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THE AMELIORATIVE ROLE OF GINGER ADMINISTRATION AGAINST GABAPENTIN-INDUCED HEPATOTOXICITY IN RAT FETUSES

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ABSTRACT

Objective: To investigate the possible side effects of prenatal exposure to the antiepileptic drug gabapentin (GBP) on the liver during the organogenesis phase of the rat embryonic development and to examine the possible ameliorative role of ginger. **Methods:** Three integrated approaches namely, histological, immuno-histochemical and ultrastructural were adopted. **Results:** Fetuses maternally injected with GBP displayed congested central vein, disruption of normal architecture and vacuolation of the hepatocytes. The expression of Bcl-2 in the cytoplasm of hepatocytes was remarkably decreased, while the expression of Caspase-3 was increased. At the ultrastructural level, the hepatocytes had irregular shaped and shrunken nuclei. The cytoplasm appeared rarefied, vacuolated and with disrupted organelles. Oral injection of ginger after GBP resulted in evident amelioration at all the investigated levels. **Conclusions:** The use of GBP as antiepileptic drug should be treated with caution during pregnancy and ginger is recommended to be taken in parallel for its ameliorative role in this regard.

KEYWORDS: Gabapentin, Adverse effects, Ginger, Amelioration, Fetal development, Liver.

1. INTRODUCTION

It is a well-documented fact that epilepsy affects millions of people all over the world. Its seriousness lies in the fact that most of the epileptic women need to continue taking medication even during pregnancy, since uncontrolled seizures may have a deleterious effect on the women as well as their fetuses.[1,2] There is a potential risk that continuous exposure of epileptic women to the drug might result in considerable accumulation of the drug in the embryo during the preimplantation period, by which time the pregnancy might not have been recognized.[3] Antiepileptic drugs (AEDs) are also frequently used for other indications, such as migraine, pain syndromes, and psychiatric disorders. For optimum use of AEDs, a thorough understanding of the teratogenic effects of AEDs and knowledge of the differences in risks between various treatment options are urgently needed.[1] Gabapentin (GBP) was originally introduced for the treatment of epilepsy but has achieved greater popularity as an adjunctive therapy for chronic pain.[4] It was originally developed as a chemical analogue of γ-aminobutyric acid (GABA) to reduce the spinal reflex for the treatment of spasticity and was found to have anticonvulsant activity in various seizure models.[5] It was also claimed to be beneficial in several other clinical disorders such as prophylaxis of migraine.[6] It is very water soluble and its half-life is five to seven hours in humans.[7] It is relatively a new agent of a typical antiepileptic class and categorized as 'C' considering its possible teratogenic risk by FDA.[8] Due to its low molecular weight (171 Da) and poor binding capacity to plasma protein, it crosses easily the placental membrane and blood–brain barrier.[5] Ohman et al.[9] studied the pharmacokinetics of GBP during delivery, lactation, and in the neonatal period, and reported an active transplacental transport of GBP, with accumulation in the fetus as an important consequence. However, despite expanding data on the usage of GBP, there is little information, so far, on its teratogenic effects.[6,10]

Ginger has a long history of medicinal use in traditional medicine for conditions such as headaches, toothache, colds, improve circulation of the limbs and helps in lowering blood cholesterol.[11] In addition, ginger extract also possesses antioxidant activity.[12,13] Furthermore, ginger has anti-cancer, anti-inflammatory properties as well as anti-nausea/vomiting properties.[14] *In vitro* studies showed that ginger can act as a therapeutic agent for scavenging of nitric oxide (NO) and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, proxy nitrite.[15]

Some studies have dealt with the effect of ginger on hepatic damage. Protective effects for ginger extract on the hepatotoxic effects of both CCl_4 and acetaminophen have been reported.[16] Sakr et al.[17] showed that treatment of rats with both Adriamycin and ginger improved the Adriamycin induced liver histopathological changes. Barakat and Maha[18] also showed the ability of

ginger to protect the liver against the oxidative stress and hepatocellular injury that follows a supra therapeutic dose of acetaminophen. Balubaid et al.[19] studied the therapeutic effect of ginger on tetracycline treated pregnant rats and their embryos during the second period of pregnancy and reported that ginger treated mothers, showed better hepatic histological and ultrastructure architecture. Other investigators concluded that the aqueous extract of ginger showed an ameliorative effect against cadmium bromide induced hepatotoxicity.[20] Poorrostami et al.[21] found that hydroalcoholic extract of ginger improves liver function and histopathology in lamotrigine-induced hepatotoxicity in epileptic female rats.

