CYTOTOXICITY CHANGES OF CISPLATIN DRUG IN THE PRESENCE OF MAGNETIC FIELDS

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Abstract: The aim of the present work is to study the cytotoxicity of cisplatin in the presence of magnetic fields. For this purpose an exposure system capable of producing static and alternating magnetic fields in horizontal area of 30×20 cm^2 was built. Eighty mice were grouped equally into four groups namely GI, GII, GIII and GIV. Animals of group GI were used as control (sham exposed), animals of GII were exposed to a combined static and alternating magnetic fields of 3.6 mT for 35 min/day, 6 day/week for 21 days, animals of GIII were injected with cisplatin (3 mg/kg) and sham exposed to magnetic fields, the final group GIV were injected with cisplatin (3 mg/kg) and exposed to magnetic fields as GII. The induced changes in spleen and kidney were carried out through the calculation of the relative spleen weights and determination of DNA content of the kidney and its ultrastructure changes. The results indicated a decrease in the relative spleen weights with a decrease in the growth rates of mice. On the other hand there is a decrease in the DNA content of the kidney tissue as a result of combination of magnetic fields with cisplatin indicating increase in DNA break down. Ultrastructure changes of kidney tissues were observed. It was concluded that magnetic field increases the cytotoxicity produced by cisplatin, which can be taken as guide for the oncologist to apply to cancer patients.

Key words: cytotoxicity, cisplatin, static and alternating magnetic fields, DNA, kidney, spleen and ultrastructure.

INTRODUCTION

Cisplatin is an anticancer drug that falls into the class of DNA-damaging agents. It is usually administrated intravenously. Once in the blood stream, it diffuses across the plasma membranes into the cytoplasm where cisplatin transforms into the active form ($(NH3)2Pt^{++}$). The binding of ($(NH3)2Pt^{++}$) to DNA leads to change in the DNA structure [11], and prevent DNA repair mechanism within the cell, consequently DNA replication and cell division will not occur. Blocking of replication sets off signals within the cell that lead to programmed cell death [19].

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Magnetic fields are thought to interfere with cytoplasm enzyme functions by modifying permeability, ion transport, and/or ligand receptor interactions at the cell membrane surface [10]. Recent evidence suggests that cell processes can be influenced by the combination of MFs and drugs [16]. Indeed, coupling MF exposure with possible chemotherapy against cancers is a fascinating new area which is currently evolving [8].

Cadossi *et al*. [3], investigated the effect of a combination of cyclophosphamide (CY) and pulsed electromagnetic field (PEMF). The data demonstrate a synergic potentiating effect of PEMF over the damage induced by intra-peritoneal (i.p.) injection of CY. Tofani *et al*. [18], found that the survival time of mice bearing murine lewis lung carcinomas (LLCs) or B16 melanotic melanomas and treated with cisplatin and exposed to MF was significantly longer than that of mice treated only with cisplatin or only exposed to MF, showing that MF act synergic with the pharmacological treatment. Static and alternating MFs were suggested to selectively act on cell signaling, through their effects on charged matter motion, thus influencing cell survival mechanism in transformed cells [15], which have differently depolarized cell membranes compared with normal cell [9].

These results encouraged us to further investigate possible synergism between MFs and a chemotherapeutic agent (cisplatin) in terms of some biophysical and biochemical properties of kidney and spleen of mice injected with cisplatin and exposed to a static magnetic field superimposed to an alternating magnetic one.

MATERIALS AND METHODS

EXPOSURE FACILITY

Animals were exposed to homogeneous MF generated by a device of two coils that produces static and alternating MFs (Fig. 1). The first coil was connected with a circuit providing DC current, while the second coil with a circuit providing an alternating AC current at 50 Hz. This device can produce MF with minimal heating and field variations over the exposure area. By varying the input current to each coil and measuring the rise in temperature in each coil, it was found that there is no any increase in temperature when we used a current of 0.25 amperes through the DC coil and 0.15 amperes through the AC coil. As a precautionary measure, thermocouple probe (T-type, copper-constantan) was embedded in the windings of each coil for continual temperature measurements, while the coils are operational.

The MF intensity of alternating MF was measured using homemade search coil (0.1 mm in diameter and 100 turns), while the field intensity of static MF was measured using gauss meter (Laboratorio elettrofisico, Nervinao, Italy, model CTM2000 with a 3.5×12 mm^2 Hall probe). The Gauss meter probe was calibrated to provide measuring accuracies of $\pm 0.5\%$ for the DC field component, so that obtained reading are in mT.

