

Morphological, biochemical, histological, and ultrastructural protective effects of misoprostol on cisplatin induced-hepatotoxicity in adult male rats

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ABSTRACT

الأهداف: فحص التأثير الوقائي المحتمل للميسوبروستول على تأثير السيسبلاطين الضار على الكبد.

الطريقة: اشتملت الدراسة على 4 مجموعات متساوية من الفئران الذكور البالغة كلا منهم 6 فئران من فصيلة الويستر. وتلك المجموعات هي الشاهد، ومعالجة بالسيسبلاطين، ومعالجة بالميسوبروستول، ومعالجة بكلا من الميسوبروستول والسيسبلاطين. وقد تم حساب وزن الجسم، وزن الكبد، ونسبة وزن الكبد الى وزن الجسم. وتم أخذ عينات دم من قلوب الفئران لتقدير مستوى كلا من الصبغة الصفراء، وإنزيم أسبرتات أمينوترانسفيراس، وإنزيم الألانين أمينوترانسفيراس والزرال في خثر الدم. وتم إعداد عينات كبد للفحص بكلا من الميكروسكوب الضوئي والميكروسكوب الإلكتروني. أجريت هذه الدراسة خلال الفترة من يونيو 2012م إلى إبريل 2013م بقسم التشريح، كلية الطب، جامعة الزقازيق، الزقازيق، مصر وجامعة الملك عبد العزيز، جدة، المملكة العربية السعودية.

النتائج: إن حقن السيسبلاطين جرعة واحدة بمعدل 7.5 ملليجرام/كجم عن طريق الغشاء البريتوني أدى الى ارتفاع ملحوظ في مستويات كلا من الصبغة الصفراء، إنزيم أسبرتات أمينوترانسفيراس، إنزيم الألانين أمينوترانسفيراس ونقص واضح في مستوى الزلال في خثر الدم مع نقص وزن الجسم، وزن الكبد ونسبة وزن الكبد الى وزن الجسم. إن إعطاء الميسوبروستول مع السيسبلاطين أدى إلى تحسن معظم القياسات السابقة. إن فحص العينات بكلا من الميكروسكوب الضوئي والإلكتروني أوضح وجود تكسير في خلايا الكبد المحيطة بالوريد المركزي، وتليف حول المضمار البائي، واتساع واحتقان للوريد المركزي والجيوب الدموية، نقص في محتوى الجليكوجين، تكسير في الأجسام العصبية، واتساع فقعي بالشبكة الأندوبلازمية الخشنة وتغيرات نووية في الفئران المعالجة بالسيسبلاطين. ولقد لوحظ تحسن في العديد من التغيرات سابقة الذكر عند أخذ الميسوبروستول مع السيسبلاطين.

خاتمة: دلت نتائج هذا البحث على أن الميسوبروستول ممكن أن يكون له تأثير واقعي ضد التأثير الضار للسيسبلاطين على الكبد.

Objectives: To investigate the possible protective effect of misoprostol on cisplatin-induced hepatotoxicity.

Methods: Four-equal sized groups (control, cisplatin-treated, misoprostol-treated, combined misoprostol, and cisplatin-treated) adult male Wistar rats (6 each) were used in this study. Body weight, liver weight, and liver weight/body weight ratio was calculated. Blood samples were obtained from the hearts of rats to determine the levels of total serum bilirubin (TSB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin. Liver specimens were prepared for both light and electron microscopes. The study was carried out between June 2012 and April 2013 at the Anatomy Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, and the Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Results: A single cisplatin dose (7.5 mg/kg intraperitoneally) resulted in significant elevation of AST, ALT, and TSB serum levels, and a significant reduction of serum albumin level, body weight, liver weight, and liver weight/body weight ratio. A combination of misoprostol (200 µg/kg/day) with cisplatin improved most of the previous parameters. Examination of specimens by both light and electron microscopes revealed pericentral hepatic necrosis, periportal fibrosis, dilatation, and congestion of central vein and blood sinusoids, diminished glycogen content, degenerated mitochondria, vesicular dilated rough endoplasmic reticulum, and nuclear changes in cisplatin-treated rats. Oral intake of misoprostol with cisplatin improved many of these changes.

Conclusion: The results indicate that misoprostol may have a protective effect on cisplatin-induced hepatotoxicity.

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Cisplatin (CP) is one of the main drugs used in the treatment of ovarian, testicular, lung, bladder, and cervical cancers.¹ Despite its clinical importance, cisplatin has undesirable side effects when administered at high doses.² A limited number of studies have investigated the effect of cisplatin on hepatic ultrastructure,³⁻⁵ and several antioxidants have been reported to ameliorate the hepatotoxic effects of cisplatin.⁶⁻⁸ Misoprostol is a synthetic methyl prostaglandin E1 (PGE1) analogue that is used in the treatment of peptic ulcer as it increases secretion of the protective mucus that lines the gastrointestinal tract and increases mucosal blood flow.⁹ Recently, the potential antioxidant and antiapoptotic effects of misoprostol have been confirmed in different studies through its role as a reactive oxygen species (ROS) scavenger.¹⁰ In addition, the effect of misoprostol on cisplatin-induced changes in lipid peroxidation products, and the activity of antioxidant enzymes in the rat kidney has been investigated to determine the extent of tissue damage due to oxidative stress.⁷ The present study aims to explore the possible protective effect of misoprostol on cisplatin-induced hepatotoxicity in adult male rats.

Methods. Animals. Twenty-four adult male Wistar albino rats (10-12 weeks of age) were used in the present study. The rats were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. For one week, the rats were conditioned at room temperature. The rats were provided with a commercial balanced diet and tap water ad libitum throughout the experimental period.

Drugs. Cisplatin was obtained in the form of commercial Egyptian Unistin Vial (Egyptian International Medical Company (EIMC) United Pharmaceuticals, Cairo, Egypt). Misoprostol (in the form of Misotac 200 µg/tablet) was purchased from SIGMA Pharmaceutical industries, Cairo, Egypt.