Liver is among the most important organs in the body serving many vital processes. Liver is vulnerable to drug-induced toxicity mainly because of its role as a primary organ of drug elimination and its subsequent exposure to potential toxins.[22] Consequently and based on the fact that treatment with the AEDs is associated with adverse side effects, the present study was designed firstly to investigate the possible hepatotoxic side effects of prenatal exposure to GBP through maternal administration of GBP during the organogenesis phase of the embryonic development in rat fetuses. Secondly, to examine the possible ameliorative role of ginger against the toxicity induced by GBP administration in the same experimental model.

2. MATERIALS AND METHODS

2.1. Animals and treatment

All the experiments were done in compliance with the guide for the care and use of laboratory animals approved by Faculty of Science, Menoufiya University, Egypt. Healthy mature virgin females and fertile males 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 females with males at a ratio of one male with two females overnight. Females were checked daily in the morning for the presence of a copulatory plug and the presence of sperms in unstained native vaginal smears. Therefore, vaginal smears were carried out to give a precise determination of the onset of gestation. The day at which vaginal smear was positive has been considered as the day zero of pregnancy. Day 20 was determined as the end point for experimentation. A total of 36 rats (24 females and 12 males) were used for the present study. 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.

A total number of 36 fetuses were included in the investigation.

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.[23]

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.[24] Ginger extract was daily administrated orally, one hour after GBP injection, by gavage tube at a dose of 200 mg/kg body weight[25] during the organogenesis phase of gestation.

2.4. Investigated parameters

2.4.1. Histological examination

For light microscopical examination, the liver of rat fetuses of different groups was 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. 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 rat liver was fixed in 10 % formalin for 24 hours and followed the same steps of histological examination up to sectioning except that the sections were mounted on positive charged slides. Briefly, before the incubation with antibodies, endogenous peroxidase activity was quenched, slides

washed and then incubated in a blocking solution of hydrogen peroxide 1% in methanol, in darkness for 15 min. Antigen retrieval occurred with citrate buffer 10 mM, pH 6. After cooling, sections were rinsed in tap water and then phosphate buffer saline 1 M. Primary antibodies for Bcl-2 and Caspase-3, diluted 1:150 in PBS, were applied for 1 h at 37 ˚C. Secondary biotinylated antibody diluted 1:100 in PBS was applied for a period of 30 min at 37˚C. Streptavidin–biotin or avidin–biotin peroxidase (ABC/ HRP) was applied for 10 min at room temperature. Bound antibody complex was visualized by the reaction of 3,3-diaminobenzidine substrate (DAB, Wako pure chemical industries, Ltd) and counter stained with hematoxylin. Sections were then dehydrated through ascending grades of ethanol, cleared in xylol and cover slipped with DPX. The criterion for a positive reaction confirming the presence of Bcl-2 or Caspase-3proteins was a dark, brownish, intracytoplasmic precipitate. For the negative control, the primary antibody was omitted to guard against any false positive results that might develop from a non-specific reaction. Negative control sections were done by substituting Bcl-2and Caspase-3 primary antibodies by normal goat serum. All stained slides were viewed using Olympus microscope and images were captured by a digital camera (Canon Power Shot A620). Brightness and contrast of the images were adjusted using Adobe Photoshop software (Adobe Systems, San Jose, CA).