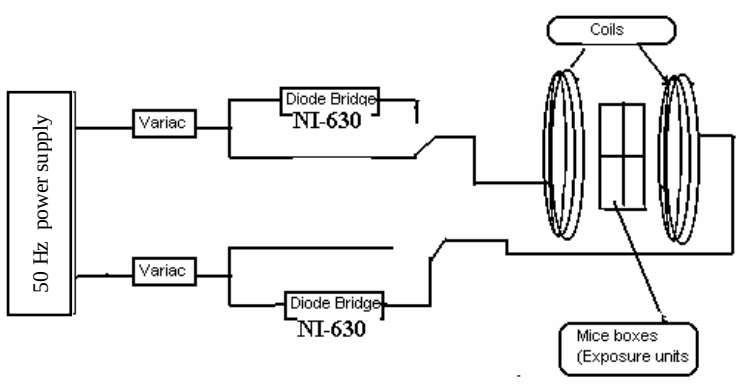

Fig. 1. Schematic drawing of the coils and their electrical connections.

An animal exposure unit is made of plexiglas, and consists of eight boxes. This unit is placed between the two coils. The dimensions of the box (2×5×10 cm) allow mice a certain degree of movement to avoid possible stress. In any case, for each experiment a sham-exposed mice group is handled and treated in the same conditions as the exposed ones.

In each run, eight mice were exposed to combined AC/DC MFs, provided daily (6 days/week) for 35 minutes, for three successive weeks. Exposure began on the same day of injection. Animals were circulated every day in the exposure cages to obtain approximately equal final dose of the magnetic field exposure. The average total intensity of the field is 3.6 mT.

EXPERIMENTAL ANIMALS AND THEIR GROUPS

Six week-old adult male albino mice weighing 25–30 g were purchased from the Faculty of Medicine, Alexandria University, Egypt. Eighty mice were used, each ten animals were housed in a cage in a well-ventilated room (25±2 °C), with a relative humidity of (43±3) and maintained on 12 h light / 12 h dark cycle. Normal diet and water were available *ad libitum*. The animals were adapted for two weeks to these conditions before the experiment. Cisplatin (DPL, Faulding and Colimited-Australia) dissolved in 9% saline to produce the selected concentration (3 mg/kg), forty mice were injected once with 1 ml cisplatin intraperitoneal. Animals were then divided into four equal groups, namely GI, GII, GIII, and GIV, each group contained twenty mice. Animals of group GI were injected with saline and used as control (sham exposed), animals of GII were injected with saline and exposed to a combined static and alternating magnetic field of 3.6 mT for 35 min/day, 6 day/week for 21 days, animals of GIII were injected with cisplatin (3 mg/kg) and sham exposed to magnetic fields, the final group GIV were injected with cisplatin (3 mg/kg) and exposed to magnetic fields as GII.

After the exposure period, animals were sacrificed by cervical decapitation, spleens and kidneys were removed and washed with saline. Fresh specimens were taken from the kidney of all studied groups and processed for electron microscopic study.

SPLEEN WEIGHT STUDY

Just before sacrifice of each group of our experiment, body weight of mouse was recorded. After sacrifice, spleens were dissected and washed with saline, then left on Wattman filter paper 15 min, then weighted accurately, and the relative spleen weight was calculated.

$$\text{Relative spleen weight} = \frac{\text{Spleen weight after drying}}{\text{Mouse weight}} \quad (1)$$

DETERMINATION OF DNA OF KIDNEY TISSUE

DNA of kidney tissues were isolated on agarose gel electrophoresis [1], using QIAamp®DNA mini kit (QIAGEN GmbH; Germany). The electrophoresis was run at 100–120 mA for 30 minutes. After the termination of the run, the bands were visualized by using UV plate, and were photographed using a digital camera. The bands were scanned (EPSON Scanner GT-9600) and the data were analyzed using LabWorksTM Image & acquisition and analysis software (Ultra-Violet product, Ltd. Cambridge, UK).