Experimental design. The rats were divided into 4 equal groups of 6 rats each. The rats of each group were housed in separate plastic cages at a temperature of 25±2°C with a 12h light/dark cycle and a relative humidity of 50-60%. The rats in group I (control) received oral distilled water for 9 days and a single intraperitoneal (i.p.) injection of normal saline on the fifth day. The rats in group II (cisplatin-treated rats) received a single i.p. injection of cisplatin

7.5mg/kg body weight on the fifth day. The rats in group III (misoprostol-treated rats) received oral misoprostol 200µg/kg body weight/day for 9 days, and a single normal saline i.p. injection on the fifth day. The rats in group IV (combined misoprostol and cisplatin-treated rats) were treated with oral misoprostol 200µg/kg body weight/day for 9 days in combination with a single i.p. cisplatin injection of 7.5mg/kg body weight on the fifth day. The animals of all groups were weighed on the first, third, fifth, seventh, and ninth days of the study. On the tenth day, the rats were anesthetized by ether then abdominal and thoracic dissections were carried out. Blood samples were collected directly from the heart by intracardiac puncture and poured into a separate test tube. The livers of the rats were excised, washed with normal physiological saline, cleaned from fat, and weighed.

Assessment of liver functions. The blood samples were centrifuged at 3000 rpm for 15 minutes, their sera were separated and used within 48 hours for estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total serum bilirubin (TSB) by standard laboratory methods.¹¹

Relative liver weight as a ratio of body weight (hepatosomatic index). The livers were washed with ice-cold saline, dried on filter paper, and weighed. The liver weight/body weight ratio was calculated according to the following formula, organ ratio (%) = organ weight X 100/body weight.⁸

Light microscopic examination.¹² The liver sections were fixed in 10% neutral-buffered formalin solution for 48 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylol, and embedded in paraffin blocks. Serial sections (3-5µm) were cut using a microtome (Leica RM 2125, Leica Biosystems Nussloch GmbH, Germany). The sections were washed in a water bath and left in the oven for dewaxing. The sections were stained with hematoxylin and eosin, Masson's trichrome, and Periodic acid-Schiff (PAS). The PAS and Masson's trichrome stains were used to determine the amount of glycogen and collagen connective tissue contents in the specimens. The slides of the stained sections were mounted with Di-N-Butyle Phthalate Xylene and covered with cover slips.¹² All sections were examined using a light microscope (Olympus BH-2, Olympus, Tokyo, Japan).

Electron microscopic examination. From each animal, liver specimens (1mm³) were cut and immersed in 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C. After rinsing in buffer, the samples were post-fixed in 1% osmium tetroxide for 2 hours at 4°C. Dehydration of the specimens was carried out via ascending grades of

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ethanol after washing.^{5,6} Then, they were treated with a propylene oxide solution and embedded in a mixture of 1:1 Epon-Araldite for one hour. Polymerization was performed in the oven at 65°C for 24 hours. One µm sections were cut with a glass knife on a LKB-2000S ultramicrotome, mounted on glass slides and stained with buffered toluidine blue. Appropriate areas were selected with the light microscope. Ultra thin sections (50-60nm) were cut with a diamond knife on a LKB ultramicrotome, then mounted on copper grids, double stained with uranyl acetate and lead citrate to examine under the electron microscope (Joel 100CX TEM, Joel, Tokyo, Japan).

Statistical analysis. All data were expressed as mean ± standard deviation (SD). The data were analyzed using the Statistical Package for Social Sciences software, version 16 (SPSS, Chicago, IL, USA). The significance of differences between the mean values were calculated using the unpaired Student's t-test. The values were considered to be significant at $p < 0.05$.

The study was performed after the approval of the Medical Ethical Committee of the Faculty of Medicine, King Abdulaziz University, and followed the recommendations of the National Institutes of Health's Guide for Care and Use of Laboratory Animals.

Results. Effect of cisplatin and/or misoprostol on body weight of rats. There was a significant ($p < 0.0008$)

decrease in the body weight of cisplatin-treated rats compared with control rats (Table 1). However, a combination of misoprostol with cisplatin caused a significant ($p < 0.0001$) increase in body weight of rats when compared with cisplatin-treated rats. Rats treated with misoprostol alone recorded a significant increase of their body weights when compared with both control ($p \leq 0.0130$) and cisplatin-treated ($p < 0.0001$) rats.

Effect of cisplatin and/or misoprostol on liver wet weight and liver weight/body weight ratio of rats. The liver wet weight and liver wet weight/body weight x 100 ratio (hepatosomatic index) of the different groups were recorded and is shown in Table 2. A significant decrease in liver wet weight was noticed in cisplatin-treated rats compared with misoprostol-treated and control rats ($p < 0.0001$). There was a significant increase of the liver wet weight in combined misoprostol and cisplatin-treated rats ($p < 0.0001$) when compared with the control rats, but a non-significant increase was reported when compared with cisplatin-treated rats. We observed a significant reduction ($p < 0.001$) in the liver wet weight/body weight ratio in cisplatin-treated rats when compared with control and misoprostol-treated rats. However, a non-significant reduction of the liver wet weight/body weight ratio was recorded in combined misoprostol and cisplatin-treated rats.

Effect of cisplatin and/or misoprostol on liver biomarkers of rats. Serum alanine aminotransferase

Table 1 - Effects of cisplatin and/or misoprostol on body weight (gm) in rats (Mean ± SD).

Group	Day One	Day 3	Day 5	Day 7	Day 9	P-value	T-value
Control	183.3 ± 4.3	188.5 ± 3.9	193.2 ± 3.7	199.3 ± 3.4	205.7 ± 3.6 ^a	0.0001 ^a	9.7112 ^a
Cisplatin	180.7 ± 6.8	172.3 ± 4.2	165.2 ± 4.4 ^{ab}	The rats were decapitated on 6 th day after cisplatin injection		0.0008 ^a 0.0001 ^b	4.7132 ^a 17.5278 ^b
Misoprostol	180.8 ± 1.6	185.3 ± 1.65	189.7 ± 1.7	193.7 ± 1.7	200.7 ± 1.9 ^{abc}	0.0001 ^{ac} 0.0130 ^b	19.6078 ^a 3.01410 ^b 18.3684 ^c
Misoprostol-cisplatin	181.8 ± 2.04	186.8 ± 2.2	191.3 ± 2.2	188.7 ± 2.3	185.5 ± 2.2 ^{abc}	0.0135 ^a 0.0001 ^{bc}	2.9947 ^a 11.6702 ^b 10.2056 ^c

Data are expressed as mean ± SD (n=6). Body weight is expressed in gram (gm), ^aMeans in the same group are significantly different at $p < 0.001$, ^bSignificantly different from control at $p < 0.001$, ^cSignificantly different from cisplatin-treated rats at $p < 0.0001$.

Table 2 - Effect of cisplatin and/or misoprostol on liver wet weight and liver weight/body weight ratio in rats.