Digital images were analyzed by a semi-quantitative scoring system (Figi-Image J software, Java based application for analyzing images). The brown stained immuno-histochemical expressions of Bcl-2 and Caspase-3 positive cells were analyzed; the percentage colored stained area (area fraction) per field area was determined by measuring six randomly photographed high-power fields (X400 magnifications).[26]

2.4.3. Ultrastructural investigation

For ultrastructural investigation, which has been done using transmission electron microscope, specimens of fetal liver of both control and experimental groups were separated and immediately fixed for 4 hours at room temperature in 2.5% Glutaraldehyde and 2% paraformaldehyde in 0.1 cacodylate buffer (PH. 7.4). After rinsing in cacodylate buffer, samples were post fixed in buffered solution of 1% osmium tetra-oxide for three hours at 4 °C. This was followed by dehydration in ascending grades of ethanol and embedded in epoxy resin. Ultra-thin (50 nm) sections were cut, mounted on formvar-coated grids and stained with uranyl acetate for10 minutes. Sections were then stained with freshly prepared lead citrate for 10 minutes and washed with distilled water. Examination of grids was done by using JEOL electron microscope, Electron Microscope Unit, Tanta University, Egypt.

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 livers 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 three categories, i.e. $P<0.0001$, $P<0.001$ and $P<0.05$ according to P values.

3. RESULTS

3.1. Histological investigation

The liver of control fetuses displayed normal histological features. It consisted of cords of polyhedral hepatocytes with acidophilic cytoplasm. The hepatocytes were seen radiating from the central vein to the periphery of the hepatic lobule and alternating with blood sinusoids. The liver was permeated by rare megakaryocytes and conserved sinusoid capillaries (Fig. 1A). The liver of the fetuses maternally administered ginger exhibited normal histological structure similar to that of the control group (Fig. 1B).

Fetuses maternally injected with GBP alone showed widened, congested and elongated central veins (Fig. 1C&D). There was an evident disruption of normal architecture pattern of the classical lobule structure along with severe hemorrhage infiltrating many areas, especially the sinusoids, through which blood had escaped, producing hemorrhagic foci (Fig. 1D). Furthermore, GBP induced hepatocytes necrotic changes in the form of pale vacuolated cytoplasm and small dense pyknotic nuclei characterized by condensed chromatin (Fig. 1C-E). In addition, enlarged portal areas with periportal mononuclear cellular infiltrate, dilated branches of portal vein and proliferated bile ductiles surrounded by large area of inflammatory and degenerative cells were observed (Fig. 1E).

Histological examination of liver of fetuses maternally injected with GBP followed by ginger displayed a clear decline in the severity of circulatory disturbance and hemorrhage with a hepatic architecture more or less similar to the control group, however, few cells appeared with vacuolated cytoplasm and pyknotic nuclei. Slightly congested dilated hepatic sinusoids were seen (Fig. 1F).

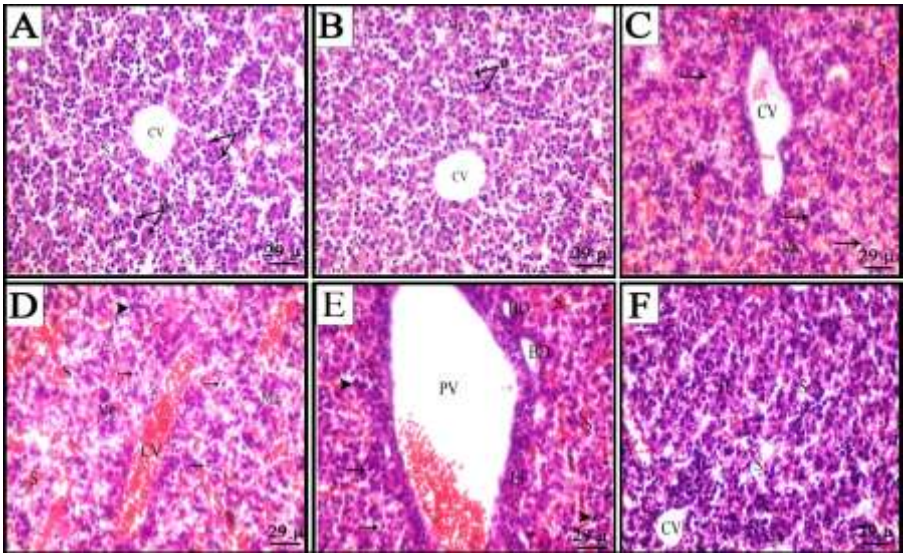

Figure (1): Photomicrographs of transverse sections of fetal liver. (A) Control (B) Ginger (C-E) GBP group (F) GBP+ginger group. Scale bar= 29µ.

