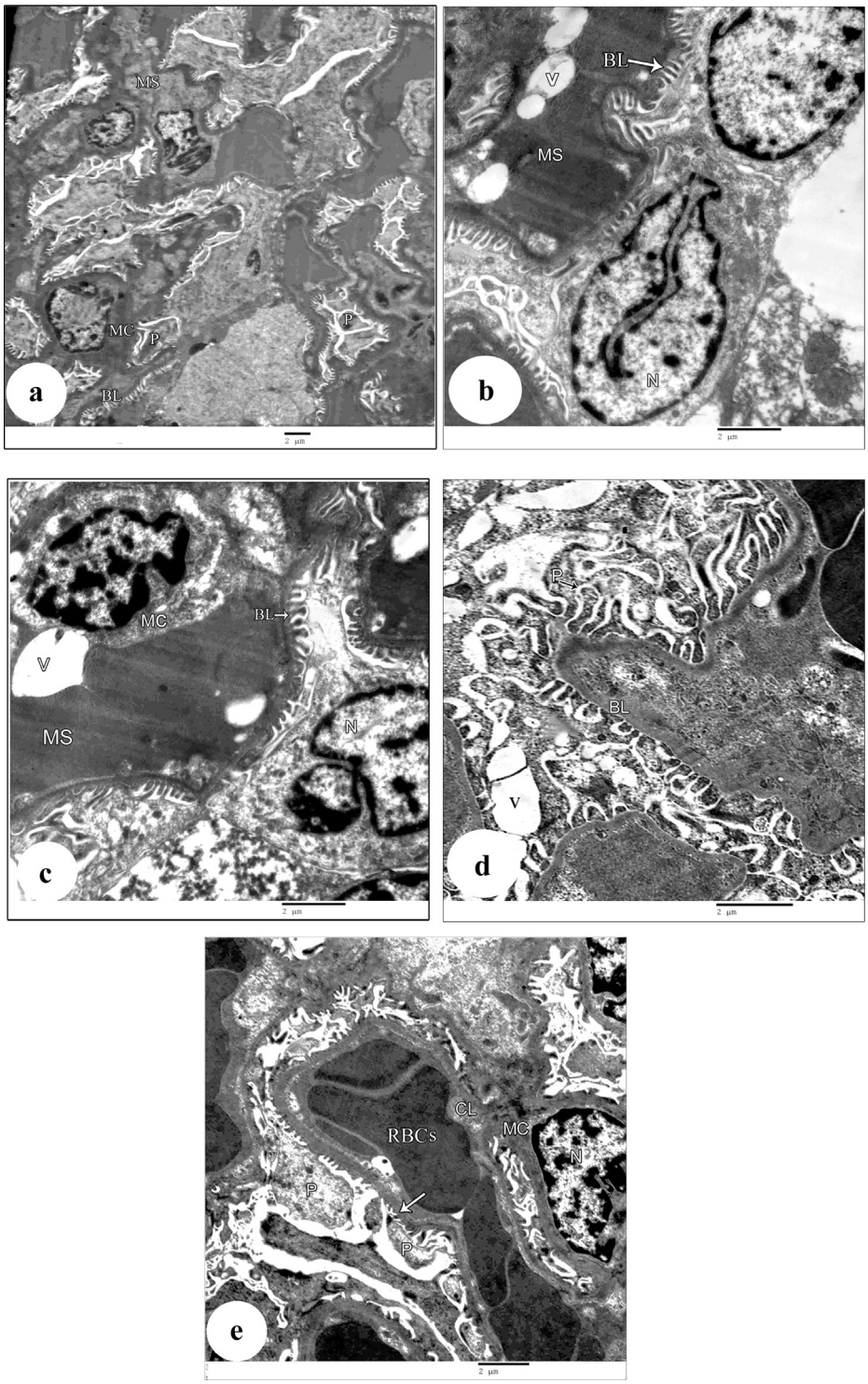

Fig. 5 – (a) Electron micrograph of kidney section of control mice showing podocytes (P) resting on the basal lamina (BL) and surrounding the capillary loops. Each capillary loop is lined by endothelial cells. Mesangial cells (MC) and Mesangial substance (MS) support the capillary loops. Scale bar = 2 μm. (b) Electron micrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing renal corpuscle with marked degeneration of almost structures of glomerulus including some vacuoles (V) in mesangial substance (MS), segmented nucleus (N) and irregular basal lamina (BL). Scale bar = 2 μm. (c) Electron micrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing vacuoles (V), irregular heterochromatic nucleus (N), dark cytoplasm of mesangial cell (MC), the expansion of mesangial substance (MS) and basal lamina (BL). Scale bar = 2 μm. (d) Electron micrograph of kidney section of mice given valproic acid (100 mg/kg) for 3 weeks showing thick and irregular of basal lamina (BL), podocytes (P) and vacuoles (V). Scale bar = 2 μm. (e) Electron micrograph of kidney section of mice treated with valproic acid (100 mg/kg) and royal jelly (100 mg/kg) for 3 weeks showing basal lamina (↓), mesangial cell (MC), podocyte (P), capillary lumen (CL) and red blood cells (RBCs). Note the appearance of nucleus (N) with dark clumbs of heterochromatin. Scale bar = 2 μm.

(Defoort et al., 2005). However further evidences indicated that valproic acid may contribute to genomic instability, because a growing body of evidence demonstrates that valproic acid alters chromatin structure because it can function as a histone deacetylase inhibitor, leading to the acetylation of histone tails (Wu et al., 2010). Also, VPA further induced a depletion of several members of the structural maintenance of chromatin (SMC) proteins, SMC-associated proteins, DNA methyltransferase and heterochromatin proteins (Marchion et al., 2005). This change in the chromatin structure relaxes the conformation of DNA, making it more susceptible to DNA damage, including double-strand breaks (Coyle et al., 2005).

The present study revealed marked histological changes in the kidney of VPA-treated mice. These changes were apparent in both renal corpuscles and tubules. The most prominent signs of devastation observed in the renal corpuscles were glomerular atrophy, mesangial proliferation and corpuscular adhesion with glomerular tufts. Besides, other corpuscles were shrunk with partial degeneration of glomerular tuft. The kidney tubules were also affected as indicated by the partial desquamation of epithelial cells lining the tubules, nuclear degeneration manifested by pyknosis and intertubular invasion by inflammatory cells. Moreover, karyomegally and hemorrhage were also observed. Similar results were described by (Ching-Yuang and Chiang, 1988). Vallon et al. (2003) also found that, the increase in the size of the