Item	Control	Cisplatin	Misoprostol	Misoprostol-Cisplatin	P value
Liver Weight (gm)	9.2 ± 0.16 ^b	6.9 ± 0.21 ^a	9.01 ± 0.15 ^{†,b}	7.13 ± 0.16 ^{a†}	$p < 0.0001^{ab}$
Final Body Weight	205.7 ± 3.6 ^b	165.2 ± 4.4 ^a	200.7 ± 1.9 ^{a,b}	185.5 ± 2.2 ^{a,b}	$p < 0.0001^{ab}$
Liver weight/body weight Ratio (%)	4.46 ± 0.16 ^b	4.15 ± 0.18 ^a	4.49 ± 0.002 ^{†,b}	3.85 ± 0.15 ^{ab}	$p < 0.0001^a$ and $p = 0.0103$. $p = 0.0009^b$ $p = 0.0106$

Data are expressed as mean±SD (n=6); Body weight and liver weight are expressed in gram (gm). Liver weight/body weight ratio= liver weight (gm) X100/body weight (gm). ^aSignificantly different from control group at $p < 0.0001$. ^bSignificantly different from cisplatin-treated rats at $p < 0.001$, [†]no significant difference from control ($p < 0.05$), [‡]no significant difference from cisplatin-treated rats ($p < 0.05$).

(ALT), aspartate aminotransferase (AST), and total serum bilirubin (TSB) levels increased significantly ($p<0.001$) in the rats treated with cisplatin when compared with control, misoprostol-treated, and combined misoprostol with cisplatin-treated rats. However, serum albumin significantly decreased ($p<0.009$) in cisplatin-treated rats compared with control, misoprostol-treated, and combined misoprostol with cisplatin-treated rats (Table 3).

Light microscopy. Examination of the centrilobular zone of the rat hepatic lobule revealed well-organized radiating cords of hepatocytes around the central vein with narrow sinusoidal space in-between in control rats. The blood sinusoids were lined by small flat endothelial cells and large Kupffer cells (Figure 1A). In cisplatin-treated rats, mild parenchymal disorganization, focal areas of necrotic hepatocytes, scattered hepatocytes with apoptotic nuclear changes, congestion and dilatation of

central vein, and blood sinusoids were observed in the centrilobular zone (Figure 1B). In misoprostol-treated rats, well-organized radiating cords of hepatocytes around the dilated congested central vein were seen, and dilatation and congestion of the blood sinusoids, increased number of binucleated hepatocytes and Kupffer cells were noticed in the centrilobular zone (Figure 1C). In the combined misoprostol and cisplatin-treated rats, mild congestion of both central vein and blood sinusoids with a relative increase in the number of binucleated hepatocytes and Kupffer cells were noticed in the centrilobular zone (Figure 1D).

The periportal zone of the hepatic lobule in control rats showed a normal portal tract surrounded by radiating cords of hepatocytes with the blood sinusoid in between. The portal tract was composed of bile ductule, hepatic arteriole, and portal venules with connective tissue fibers all around (Figure 2A).

Table 3 - Effect of cisplatin and/or misoprostol on liver biomarkers in rats.

Rats group	TSB ($\mu\text{mol/L}$)	Albumin (g/L)	ALT (U/L)	AST (U/L)
Control	1.18 \pm 0.32 ^b	4.67 \pm 0.62 ^b	41.15 \pm 6.38 ^b	76.45 \pm 6.38 ^b
Cisplatin	5.57 \pm 0.77 ^a	3.38 \pm 0.35 ^a	61.65 \pm 3.99 ^a	167.9 \pm 12.45 ^a
Misoprostol	1.25 \pm 0.24 ^b	4.23 \pm 0.36 ^b	40.43 \pm 2.72 ^b	51.98 \pm 2.94 ^{a, b}
Misoprostol-cisplatin	2.7 \pm 0.18 ^{a, b}	4.02 \pm 0.23 ^{a, b}	77.05 \pm 6.47 ^{a, b}	120.18 \pm 13.51 ^{a, b}

Data are expressed as mean \pm SD (n=6). TSB - total serum bilirubin ALT - serum alanine aminotransferase, AST - serum aspartate aminotransferase.

^aSignificantly different from control group at $p<0.001$; ^bSignificantly different from cisplatin-treated group at $p<0.001$.

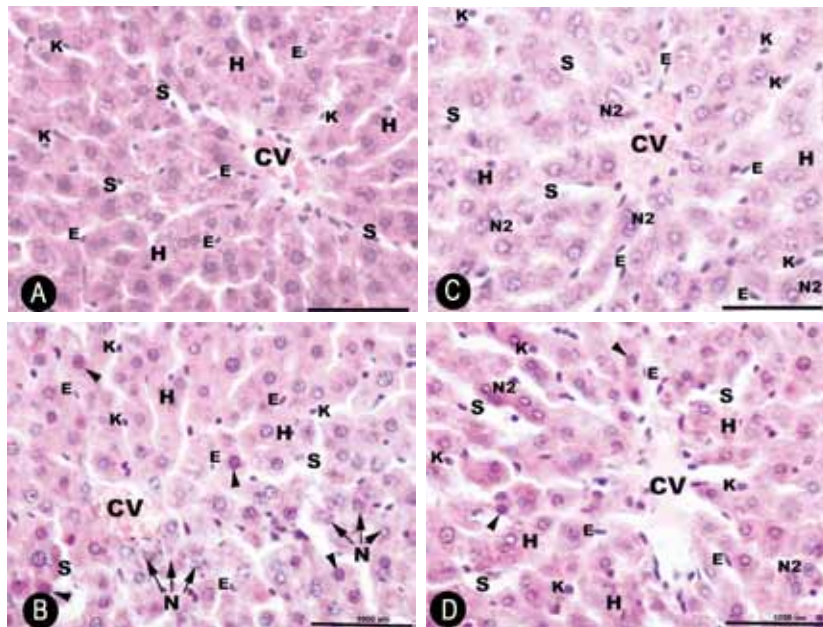


Figure 1 - Light micrograph of the centrilobular zone of a male rat liver showing A) normal parenchymal architecture of radiating hepatocyte-cords (H) around a central vein (CV) with narrow sinusoids (S) in-between in the control group. Endothelial cells (E) and Kupffer cells (K) line the blood sinusoids; B) congested central vein (CV), dilated blood sinusoids (S), focal necrotic area (N) and apoptotic nuclei (arrow head) are seen in the cisplatin-treated group; C) congested dilated central vein (CV) and blood sinusoids (S), increased number of both binucleated hepatocytes (N2) and Kupffer cells (K) are seen in misoprostol-treated rats; D) mild dilated central vein (CV) and blood sinusoids (S) with few binucleated hepatocytes (N2) and Kupffer cells (K) are observed in combined misoprostol with cisplatin-treated rats (Hematoxylin and eosin x 600).