3.2. Immuno-histochemical investigation

Liver sections of control and ginger group rat fetuses subjected to Bcl-2 primary antibodies displayed strong positive immunoreaction where Bcl-2 protein was expressed as small brown-yellow staining in the cytoplasm of most of the hepatic cells (Fig. 2A&B) and showed insignificant relationship (52.4%; 60.13%, respectively) (Table 1; Fig. 2). The expression of Bcl-2 in the cytoplasm of hepatocytes was highly significantly decreased (7.41%) in the liver of fetuses maternally injected with GBP compared with that of the control group (Fig. 2C&D). Liver sections from GBP and ginger groups, however, exhibited moderate expression and reaction to Bcl-2 antibodies (Fig. 2E&F) with significant increase when compared with GBP group (22.2%). The immune-expression of the proapoptotic antigen Caspase-3 in the liver of the rat fetuses was the opposite to that of the Bcl-2 antigen. Hepatocytes of rats from control and ginger groups showed very weak immunoreaction of the Caspase-3 protein in their cytoplasm (Fig. 2G&H) with no significant difference in the mean area expression (7.66%; 8.07%). Hepatocytes of GBP group showed very strong immunoreaction to Caspase-3 antigen and showed high significant increase in the mean area percentage of expression (33.62%) and almost all the hepatocytes were immune-stained (Fig. 2I-K). Co-administration of ginger followed by GBP resulted in moderate expression of Caspase-3 antigen in the cytoplasm (Fig. 2L) and low significant expression increase when compared with control group (15.18%).

Table. 1. The mean area % of Bcl-2 and Caspase-3 expression in the fetal rat liver of control and experimental groups.

Groups	Control	Ginger	GBP	GBP + Ginger
Bcl-2	60.13 ± 1.5	52.4± 2.1	7.41 ± 0.96**	22.2± 0.9*a
Caspase-3	7.66 ± 0.29	8.07 ± 0.43	33.62 ± 1.21**	15.18 ± 0.32*a