tubules resulted in increased proximal reabsorption, which in turn caused glomerular hyperfiltration via hyperabsorption. The hyperfiltration increased glomerular capillary pressure; the glomerular hypertension caused endothelial mesangial and podocyte injuries with the increased filtration of proteins to tubular lumen resulting in tubulointenstial inflammation (Sanchez-Lozada et al., 2004), which was also seen in the present histological and ultrastructural study.

Renal tubular dysfunction characterized by glucosuria, phosphaturia, aminoaciduria and renal tubular acidosis in an epileptic child receiving VPA was proved by Lenoir et al. (1981), they suggested that these findings were due to the direct nephrotoxic effect of VPA. They showed giant mitochondria in proximal tubules and abnormal, round granular inclusions in the cytosol. In addition, Hawkins and Brewer (1993) also showed evidence of renal tubular dysfunction in a 10-year-old child who had psychomotor retardation. They described multifocal interstitial nephritis characterized by mononuclear cell infiltration around the glomeruli on renal biopsy material. They also showed that the proximal tubular epithelium was inflamed and contained giant eosinophilic inclusions and vacuoles. Mitochondria were excessively enlarged and the cytoplasm contained lipid droplets but no immune deposits, these results are in agreement with the present study.

Ultrastructural examination of the current investigation showed dark cytoplasm and long processes of mesangial substance expansion of VPA compared to control mice. This result is in agreement with El-Sayyad et al. (2013), who reported focal expansion of mesangial region and the podocytes showed either nuclear chromatin condensation of chromatin or exfoliated in many areas, and the slit membrane structure was deteriorated. Partial deteriorations of foot processes of podocytes were detected. All of the mentioned findings illustrated the impairment of renal function as a result of disease and drug severe toxicity (El-Sayyad et al., 2013).

The observed findings supported the work of many authors in patients treated with sodium valproate, carbamazepine and phenobarbital (Verrotti et al., 2000; Unay et al., 2006) and experimental mice treated with sodium valporate (Eluma et al., 1984). The observed findings of nephrotoxicity as illustrated by cytological alterations of mitochondria as well as the recent studies of sodium valporate (Pourahmad et al., 2012) may be attributed to the increased production of free radicals and/or a decreased free radical scavenging capacity. There is another hypothesis suggesting an involvement of lipid peroxidation (Olson et al., 1986). The involvement of peroxidative injury in sodium valproate induced renal tubular disorder is based on several lines of evidence. Valproate treatment decreased the rate of oxidized glutathione released into the bile (Olson et al., 1986).