In cisplatin-treated rats, proliferation of bile ductules and dilatation of the portal venule was noticed in the periportal area (Figure 2B). In misoprostol-treated rats, a relative increase in binucleated hepatocytes and Kupffer cell number with congested portal venules was seen in the periportal zone (Figure 2C). In combined misoprostol and cisplatin-treated rats, normal histological architecture was seen in the periportal zone (Figure 2D).

Normal distribution of collagen fibers was observed within the portal area in control rat liver specimens (Figure 3A). However, an excessive amount of the collagen fibers was noticed within the portal tracts in cisplatin-treated rats (Figure 3B). A normal distribution of the collagen fibers was observed within the portal tracts in the liver specimens of misoprostol-treated rats (Figure 3C), and combined misoprostol and cisplatin-treated rats (Figure 3D). An even distribution of glycogen was noticed in the hepatocytes of the control rats (Figure 4A). A small amount of glycogen was seen in a few centrilobular hepatocytes of the cisplatin-treated rats (Figure 4B). In misoprostol-treated rats, an excessive amount of glycogen was observed within the hepatocytes of both lobular zones (Figure 4C). A moderate amount

of glycogen content was observed in the periportal and centrilobular hepatocytes in the combined misoprostol and cisplatin-treated rats (Figure 4D).

Electron microscopy. In the control rats, normal polygonal hepatocytes each with a central oval-shaped euchromatic nucleus were seen (Figure 5A). The nucleus had a central electron-dense nucleolus and scattered variable-sized masses of condensed heterochromatin within its nucleoplasm and on the inner aspect of the nuclear envelope around the nuclear pores (Figures 5A & 5B). The biliary surface of the hepatocytes showed tight junctions at the ends of the bile canaliculi and many long microvilli projecting within the biliary lumen (Figure 5B). Scattered groups of round and oblong-shaped mitochondria, parallel rough endoplasmic reticulum cisternae, few lysosomes, numerous free ribosomes, and variable-sized glycogen masses were seen within the cytoplasm of the hepatocyte in control rats (Figures 5B & 5C). The space of Disse contained numerous microvilli originating from the sinusoidal surface of the hepatocyte. The endothelial cells lining the blood sinusoids had spindle-shaped nuclei and fenestrated cytoplasmic processes, while

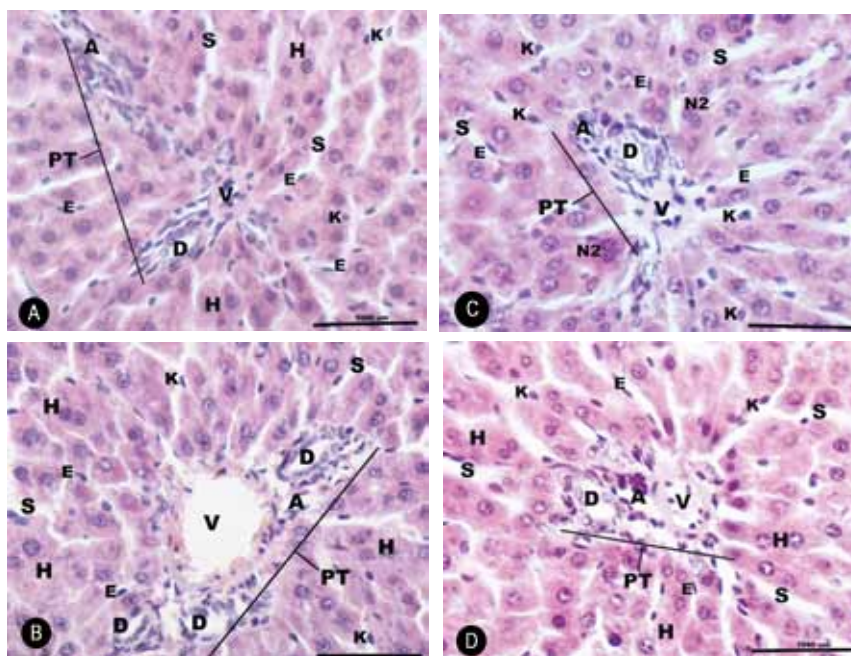


Figure 2 - Light micrograph of the periportal zone of a male rat hepatic lobule showing A) the cords of hepatocytes (H) with narrow blood sinusoids (S) in-between surrounding the portal tract (PT) in the control group. The portal tract consists of bile ductule (D), hepatic arteriole (A) and portal venule (V) with collagen fibers all around. The blood sinusoids are lined by flat endothelial cells (E) and large Kupffer cells (K); B) dilated portal venule (V) with collagen fibers all around. The blood sinusoids are lined by flat endothelial cells (E) and large Kupffer cells (K); C) small-sized portal tract (PT) with normal structure of the bile ductule (D), hepatic arteriole (A) and congested portal venule (V) and proliferated bile ductules (D) are seen within the portal tract of the cisplatin-treated rats group; D) normal structure of portal tract (PT) with relative reduction of binucleated hepatocytes (N2) and Kupffer cell (K) number are noticed in combined misoprostol with cisplatin-treated rats group (Hematoxylin and eosin x 600).