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

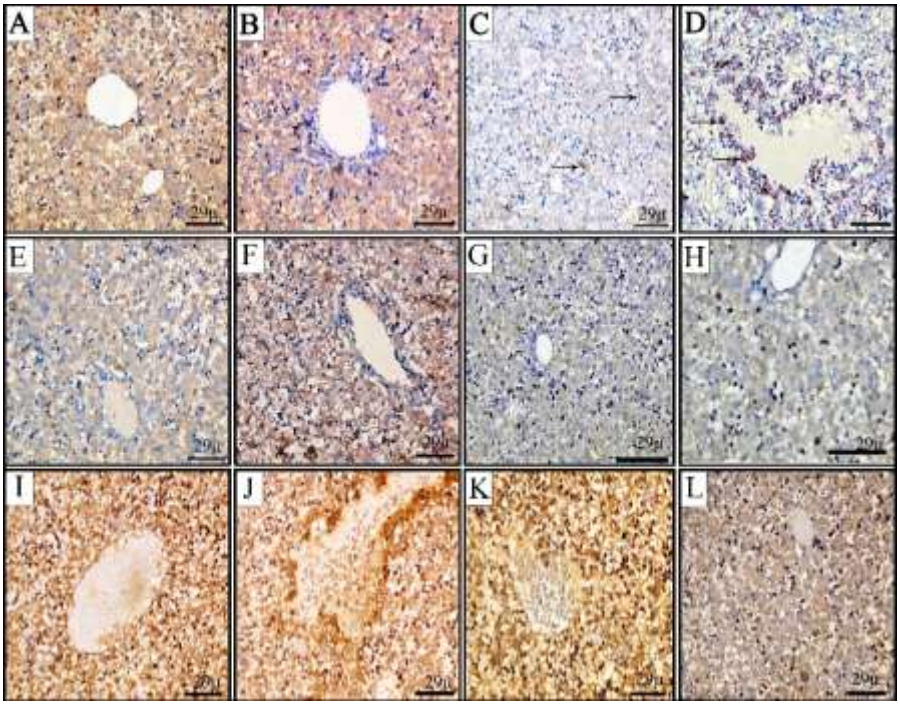

Figure. (2): Photomicrographs showing immuno-histochemical localization of Bcl-2 (A-F) and Caspase-3 (G-L) antigens in liver sections of 20-day old rat fetuses. (A&G) Control group (B&H) Ginger group (C, D, I, J & K) GBP group (E, F& L) GBP+ginger group. (Scale bar= 29µ).

3.3. Ultrastructure investigation

Ultrastructural examination of fetal liver from control mothers revealed that the hepatocytes possessed a large rounded achromatic nucleus with a prominent electron-dense nucleolus. The nuclei displayed fine arrangement of variable-sized masses of condensed heterochromatin within its nucleoplasm and on the inner side of the well identified, double membrane regular nuclear envelope interrupted by nuclear pores. The granular cytoplasm possessed normal arrangement of numerous spherical or slightly elongated mitochondria with prominent cristae. The rough endoplasmic reticulum (rER) was well represented as thin, parallel stacks of flattened cisternae surrounding the nucleus or arranged between and around the mitochondria. Its outer surfaces were studded with ribosomes (Fig. 3A). The hepatocytes of 20-day old rat fetuses maternally administered ginger exhibited the same ultrastructure profile of hepatocytes compared with control (Fig. 3B).

TEM examination of fetal hepatocytes, maternally injected with GBP confirmed the histological changes and provided more details on the adverse effects of GBP at the ultrastructural level. The hepatocytes displayed deep modifications that were irreversible and led to their destruction on both the nuclear and cytoplasmic level. There were different kinds of nuclear damages, many of the nuclei lost their spherical form, and their outline was irregular (Fig. 3C, D&F) and some of them appeared shrunken and electron dense with condensation and margination of heterochromatin resulting in the formation of sharply circumscribed masses of chromatin at its periphery (Fig. 3E). In such hepatocytes, the cytoplasm lacked its compartmentation and appeared to be rarefied and electron lucent (Fig. 3D, H&I), vacuolated (Fig. 3D&E) and with a ruptured cell membrane (Fig. 3G&I). Mitochondria were often swollen, with bright matrix and cristae destruction, some of them were damaged with ruptured outer membrane and vacuolation of the inner compartment (Fig. 3D, G, H&I). The cisternae of the rER showed fragmentation in the form of short segments and reduction of their attached ribosomes within a rarefied cytoplasm (Fig. 3D). Numerous lysosomes were spread in the cytoplasm of some hepatocytes (Fig. 3H). In some cases, numerous fat globules of varying sizes were distributed throughout the cytoplasm (Fig. 3E). Some hepatocytes with cytoplasmic hydrolysis were detached from the cellular cords and destructed in the sinusoidal space (Fig. 3F). Kupffer cells with peculiar chromatin condensation were noticed in the lumen of sinusoid capillaries (Fig. 3J) while very distinct Kupffer and macrophage cells were evident between the hepatocytes (Fig. 3E). Hemorrhage was also evident between hepatocytes (Fig. 3F&G) and in the sinusoids (Figs. 3E&J).

Maternal injection of GBP followed by ginger administration led to an evident improvement in the ultrastructure of fetal hepatocytes which appeared nearly similar to the control (Fig. 3K&L), although, there was fragmentation of the microvilli of the adjacent bile ductile (Fig. 3L).