RESULTS

SPLEEN WEIGHT

Eight mice at age of 6 weeks were sacrificed at this age to determine the relative spleen weight. Half of the animals in each group were sacrificed after 10 days (i.e. at age of 9.4 weeks), while the rest were sacrificed after 21 days (i.e. at age of 11 weeks). Table 1 collects the obtained data. The relative spleen weights (RSW) for each group are shown as a mean ± standard deviation. Also, the mice growth rates in each group are tabulated at the specified age. Figure 2 illustrates the changes in RSW among the groups. Figure 3 illustrates the change in the growth rates of mice in each group. The results showed that there was a decrease in RSW among animals exposed to MFs. Noticeably from Fig. 2 that there is a significant decrease in the value of RSW for groups GII, GIII and GIV, respectively, reaching a lowest value in group G IV. Similarly, it is clear from Fig. 3 that the growth rate in each group decreased sharply and reached its lowest value in group G IV.

Table 1

Effect of MF 3.6 mT exposure and/or cisplatin injection on the relative spleen weight (RSW) as a function of time (data represented as a mean ± SD)

Groups		6 weeks	9.4 weeks	11 weeks
G I: Saline+ unexposed	Mean mouse weight (g)	23.837±0.73	28.624±1.291	33.91±0.642
	Mean spleen weight (g)	0.057±0.004	0.723±0.005	0.088±0.0009
	RSW × 10^{-3}	0.24±0.010	0.253±0.005	0.26±0.0063
	Growth rate (g/week)**	0.1193	2.698	3.9113
G II: Saline + exposed	Mean mouse weight (g)		28.403±1.135	32.839±0.5076
	Mean spleen weight (g)		0.20±0.0089	0.191±0.0065
	RSW		0.20±0.0089*	0.191±0.0065*
	Growth rate (g/week)**		2.3148*	3.2297*
G III: Cisplatin + unexposed	Mean mouse weight (g)		27.093±0.8693	30.879±0.276
	Mean spleen weight (g)		0.054±0.0024	0.059±0.0024
	RSW		0.20±0.0089*	0.191±0.0065*
	Growth rate (g/week)**		1.91516*	2.8166*
G IV: Cisplatin + exposed	Mean mouse weight (g)		26.517±0.485	28.765±0.269
	Mean spleen weight (g)		0.048±0.00089	0.0467±0.0014
	RSW		0.18±2.36E–09*	0.162±0.0041*
	Growth rate (g/week)**		1.20852*	1.6034*

* Significant at $p < 0.05$.

** Growth rate was calculated from the corresponding curve for each group.

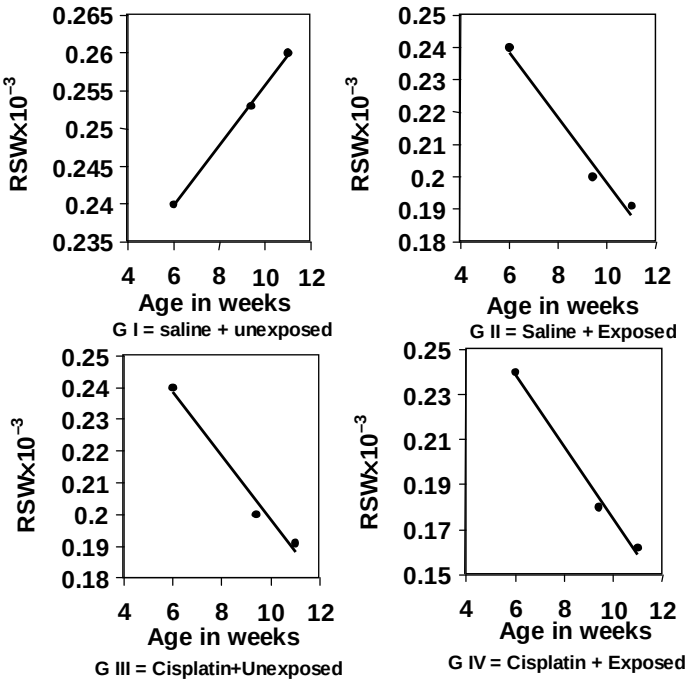

Fig. 2. The changes in the relative spleen weight versus age of mice in weeks among groups after exposure to 3.6 mT MF and cisplatin injection.