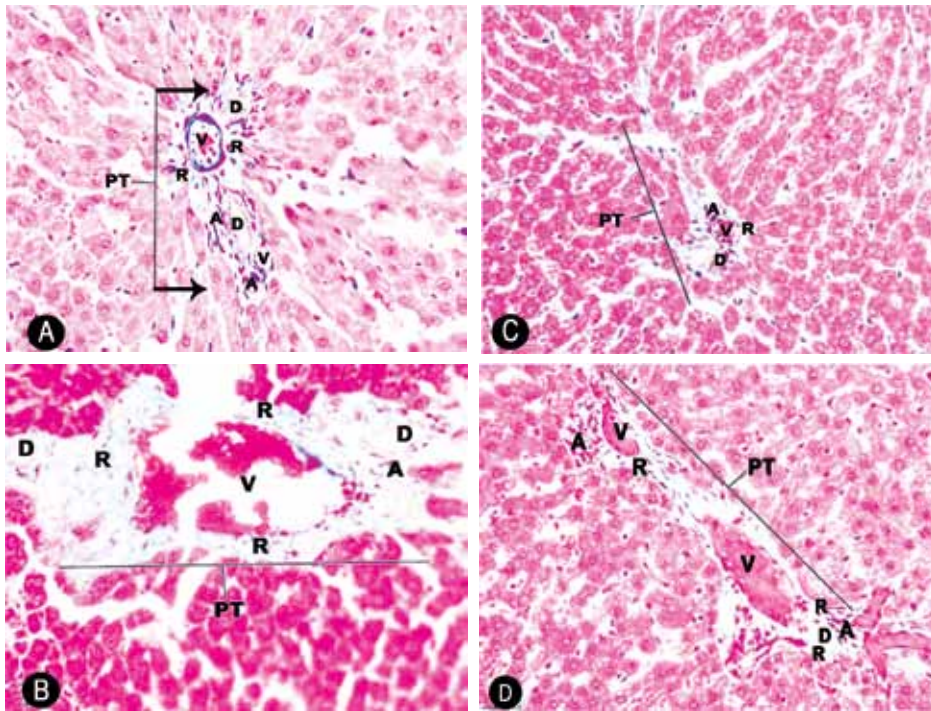


Figure 3 - Light micrograph of Masson trichrome-stained sections of a male rat liver showing A) little amount of collagen fibers (R) around the structures of the portal tract (PT) of the control group; B) an excessive amount of the collagen fibers was noticed around the structures of the portal tract (PT) of cisplatin-treated rats; C) Little amount of collagen fibers (R) is seen around the structures of the portal tract (PT) of misoprostol-treated rats; D) Little amount of collagen fibers (R) is present around the components of portal tract (PT) of combined misoprostol with cisplatin-treated rats. V: portal venule, A: hepatic arteriole, D: bile ductule (Masson trichrome stain x 400).

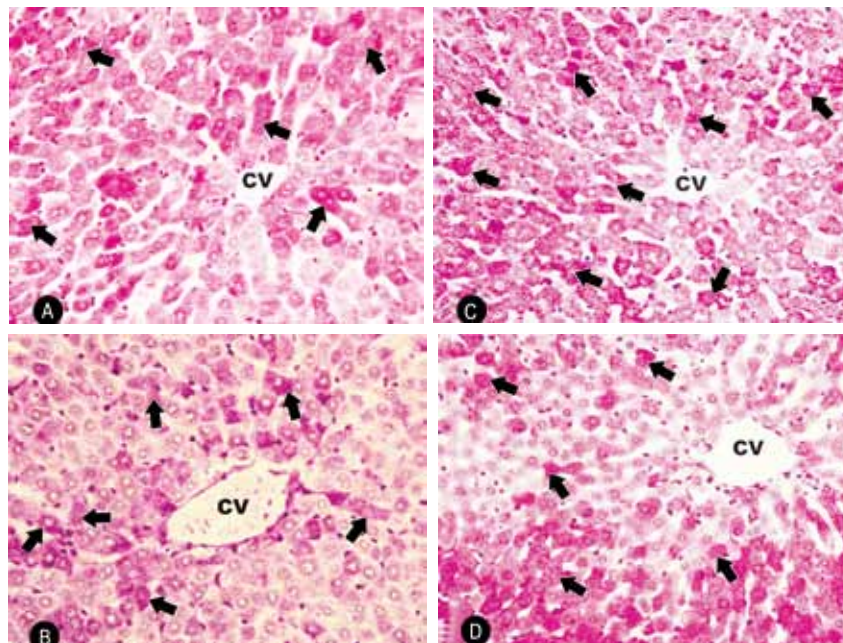


Figure 4 - Light micrograph of PAS- stained sections of a male rat liver showing A) even distribution of glycogen contents (arrow head) within the parenchymal hepatocytes of the control group; B) marked reduction of glycogen content (arrow head) within the hepatic cytoplasm in cisplatin-treated rats group; C) an excessive amount of glycogen content (arrow head) within the parenchymal hepatocytes of misoprostol-treated rats group; D) little increase of glycogen content (arrow head) within the periportal hepatocytes of combined misoprostol with cisplatin-treated rats group (Periodic acid–Schiff stain x 400).

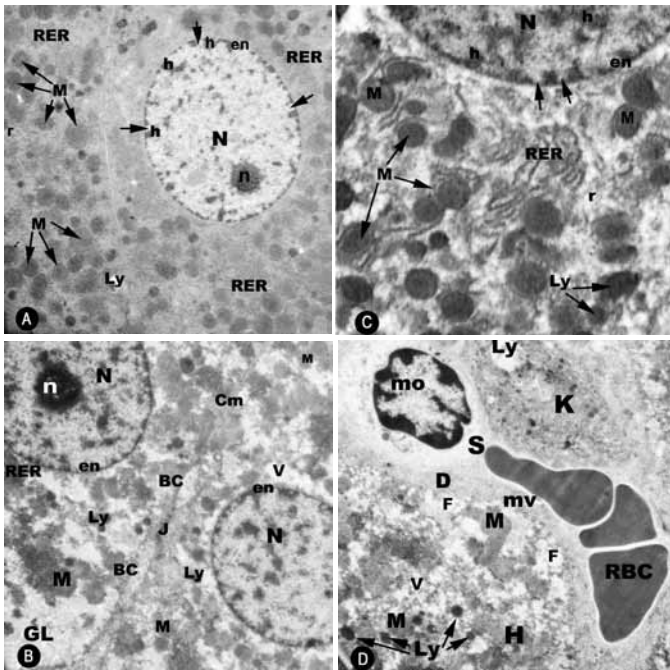


Figure 5 - Electron micrographs of the liver of a control rat showing the normal structure of a hepatocyte. A) the hepatocyte has a central oval nucleus (N) with electron dense nucleolus (n). The cytoplasm contains numerous oblong and round-shaped mitochondria (M), free ribosomes (r), few lysosomes (Ly) and rough endoplasmic reticulum cisternae (RER). B) Glycogen masses of variable size (GL) are seen between the mitochondria (M). The biliary surface of the hepatocyte membrane (Cm) has junction complexes (J) at the ends of the bile canaliculus (BC). C) The rough endoplasmic reticulum cisternae (RER) are distributed between and around the mitochondria (M). Condensed heterochromatin masses (h) are seen within the nucleoplasm and deposit on the inner aspect of the nuclear envelope (en) around the nuclear pores (arrow). D) The blood sinusoid is lined with large Kupffer cell (K) and contains red blood cells (RBC) and monocyte (Mo). The sinusoidal surface of the hepatocyte has numerous microvilli (mv) projecting into the Disse space (D).