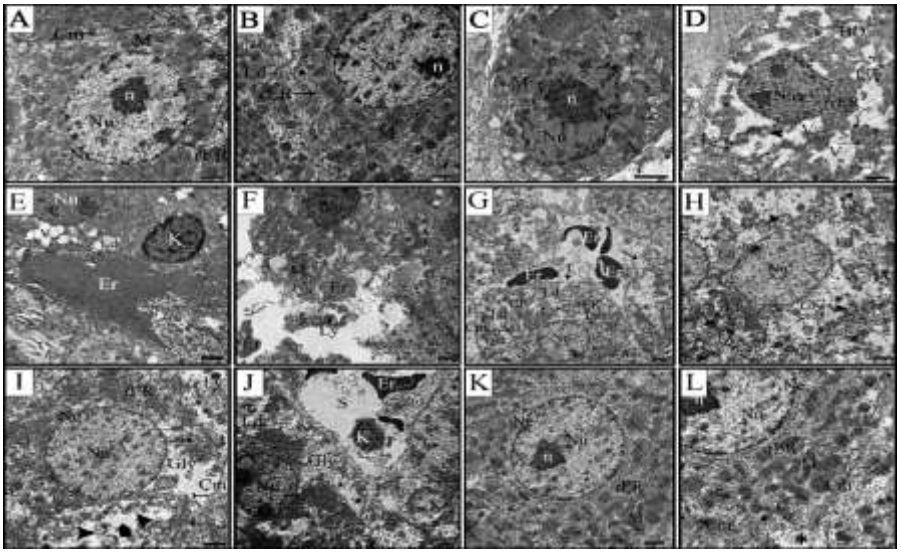

Figure. (3): Transmission electron photomicrographs of liver sections of rat fetuses. (A) Control group(B) Ginger group (C-J) GBP group(K&L) GBP+ginger group. Scale bar A-K = 2µm, L= 500nm.

4. DISCUSSION

It has been known that administration of higher dosages of AEDs, higher concentrations of these drugs in the blood, and polytherapy are all associated with high risks for both anatomical and behavioral teratogenesis in the embryo.[27] For some AEDs, especially the old generation ones, the risks are widely studied and outcomes for the fetus are more or less clear.[27] Of the older AEDs, carbamazepine and valproate together bring a threat of hepatic toxicity and have been associated with fetal anomalies.[28] However, there is no sufficient knowledge concerning teratogenic effect of newer AED's.[29] Although the detrimental effects of the antiepileptic drug GBP on liver have been described in some clinical reports, these side effects are not extensively studied.[30] GBP was found to be accumulated in the fetal tissues.[9] In the present study, maternal injection of GBP during the organogenesis phase induced various histological, immuno-histochemical and ultrastructural changes in the liver of the developing rat fetus.

Light microscope investigation of the present study revealed that the liver of maternally treated fetuses with GBP showed marked congestion of blood vessels and blood sinusoids. There was loss of liver parenchyma architecture as well as vacuolated cytoplasm and pyknotic cells with shrunken nuclei. Similar findings were reported in patients subjected to valproate and lamotrigine-treatment.[31,32] Topiramate injection induced similar results in the study of Huang et al.[33] who found that diffuse necrosis of hepatocytes distributed in the portal area were found in topiramate injected rats accompanied by granular degeneration of some hepatocytes near the central veins. These results are also consistent with El-Sayyad et al.[34] and Jassim[35] who found that administration of valproate and lamotrigine altered the liver structure of epileptic rat mothers. It was demonstrated that chronic treatment with GBP may alter liver homeostasis.[36,37] Abd-Allah et al.[30] found that daily treatment with GBP for four weeks elevated serum transaminases activities reflecting liver injury. In a case study, Richardson et al.[38] found that supplementation of GBP for two weeks induced cholestasis which was confirmed by liver function tests. After GBP intake stopped, the liver functions improved gradually.