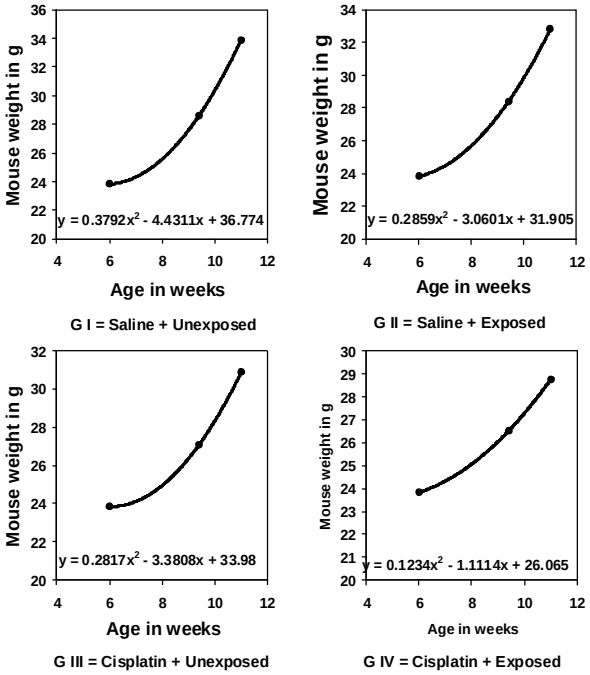

Fig. 3. The changes in the mouse weight in grams versus age in weeks for mice exposed to 3.6 mT MF and injected cisplatin of all groups. The equation describes the growth function for each group tabulated in the figure.

DETERMINATION OF KIDNEY DNA

The DNA bands analysis was repeated for each mouse in the same group, results showed reproducible shape and area for mice in the same group. Figure 4 shows the curves of DNA concentration after evaluation of bands. Table 2 shows the mean value of the area under the curves of DNA bands and the changes of this area from group to group.

Table 2

The change in the area under the curve (arbitrary units) in Figure 4

Parameters	**Saline + unexposed**	**Saline + exposed**	**Cisplatin + unexposed**	**Cisplatin + exposed**
Area (square pixels)	274	270	267	231
Mean	124.33	70.96	80.22	69.77
S.D.	12.35	3.39	5.98	5.30
Minim	85.00	64.00	68.00	58.00
Maxim	144.00	80.00	92.00	80.00

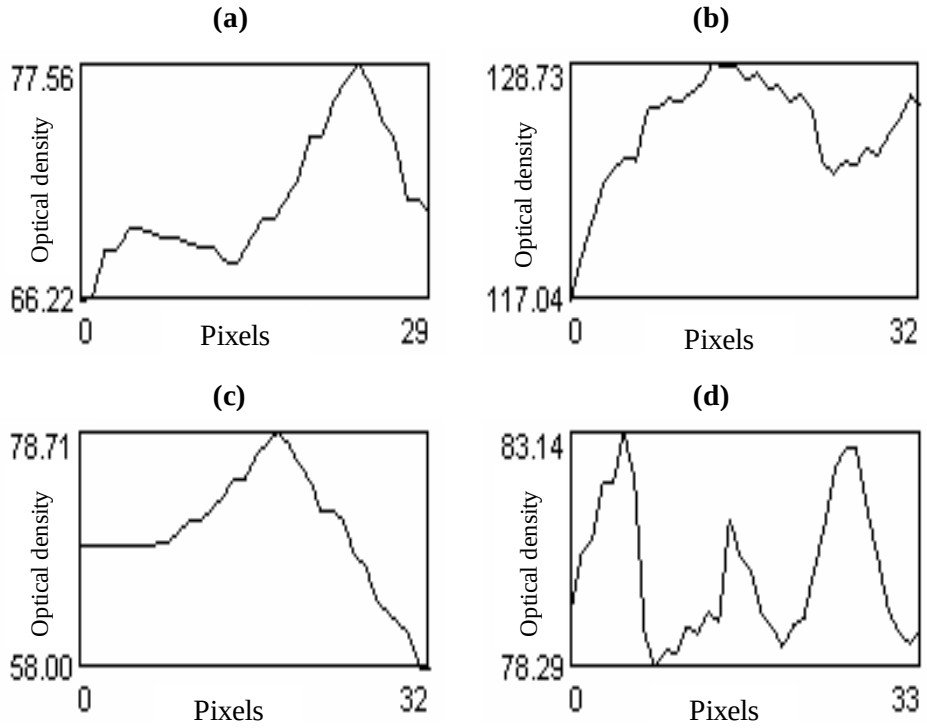

Fig. 4. The representative peaks for each band of each group. a. saline+unexposed; b. saline+exposed; c. cisplatin+unexposed; d. cisplatin+exposed.