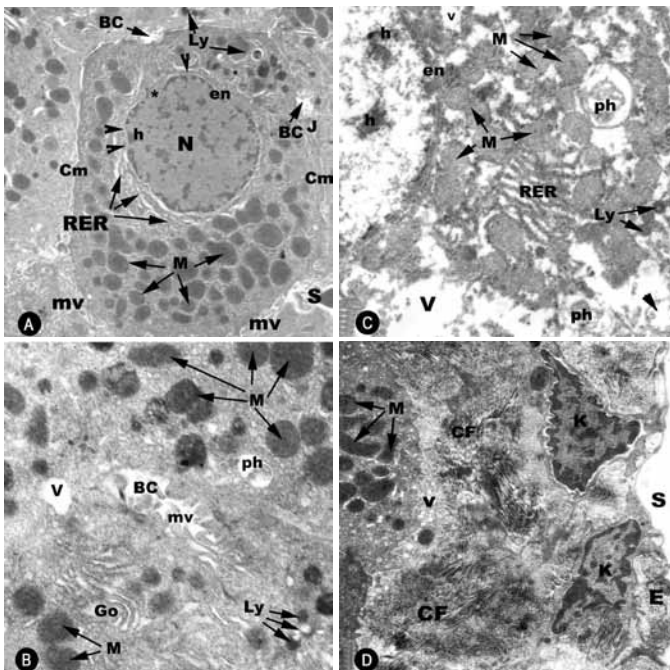


Figure 6 - Electron micrographs of the cisplatin-treated rats liver showing a A) small-sized polygonal hepatocyte having a central irregular nucleus (N) with a defect (*) in its envelope (en), little amount of condensed heterochromatin (h) on the inner aspect of the nuclear envelope around the wide nuclear pores (arrow head). The cytoplasm contains variable-sized round and oblong-shaped mitochondria (M) and rough endoplasmic reticulum (RER); B) wide bile canaliculus (BC) with few long microvilli within its lumen is seen between the 2 hepatocytes. The pericanalicular area contains Golgi apparatus (Go), lysosomes (Ly), phagosome (Ph), vacuole (V) and mitochondria; C) The juxtra-nuclear cytoplasm contains marked dilated RER cisternae between and around the mitochondria (M), large heterogeneous phagosomes (ph), many lysosomes (Ly),) large electron-lucent vacuole (V) and degenerated mitochondria (arrow head). The nuclear part showed a little amount of condensed heterochromatin (h) within the nucleoplasm only; D) Both Kupffer (K) and endothelial (E) cells are seen in the wall of the blood sinusoid (S). Many collagen bundles (CF) are present between the Kupffer cells and the hepatocytes.

the phagocytic Kupffer cell had a large triangular heterochromatic nucleus surrounded by a small amount of cytoplasm. The sinusoidal lumen contained few blood cells (Figure 5D).

In cisplatin-treated rats, the hepatocyte attained small size, polygonal shape, and a central nucleus with irregular outline (Figure 6A). The nucleus showed a

discontinued nuclear envelope, wide nuclear pores, few variable-sized heterochromatin masses within the nucleoplasm, and on the inner aspect of the nuclear envelope around the nuclear pores with an absence of the nucleolus (Figures 6A & 6C). A dilated bile canaliculus with few microvilli was noticed. The Golgi apparatus with dilated stacks, few lysosomes, atrophic

electron-dense round mitochondria, a large-sized phagosome, and heterogenous vacuoles were seen at the juxta-biliary region of the hepatocyte (Figure 6B). Vesicular dilated rough endoplasmic reticulum cisternae and electron-dense mitochondria with variable-sizes and shapes were seen within the cytoplasm of the hepatocyte as well (Figure 6C). The blood sinusoid was lined by many large irregular outlined Kupffer cells and discontinued endothelial cells. The Kupffer cells had large corrugated heterochromatic nuclei with perinuclear electron-lucent zones. Collagen bundles of different sizes and orientations were noticed within the sinusoidal wall between the hepatocytes and the Kupffer cells (Figure 6D).

In misoprostol-treated rats, the hepatocytes had a large size, regular polygonal outline, and large central oval nucleus with a peripheral electron-dense nucleolus (Figure 7A). The biliary surface of the hepatocyte showed many long microvilli, narrow lumen, and 2 tight junctions at the ends of the bile canaliculus (Figure 7B). Hepatocytes with 2 variable-sized oval-shaped nuclei were noticed as well. Many round mitochondria, single and parallel rough endoplasmic reticulum cisternae, lysosomes, and numerous free ribosomes were observed in between and all around the nuclei within the cell cytoplasm (Figure 7C). The

sinusoidal surface of hepatocytes had numerous long microvilli extending within the space of Disse. The blood sinusoid was lined by flat endothelial cells and large Kupffer cells. The endothelial cells showed a large triangular nucleus while, the Kupffer cell had an irregular-outlined nucleus with a perinuclear electron-lucent zone. The sinusoidal lumen was engorged with many blood cells (Figure 7D).

In combined misoprostol and cisplatin treated rats, the hepatocytes attained large size, polygonal shape, regular outline, and regular oval nucleus with a peripheral electron-dense nucleolus. The hepatocyte cytoplasm contained numerous round and oblong-shaped mitochondria and single rough endoplasmic reticulum cistern in between (Figures 8A & 8B). The bile canaliculus showed narrow lumen and short microvilli (Figure 8B). Few hepatocytes with 2 equal-sized, oval-shaped euchromatic nuclei with peripheral electron-dense nucleoli were also seen (Figure 8C). The blood sinusoid was lined by flat endothelial cells with a spindle-shaped nucleus, and large Kupffer cells with a corrugated outlined nucleus (Figure 8D).