Congested central veins occurred in various parts of liver sections of rats maternally treated with GBP. Congestion might be due to loss of fluid from the blood and the vessels engorged with RBC's.[39] In the current study, bile ductal proliferation was detected in the portal areas of GBP group. Michalopoulos et al.[40] and Chen et al.[41] attributed this proliferation to trans-differentiation of hepatocytes into biliary cells. According to the present study, cellular infiltration was noticed in the portal areas in liver sections of GBP group. El-Ghonaimy[42] considered this cellular infiltration as a prominent immune response of the body tissues by movement of fluids and leukocytes from the blood into the extravascular tissues.

This study provided evidence for induced apoptosis of hepatic cells of rat fetuses maternally injected with GBP during the organogenesis period. Bcl-2 expression was decreased in the cytoplasm of fetal hepatic tissue of the GBP group, while it was highly expressed in the hepatocyte cytoplasm of control group. On the contrary, Caspase-3 expression levels were elevated in the GBP group and severely decreased in the control group. These results go in hand with those of Galaly et al.[43] who showed that liver pro-apoptotic protein Bax and Caspase-3 expressions were remarkably increased in gentamicin-administered rats, while liver antiapoptotic protein Bcl-2 was obviously decreased suggesting an increased hepatocyte apoptosis. The increase in Bcl-2 expression level in the liver was observed by Akcali et al.[44] during hepatocyte regeneration after hepatectomy. Also, the

study of Pedrycz et al.[45] of Adriamycin treated rat mothers confirmed the presence of hepatocyte apoptosis evidenced by lack of the Bcl-2 protein expression. Aktuğ et al.[46] found that the expression of Bcl-2 mRNA in the diabetic spleen tissues was significantly lower and the expression of Caspase-3 was much higher than normal.

At the ultrastructure level, GBP was found to induce deleterious effects on the liver of rat fetuses. Ruptured mitochondria, rarified cytoplasm, short fragmented rER, shrunken nuclei with condensed chromatin along with vacuolation of the cytoplasm, were the main modification in the hepatocytes of GBP group. Applying of valproate and lamotrigine to epileptic rat mothers was found to increase the average of the hepatocytes damage with apparent pyknosis, vesicuolated rER and electron-dense mitochondria with lysis of their cristae.[34] These results were similar to those of Abdella et al.[47] who found that injection of valproic acid for three weeks resulted in adverse effects on the liver ultrastructure of mice.

The mitochondrial swelling and degeneration of the mitochondria cristae observed in the present study may reflect the disturbances in oxyreduction processes taking place in the organelle.[48] In the present study, some fat globules were observed in the cytoplasm of some hepatocytes. Previous researchers attributed this fat infiltration as a defense mechanism by which hepatocyte attempt to collect all toxic compounds invading the cell in these vacuoles prior to excretion.[49] Liver sinusoids became dilated and filled with blood under the effect of GBP which may presumably be attributed to sinusoidal endothelial cell damage causing hemorrhage.[50]

The mitochondria were either swollen, ruptured or vacuolated and with distorted cristae accompanied by apoptotic characteristics of most hepatic cells. In addition, the expression of Bcl-2 antigen was decreased in the cytoplasm of these cells. It is known that Bcl-2 proteins layered on the surface of the mitochondria can prevent cytochrome c from releasing into plasma and therefore, protecting the cell from apoptosis, whereas Bax can cause leaking out of cytochrome c by punching holes in the mitochondrial membrane.[51,52] From these findings, it may be hypothesized that GBP exerts its apoptotic action via the mitochondrial pathway.

Considerable attention has been given to the application of natural antioxidants in food, because of their potential nutritional and therapeutic effects.[53] Ginger, *Zingiber officinale*, is an example of botanicals which is gaining popularity amongst modern physicians.[54] Ginger contains substances that retard the rate of oxidation by scavenging free radicals or controlling the breakdown of peroxides into stable substances that do not promote further oxidation.[55, 56] Portnoi et al.[57] examined the degree of safety of using ginger as a treatment and its efficiency and effect on pregnancy sickness and noticed that there was no significant difference between treated ginger embryos' mothers and those of the control group, except an increase in the weight of the treated embryos. The outcome of the present study is in agreement with observations of previous workers who reported that ginger is safe and well tolerated.[58] According to the present study, co-administration of ginger in dose of 200 mg/Kg with GBP partially ameliorated the histological, immuno-histochemical and ultrastructural changes produced in the liver due to GBP toxicity. This was clearly evident by light and electron microscopic examination of the liver where the hepatic lobules retained their normal appearance. Previous studies using ginger had reported its significant antioxidant and anti-inflammatory activities[59] which may be the possible explanation of the evident improvements in all changes caused by GBP administration.