ULTRASTRUCTURE OF KIDNEY TISSUES

Compared to the normal cells of kidney (Fig. 5), sections of kidney tissue exposed to both static and alternating MFs and cisplatin injection showed focal area of cellular degenerations. Kidney tissues of mice exposed to MFs alone (Fig. 6) showed irregular nuclear outline, clumping and condensation of heterochromatin, mitochondria appeared swollen with disintegrated matrix and disrupted membranes. Kidney tissues of mice injected with cisplatin showing small apoptotic nuclei (Figs. 7, 8), focal area of cytoplasmic degeneration with electron dense atrophic mitochondria. Kidney tissues of mice exposed to MFs and injected with cisplatin showing apoptotic cells with swollen cytoplasm and pyknotic and atrophic mitochondria, cell membrane is discontinuous and show focal rupture (Figs. 9, 10).

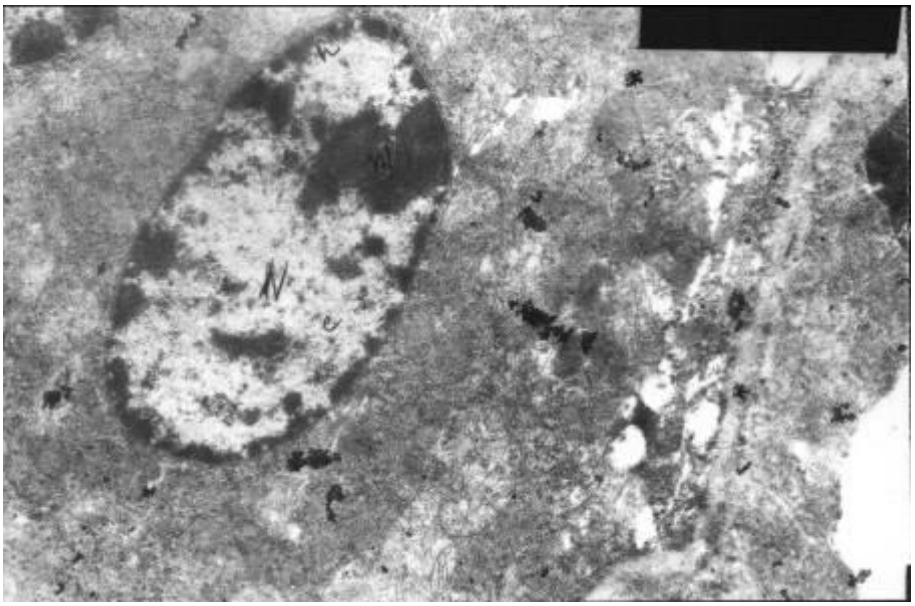

Fig. 5. Part of renal tubules of kidney tissues of control mice showing normal cell. Narrow intercellular epithelial space (*) is evident (mag. 10 K).