The present ultrastructural changes in the proximal tubule cells included destruction of the microvilli constituting the brush border, mitochondrial enlargement and increase of lysosome number, in addition to cytoplasmic vacuolation and pyknotic nuclei. Moreover, the distal tubule cells appeared with ill defined basal infoldings, electron dense mitochondria and some vacuoles. These alterations were reported also by Gossrau and Graf (1989) who reported a very severe damage to the tubules of the kidney in pregnant mice at a single dose of valproic acid (500 mg/kg i.p.) which persisted even after 48 h of drug administration. Renal biopsy indicated giant mitochondria in the proximal tubular cells and abnormal round granular inclusions in the cytosol of tubular cells, podocytes and interstitial cells which were recovered on withdrawal of valproic acid.

In the present study, Administration of RJ in concomitant with VPA caused a significant decrease in rates of clastogenetic changes compared with the VPA treated group. All types of chromosomal aberrations induced by VPA including breaks, deletions, fragments, end to end association, centric fusion, and other multiple damages were found to be reduced by RJ. The mitotic index was found to be increased over VPA group for 50 and 100 mg/kg doses, indicating their anti-cytotoxicity towards VPA.

These results were in agreement with the finding of Çavuşoğlu et al. (2009), who stated that supplementation with RJ resulted in beneficial effects against genotoxicity and oxidative damages induced by Cadmium in albino mice and had a protective effect on the chromosomal aberrations induced. Furthermore, El-Nekeety et al. (2007) determined the potential protective effect of RJ against the toxic hazards of fumonisin denoting that RJ had been proved to have oxygen radical scavenging and antioxidant properties in addition to the enhancement of glutathione peroxidase formation and the suppressive effects of lipid peroxidation and free radical generation. As well, Abd El-Monem (2011) revealed that RJ caused a significant recovery in antioxidant status and a significant inhibition of malondialdehyde production and ameliorated DNA damage and genotoxicity induced by malathion in rat cells.