Discussion. Cisplatin is one of the most common antineoplastic drugs used for the treatment of a wide variety of cancers; however, its hepatotoxic effect limits

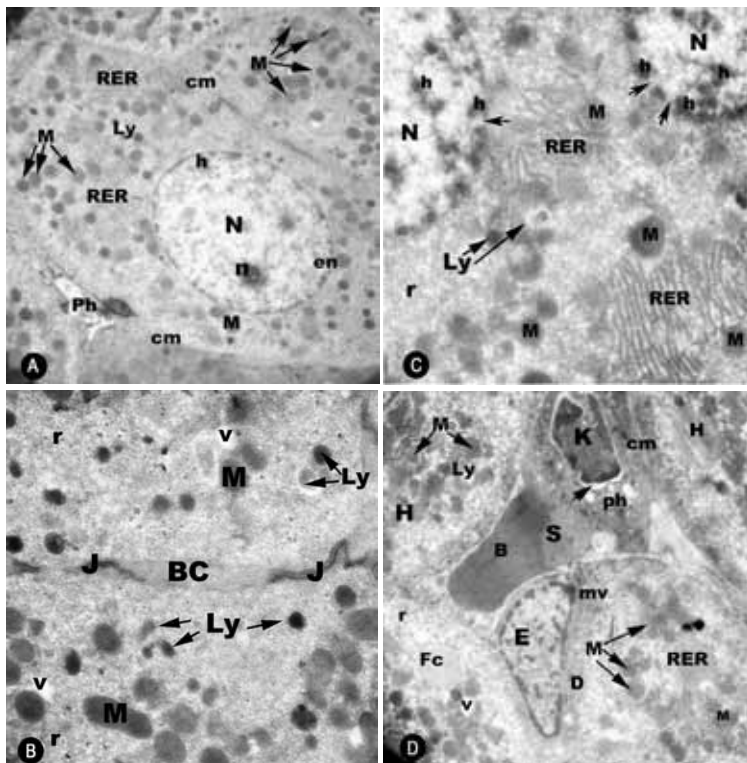


Figure 7 - Electron micrographs of the misoprostol-treated male rat liver showing A) the ultrastructure of the polygonal hepatocytes being similar to those of the normal control liver with the exception of a relatively high mitochondrial number (M). Their cytoplasm contains numerous oval and oblong mitochondria, rough endoplasmic reticulum cisternae (RER), and lysosomes (L). The cell has a central oval nucleus (N) with peripheral nucleolus (n) and masses of heterochromatin (h) within its nucleoplasm and on the inner aspect of the nuclear envelope (en); B) The cell membrane (Cm) has tight junctions (J) at the ends of the bile canaliculus (BC); C) The binucleated hepatocyte has 2 unequal oval heterochromatic nuclei (N) with electron-dense masses of heterochromatin (h) on the inner aspect of their envelopes (en) around the nuclear pores (arrow). Large groups of parallel rough endoplasmic reticulum cisternae (RER), numerous free ribosomes (r), few mitochondria (M) and many lysosomes (Ly) are seen between the 2 nuclei within the cell cytoplasm; D) The blood sinusoid (S) is lined by endothelial (E) and Kupffer (K) cells and contains many blood cells (B) within its lumen. The sinusoidal surface of the hepatocyte has numerous microvilli (mv) projecting into the Disse space under the endothelial cell. FC - fat cell.

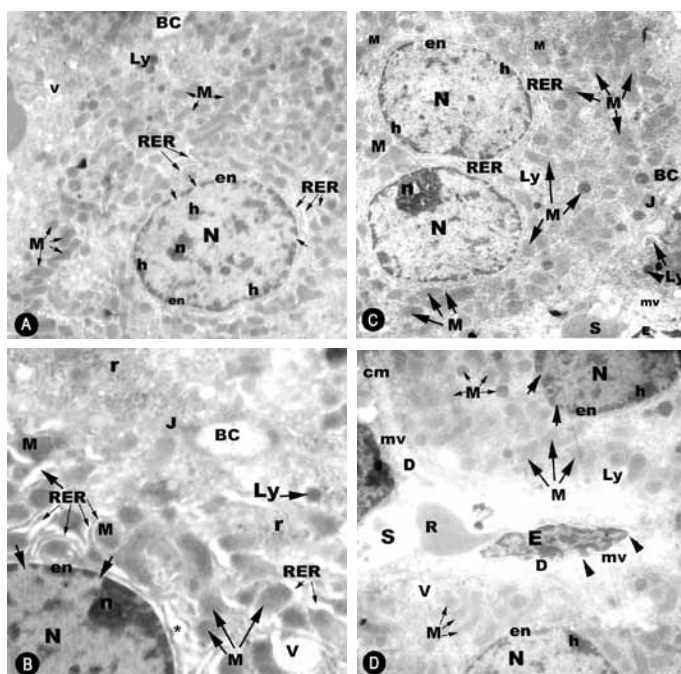


Figure 8 - Electron micrographs of the combined misoprostol and cisplatin-treated rats liver showing A & B) large polygonal hepatocyte having a central oval nucleus (N) with a well formed nuclear envelope (en), normally distributed heterochromatin masses (h) and peripheral nucleolus (n). The cytoplasm of the hepatocyte contains numerous round and oval-shaped mitochondria (M) with a single rough endoplasmic reticulum cistern (RER) in-between, numerous free ribosomes, few lysosomes (L) and electron-lucent vacuoles (V); B) The biliary surface (Cm) of the hepatocytes shows tight junctions (J) at the ends of the bile canaliculus (BC) with many microvilli (mv) projecting into the biliary lumen; C) The binucleated hepatocyte shows equal central oblong-shaped large heterochromatic nuclei (N) with regular nuclear envelopes (en) and large peripheral nucleoli (n); D) the blood sinusoid is lined by elongated endothelial cells (E) and many irregularly-outlined Kupffer cells (K). The endothelial cell has an electron-lucent perinuclear zone (arrow head) while, the Kupffer cell has a large irregularly outlined electron-dense nucleus.

clinical uses.³ Several attempts have been conducted to ameliorate the hepatotoxic effect of cisplatin by using different antioxidant agents.^{5,7,13} There is limited information on the possible protective effect of misoprostol on cisplatin-hepatotoxicity. Most earlier studies were designed to administer drugs either before or after the cisplatin injection. However, in the present study, it was hypothesized that the administration of misoprostol before and after cisplatin injection might ameliorate the toxic effect of cisplatin and/or accelerate the course of hepatic recovery.