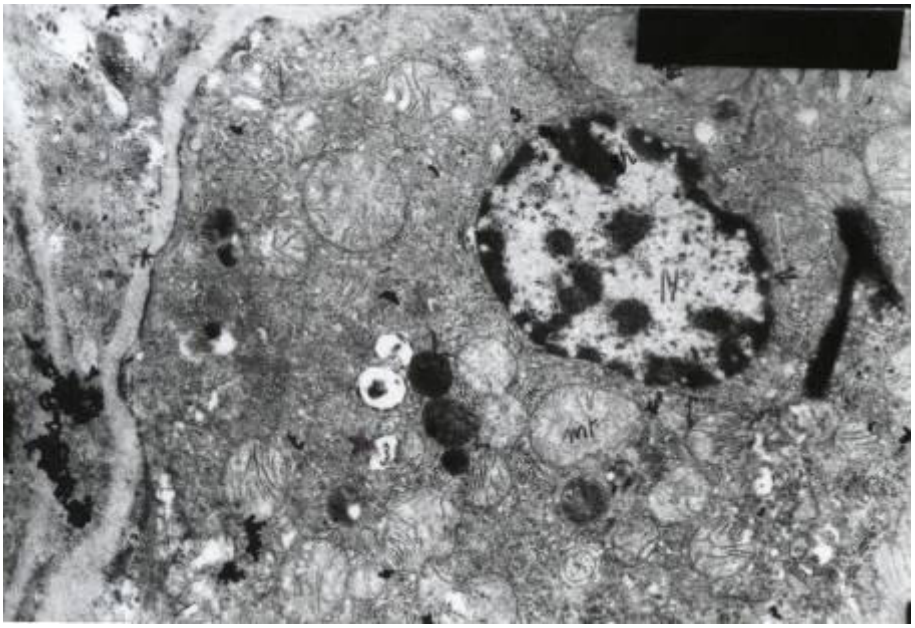

Fig. 6. Part of renal tubular epithelial cells of kidney tissues of mice exposed to MF showing a small round nucleus (N) with densely clumped heterochromatin (h) lying on the nuclear membrane with prominent nuclear pores (↓). The cytoplasm includes few large sized mitochondria (mt) having normal cristae. Numerous variable sized electron dense lysosomal bodies (L) are included. Intercellular space is slightly widened (*) (mag. 10 K).

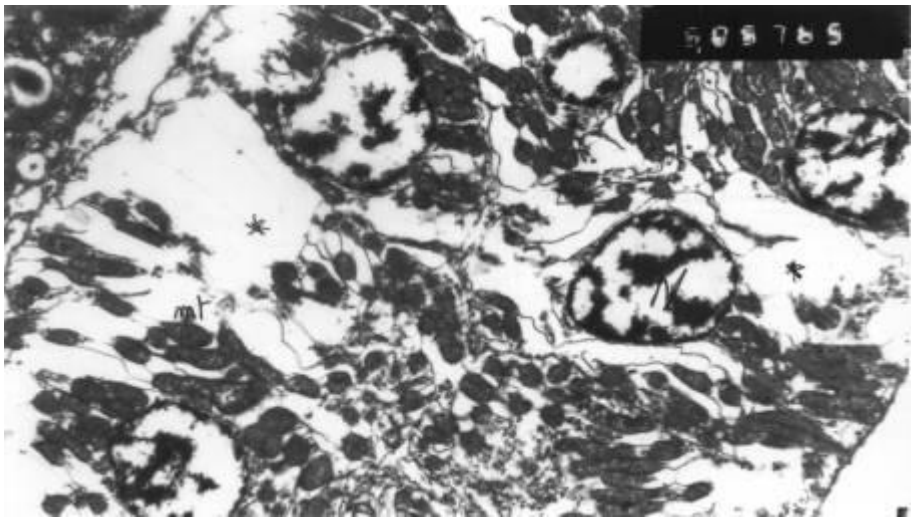

Fig. 7. Low power electron micrograph of renal tubule cells of kidney tissues of mice injected cisplatin and unexposed to MF showing small apoptotic nuclei (N) featuring coarsely clumped (aggregated) heterochromatin separated by clear spaces and thick nuclear membrane. Cytoplasm is filled up with large clear spaces (*) separating predominate small electron dense atrophic mitochondria (mt) (mag. 7.5 K).