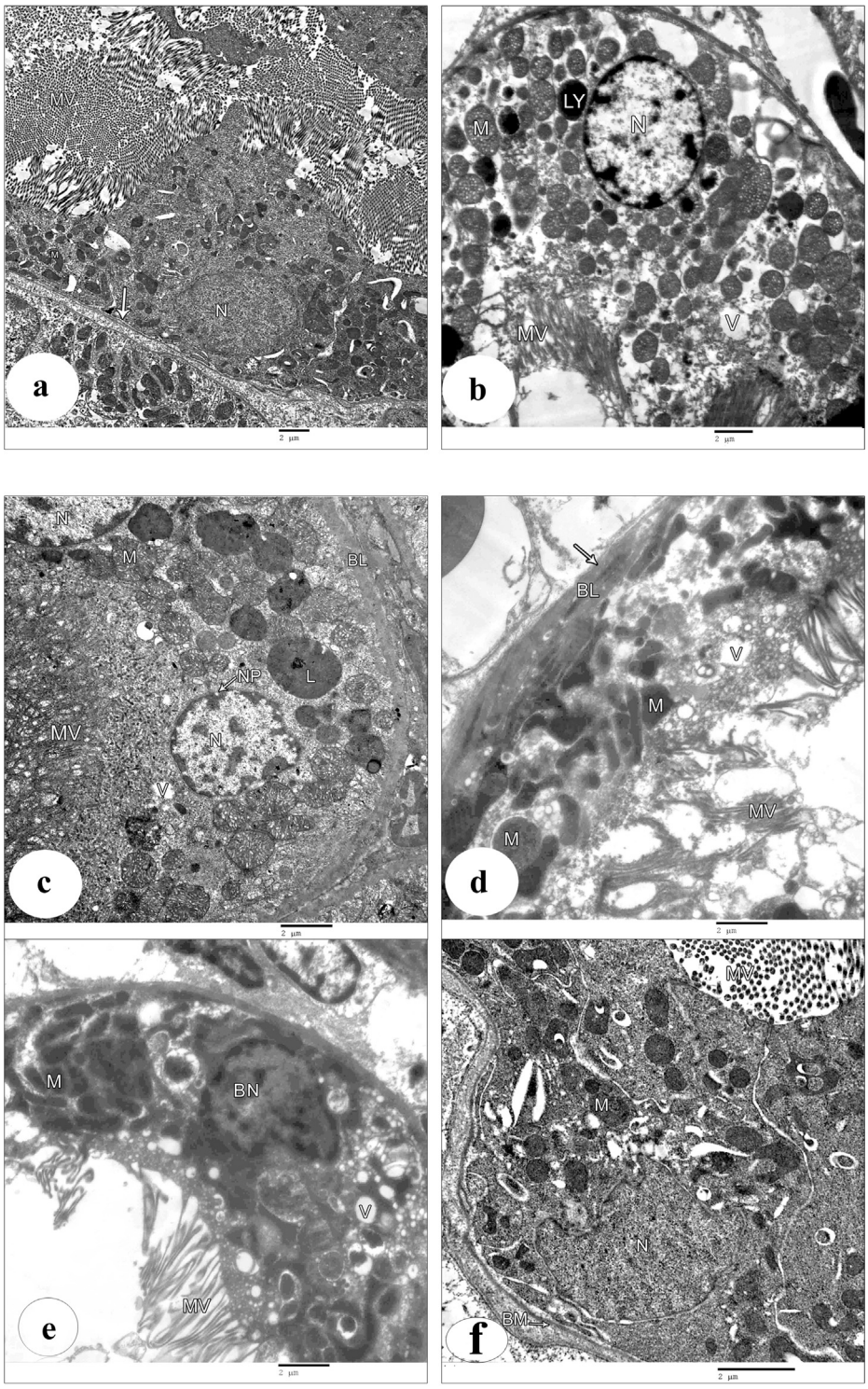

Fig. 6 – (a) Electron micrograph of a proximal tubule of control mice showing microvilli (MV), mitochondria (M), regular nucleus (N) and thin basal lamina (↓). Scale bar = 2 μm. (b) Electron micrograph of a proximal tubule of mice given valproic acid (100 mg/kg) for 3 weeks showing lysosomes (LY), mitochondria (M), nucleus (N), dissolution and degeneration of some parts of the cytoplasm and vacuoles (V) and losing part of their microvilli (MV). Scale bar = 2 μm. (c) Electron micrograph of a proximal tubule of mice given valproic acid (100 mg/kg) for 3 weeks showing thickening of the basal lamina (BL), vacuoles (V), swelled mitochondria (M), irregular nucleus (N) with nuclear pore (NP), lipid droplets (L) and microvilli (MV). Scale bar = 2 μm. (d) Electron micrograph of a proximal tubule of mice given valproic acid (100 mg/kg) for 3 weeks showing condensed mitochondria (M), destructed microvilli (MV), thickening of the basal lamina (BL), vacuoles (V) and dissolution and degeneration of some parts of the cytoplasm. Scale bar = 2 μm. (e) Electron micrograph of a proximal tubule of mice given valproic acid (100 mg/kg) for 3 weeks showing pyknotic nucleus (PN), vacuoles (V), degeneration of microvilli (MV) in