Ginger possesses hepatoprotective effects against the toxic effects of diverse class of xenobiotics.[60,61] Liver histopathological examination of ginger treated animals showed normal hepatocytes and central vein against fried cotton oil.[56] Shanmugam et al.[62] found that ginger protected the liver tissue from STZ-induced oxidative damage. Several reports[63,64] showed the protective effects of ginger extract or its constituents, through their antioxidant properties and improvement of the hepatic dysfunctions and hepatic damage that were induced by hepatotoxicants, CCl_4 and acetaminophen. Ginger offered some hepatic protection to heavy metal accumulations in the liver.[65] Studies have shown that ginger has ameliorative effect against cadmium-induced liver and kidney injury in rat models.[20]

The immuno-histochemical investigation of the present study revealed that ginger leads to suppression of apoptosis evidenced by increased expression of Bcl-2 and decreased expression of Caspase-3 in the combined ginger and GBP group compared with the GBP only. This was consistent with the study of Sakr and Badawy[66] who confirmed that ginger reduced apoptosis induced by metiram in the testis of male albino rats evidenced by decrease in Bax expression which agonists Bcl-2 expression. In the study of Abd-Allah and Sharaf El-Din[67], the results showed that rats treated with ginger powder had a decrease in apoptosis in the injured intestinal tissues, as the expression of protein levels of the pro-apoptotic Bax decreased, while those of the antiapoptotic Bcl-2 protein levels increased suggesting increased enterocyte survival. Baiomy and Mansour[68] also showed that cadmium increased the expression of Caspase-3, while ginger reduced its expression in liver hepatocytes and the kidney tubular epithelium of rabbit. Recently, Kim and Kwon[69], suggested that following oxidative stress, (6)-shogaol protects astrocytes from oxidative damage and apoptosis by attenuating the impairment of mitochondrial function proteins such as Bcl-2.

Ultrastructurally, the present study showed that the hepatocyte's mitochondria of ginger extract and GBP

treated group appeared nearly normal with intact cristae. The study of Ahmed[70] proved that the hepatic pathology caused by administration of Adriamycin was ameliorated by combined treatment with ginger where mitochondria and rER were nearly normal in Adriamycin and ginger treated group. The protective effect of ginger concomitant with DMBA treatment resulted in improvement of most ultrastructural alterations in liver tissue induced by DMBA. In the study of Ali[71], cisplatin-treated rats in combination with ginger extract exhibited remarkable improvements and inhibited most of the pathological alterations induced by cisplatin chemotherapy.

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] In addition to antioxidant effects of ginger extract, previous studies demonstrated that ginger components may exert its hepatoprotective effect by inhibiting the activity of pro-inflammatory signaling compound prostaglandin-E2 from COX-II in lipopolysaccharide-activated macrophages[73] and also by blocking the enzyme cytochrome P450-2E1.[74] In conclusion, the present data confirmed that the maternally injected GBP had adverse side effects on the liver of the rat fetuses. Furthermore, ginger can be a potential candidate agent against the hepatotoxic effect induced by 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 hepatotoxicity.

Abbreviations

BD, Bile ductile; Cm, cell membrane; CV, central vein; Er, erythrocytes; Gly, glycogen; H, hepatocyte; K, Kupffer cell; Ld, lipid droplet; Li, leucocytic infilteration; Ly, lysosome; M, mitochondria; Mg, megakaryocyte; Mv, microvilli; n, nucleolus; Ne, nuclear envelope; Nu, nucleus; PV, portal vein; rER, rough endoplasmic reticulum; S, sinusoid; V, vacuole.

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