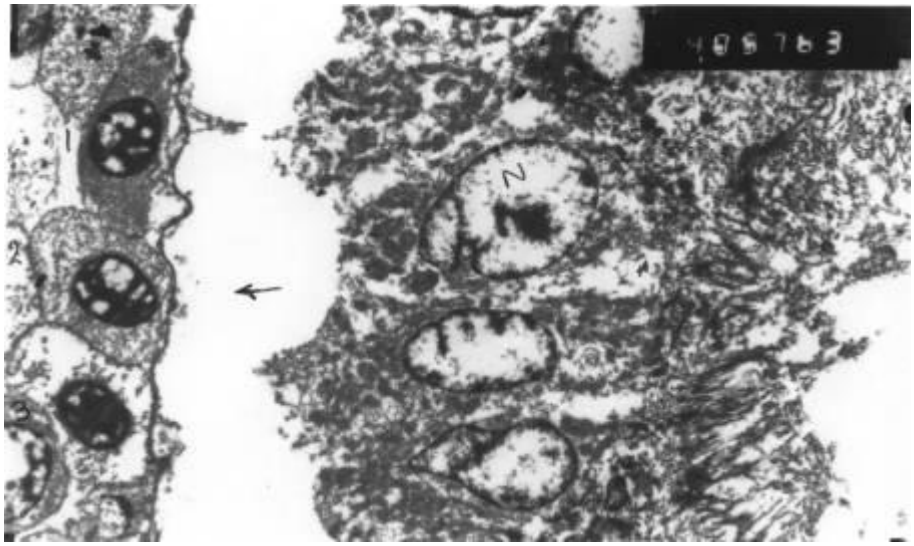

Fig. 8. Low power electron dense micrograph of both proximal and distal tubules of kidney tissues of mice injected cisplatin and unexposed to MF showing three lining cells having small dark pyknotic nuclei. Proximal tubule cells have large pale nuclei (N). Cytoplasm shows small pyknotics and atrophic mitochondria with cytoplasmic spaces (*) and irregular distorted microvilli. Basal part of epithelial cells are detached from the basement membrane (←) (mag. 6 K).

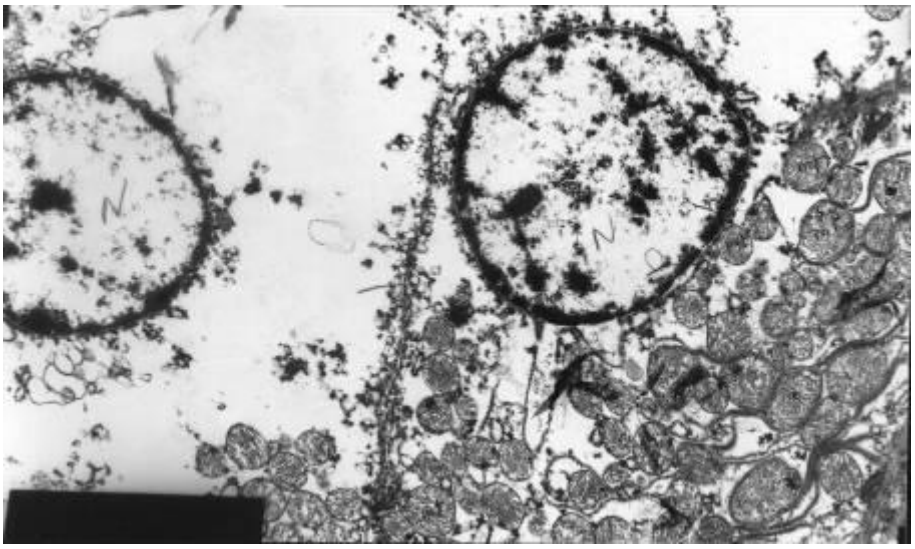

Fig. 9. Part of renal tubule cells of two epithelial cells of kidney tissues of mice injected cisplatin and exposed to MF showing large round nuclei (N) with finely clumped heterochromatin (h) on the nuclear membrane and abundant euchromatin (e). Prominent clearing up of the cytoplasmic content due to large clear areas (? water fluid) separating the mitochondria (mt) and ribosomes. Mitochondria are slightly affected as compared to normal looking RER; the intercellular space is thin and includes microvesicles (↑) (mag. 7.5 K).

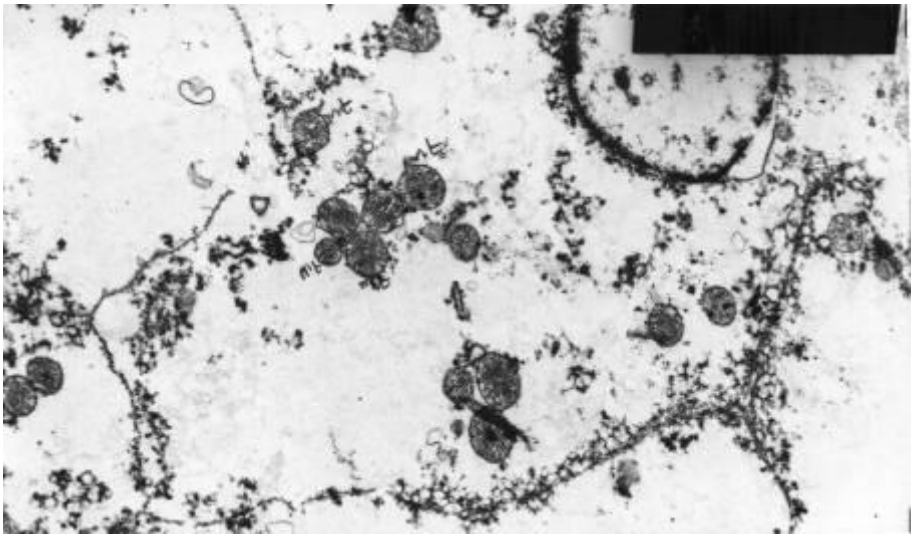

Fig. 10. Part of renal tubule cells of two epithelial cells of kidney tissues of mice injected cisplatin and exposed to MF showing a maximum swollen of the cytoplasm with few small residual mitochondria (mt) and ribosomes. Cell membrane is discontinuous and shows focal rupture (*) (mag. 12 K).