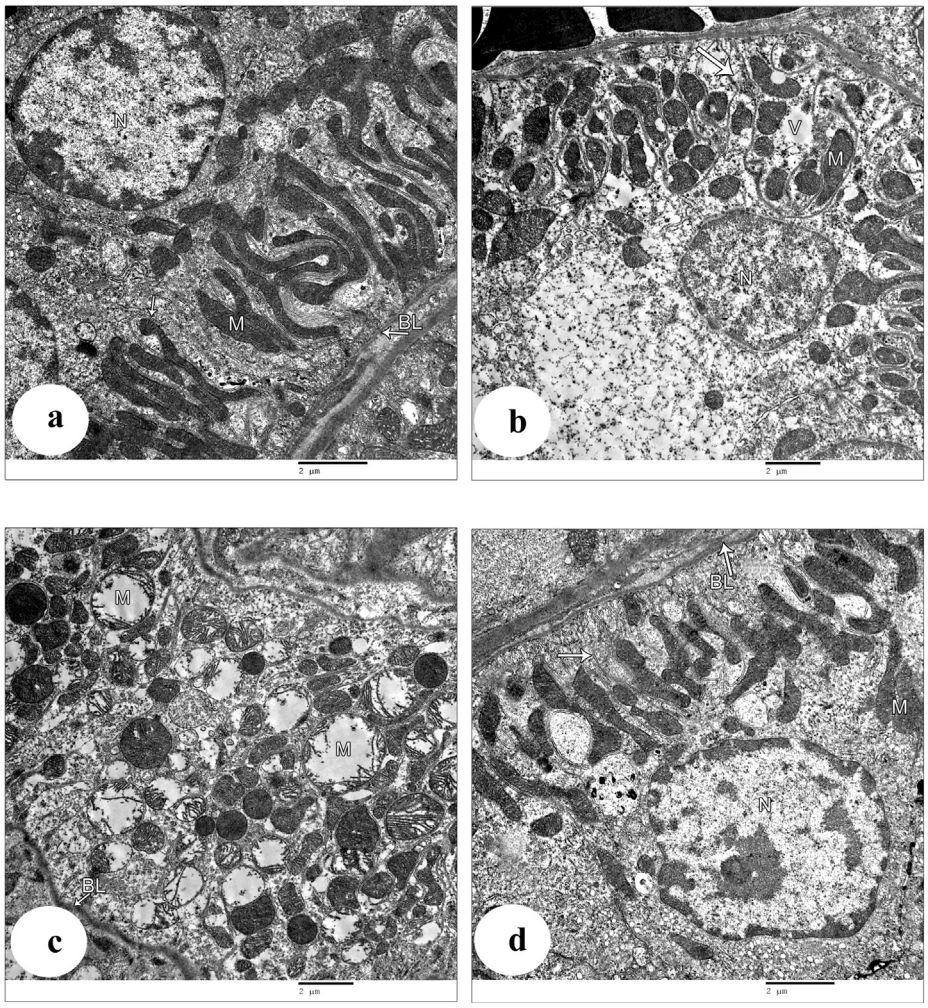

Fig. 7 – (a) Electron micrograph of a distal tubule cell of control mice showing well-developed basal infoldings (↓) with numerous mitochondria (M) in between, nucleus (N) and thin basal lamina (BL). Scale bar = 2 μm. (b) Electron micrograph of a distal tubule cell of mice given valproic acid (100 mg/kg) for 3 weeks showing vacuoles (V), irregular basal infoldings (↓), nucleus (N), mitochondria (M) and degeneration of some parts of the cytoplasm. Scale bar = 2 μm. (c) Electron micrograph of a distal tubule cell of mice given valproic acid (100 mg/kg) for 3 weeks showing completely destructed mitochondria with destructed cristae (M) and irregular basal lamina (BL). Scale bar = 2 μm. (d) Electron micrograph of a distal tubule cell of mice treated with valproic acid (100 mg/kg) and royal jelly (100 mg/kg) for 3 weeks showing restoration of the normal structure of mitochondria (M), basal infoldings (↓), basal lamina (BL) and nucleus (N). Scale bar = 2 μm.

In the present study animals treated with VPA and RJ (50 and 100 mg/kg) revealed a better preservation compared to those treated with VPA alone. It is also proved by El-Nekeety et al. (2007), who showed nearly normal structures of the renal tubules and glomeruli of rats treated with fumonisin plus RJ. In addition, RJ also decreased necrosis and lymphocytic infiltration in rats treated with RJ prophylactic with cisplatin, also RJ administration caused less degenerative alterations and amelioration in the kidney oxidative parameters. This protection may be due to antiapoptotic, antioxidant, and free radical scavenging activity of royal jelly and its components (Karadeniz et al., 2011).

5. Conclusion

Valproic Acid (VPA) has adverse effects on human health. The results demonstrated that the administration of VPA to mice at a dose of 100 mg/kg daily for 3 weeks is capable of inducing marked chromosomal aberrations, alterations in mitotic

the apical part of some cells and condensed mitochondria (M). Scale bar = 2 μm. (f) Electron micrograph of a proximal tubule of mice treated with valproic acid (100 mg/kg) and royal jelly (100 mg/kg) for 3 weeks showing normal basement membrane (BM), microvilli (MV), mitochondria (M) and irregular nucleus (N). Scale bar = 2 μm.

index and histopathological changes in kidney tissue. With using RJ combined with VPA, all the histological alteration side effects in renal tissues were greatly abated with significant reduction in chromosomal aberrations and elevation in mitotic index ($P < 0.05$). RJ at doses of 50 and 100 mg/kg appeared more potent in exerting the ameliorative effect than did the dose of 200 mg/kg.

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