In the present study, a significant reduction of rats' body weight was recorded on the fifth day after cisplatin-injection (single 7.5 mg/kg i.p.). Similarly, Chirino et al¹³ reported a significant decrease in the body weight of cisplatin-treated rats. The decrease of body weight of cisplatin-treated rats might be due to gastrointestinal toxicity and dysfunction,¹⁴ or because of the anorexic effect of the drug and increased metabolic rate, which were considered side effects of the chemotherapy.⁴ A remarkable gain in body weight was recorded in the group of rats treated with combined oral misoprostol and cisplatin in the present study. Arhoghro et al⁸ reported that post-treatment with the aqueous extract of *Cymbopogon citratus* after cisplatin injection induced significant amelioration of body weight reduction.⁸

In the present study, the hepatotoxic effect of cisplatin was characterized by a significant reduction of

liver weight and liver weight/body weight ratio. These results were in accordance with those of Lee et al¹⁵ who indicated that cisplatin treatment resulted in liver weight loss, and a significant reduction of its percentage to the total body weight in mice. The combination of misoprostol with cisplatin resulted in a non-significant resumption in the loss of liver weight or its ratio to the body weight. However, a higher significant elevation of liver weight and its weight/body weight ratio was noticed after 15 days post-treatment with the aqueous extract of *Cymbopogon citratus*.⁸ This difference might be related to the duration of drug intake after cisplatin injection, where the post-treatment period might improve the animal's appetite and decrease the high catabolic rate of cisplatin with subsequent acceleration of liver recovery.

A single dose of cisplatin (7.5 mg/kg) produced a significant elevation of serum TSB, ALT, AST, and reduction of serum albumin levels, while in the presence of misoprostol a significant improvement in the levels of liver biomarkers was noticed. A combination of misoprostol with cisplatin produced a significant decrease in liver enzymes when compared with the cisplatin-treated rats. The improvement of liver enzymes might be attributed to the stabilizing effect of misoprostol on the hepatocyte cell membrane, which prevents AST, ALT, and bilirubin leakage into the extracellular fluid.¹⁶ The reduction in the serum levels of

the liver biomarkers could be considered as an index to the regenerating activity of the damaged hepatocytes.¹⁰ Moreover, the deficiency of serum albumin might be due to the toxic effect of cisplatin on liver cells with subsequent impairment of protein synthesis and a decrease in the animal body weight. Similarly, the correlation between reduction of the animal body weight and cisplatin-hepatotoxicity was previously explained by Park et al,¹⁷ who reported that the reduction of the body weight in cisplatin intoxicated rats could be due to tissue damage and reduction of their function.

The mechanism of cisplatin-induced hepatotoxicity was previously explained as a result of the destructive effect of free radical oxidative stress with production of reactive oxygen species,¹⁸ lipid peroxidation, and depletion of glutathione,¹⁹ which has a role in the elimination of cisplatin through its effect on the non-enzymatic antioxidant system.²⁰ It has been reported that, the antioxidant effect of misoprostol did not limit lipid peroxidation *in vivo* and did not protect against the oxidant injury of tert-butylhydroperoxide *in vitro*.⁷ Conversely, the cytoprotective effect of misoprostol might be due to hemodynamic factors, increased regenerative capacity of epithelial cells, or an inflammatory response through a cytoprotective effect and reduction of the immune-mediated liver damage.^{6,9,20} Moreover, misoprostol was shown to block the apoptosis generated through the toxic D-galactosamine.²¹

The biochemical results have been confirmed by histological observations, where a single dose of cisplatin (7.5 mg/kg) induced hepatic damage manifested by pericentral disorganization, hepatic necrosis, and apoptotic changes. The combination of misoprostol with cisplatin ameliorated most of these findings. Similar findings were reported by El-Sayyad et al⁴ and Kart et al,¹⁸ who demonstrated the structural changes in the hepatic parenchyma in cisplatin-treated rats and its reversal by different antioxidants such as caffeic acid phenethyl ester or ellagic acid. This could be due to an increase in the flow of blood within the vasculature of the liver by the action of misoprostol. The histological architecture of the misoprostol-treated liver specimens revealed the ability of misoprostol to prevent hepatocellular necrosis or mononuclear infiltration with conservation of glycogen and protein contents in hepatocytes.¹⁰

This study showed an apparent increase in the amount of collagen fibers particularly around the blood vessels in the portal area and sinusoidal wall in cisplatin-treated rats. On the other hand, the presence of misoprostol showed a marked reduction in the amount of collagen

fibers within the portal tracts, and its absence in the peri-sinusoidal area. In accordance with our results, Ros et al²² found mild peri-sinusoidal fibrosis, and by electron microscope identified an increased amount of collagen fibers in the Disse space.

Our study showed marked reduction of the glycogen content within the hepatocytes in cisplatin-treated rats when compared with control and misoprostol-treated rats. The reduction of glycogen content may be due to defects in its synthesis as a consequence of the degeneration of hepatocytes and damage of mitochondria with a reduction in the amount of ATP.²³

The histopathological findings of cisplatin-treated rats were confirmed at the ultrastructural level, where size of the hepatocyte, dilated rough endoplasmic reticulum, degenerated mitochondria, disturbed nuclear envelope, reduced amount of condensed heterochromatin on the nuclear envelope around the wide nuclear pores with an absence of nucleolus, and reduction of glycogen content have been observed. In the presence of misoprostol, most of these ultrastructural changes were significantly improved. The antioxidant and cytoprotective effects of misoprostol were previously demonstrated in carbon tetrachloride-induced hepatotoxicity where, a significant decrease of the transaminases, restored histological architecture of the liver with attenuation of glycogen and DNA reduction was shown.¹⁰

Moreover, in the present study, co-administration of misoprostol with cisplatin showed a marked increase in the mitochondrial number within the hepatocyte cytoplasm. This finding reflected the decrease of the cytotoxic effects of cisplatin in conjunction with misoprostol where, the number of mitochondria was significantly increased with subsequent production of an excessive amount of glutathione that overcame the cytotoxicity of cisplatin.²⁴ Thus, an increase in number and function of mitochondria in combined misoprostol and cisplatin-treated rats might reflect the target chemotherapeutic and toxic effects of cisplatin on mitochondria; therefore, mitochondrial dysfunction could be a major mechanism of cisplatin-induced hepatotoxicity.

The question still stands, does misoprostol, which protects the liver against cisplatin-induced hepatotoxicity interfere with the anticancer activity of cisplatin. The present study did not investigate the possible effect of misoprostol on the antineoplastic action of cisplatin. This will be the topic of our next study.

In conclusion, the results of the present study revealed that, misoprostol ameliorated the biochemical, histological, and ultrastructural changes

of cisplatin-induced hepatotoxicity in rats. Misoprostol protection against cisplatin-induced hepatotoxicity may very probably be due to its antioxidant activity and cytoprotective properties or other unknown mechanisms. Further studies are needed to explore the exact mechanism underlying the cytoprotective effect of misoprostol.

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