DISCUSSION

Suitable combination of static and oscillating MFs, leading to the formation of field/tissue interaction models based on resonance mechanism. According to Liboff [13] and Blanckard-Blackman [2] theories, both the identified non-hydrated ions are partaking in protein-mediated transport as key actors. Depending on their ratio-to-mass (q/m) the ions can be made to resonate with an external oscillating MF given that the following conditions applied, based on a cyclotron resonance model,

$$f_{AC} = f_c = (1/2\,\pi)\,(q/m)\,B_{OC} \quad (2)$$

where f_{AC} is the frequency of the applied oscillating MF, f_c is the resonant frequency of a given ion with charge-to-mass ratio (q/m), and B_{OC} is the total static field parallel to the oscillating MF, the field-ion resonance will then facilitate (or possibly hinder) the transport of ions across cellular or intracellular membranes.

Our data demonstrate that mice exposed to MF after cisplatin injection show decrease in the relative spleen weights. The data suggest that the ability of the hemopoietic cells to recover after cisplatin treatment is diminished in mice exposed to MF. Such results can be interpreted as a consequence of an earlier entry of the cells into the cell cycle as a result of MF exposure in the presence of anticancer drug [3].

Our data showed that the combination of MF and cisplatin treatment increases the efficacy of cisplatin to break DNA strand. This finding may be due to the rate of conversion of cisplatin to reactive species, able to bind to DNA, that is increased by localized production of free radical due to MF exposure [18].

Lia and Singh [12] suggested that MF-induced DNA strand breaks were caused by an iron-mediated free radical process. Probably via the Fenton reaction, this converts hydrogen peroxide to the more potent and toxic hydroxy radical. Iron-induced oxidant formation is known to cause DNA strand breaks, DNA-protein cross-links, and activation of protein kinase C and alter calcium homeostasis in cells [14]. Other studies have also implicated the involvement of iron/transition metals in the effect of EMFs [20].

Mice exposed to both static and alternating MFs of nearly 3.6 mT, showed regional changes. Electron microscopic study revealed focal areas of degeneration in the kidney. The nucleus, mitochondria and plasma membrane are the most and the main organelles revealing noticeable alterations in all mice exposed to MF and/or injected cisplatin. The mitochondrial changes encountered in the present study were mitochondrial pyknosis and mitochondrial swelling. Mitochondrial swelling could be due to changes of mitochondrial membrane permeability as [4], has reported changes in the phospholipid composition of the mitochondria membrane exposed to MF which could in turn alter the permeability of their membrane. There were vacuolations in the cytoplasm, which reflected the setting of a disturbance in the electrolyte balance system of some parts of tissues especially in kidney tissues. The electrolyte disturbance occurred as a result of affection of the plasma membrane permeability by the MFs, consequently, this lead

to an increase in flux of Na+ into the cell followed by water which in turn causes distention of the rough endoplasmic reticulum (RER), cisternae and appearance of cytoplasmic vacuoles [5].

The morphological phenotype of apoptosis is characterized by rapid condensation of the cytoplasm and nuclear chromatin, resulting in DNA fragmentation and membrane blabbing followed by fragmentation of the cells into apoptotic bodies, made up of condensed cytoplasm, nuclear material and/or whole organelles surrounded by intact plasma membrane [7]. Apoptosis has been reported to be influenced by SMF exposure [17]. The induction of the apoptotic process could be linked to Ca^{2+} ion fluxes that are dependent on the effect on plasma membrane of MF exposure [6]. A synergic potentiating effect was observed between MF exposure and cisplatin.

In conclusion, the data reported in this paper seem to indicate that, the exposure to MF enhance the toxicity produced by cisplatin. However, further investigations are needed to optimize MF physical parameters, chemotherapy with cisplatin schedule and combination of both.

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