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Possible ameliorative effect of human placental extract on methotrexate-induced nephrotoxicity in albino rats

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Abstract

Background: Methotrexate (MTX) is one of chemotherapeutic drugs that induce several side effects. The present study aimed to investigate the ameliorative effect of human placental extract (HPE) against MTX-induced nephrotoxicity in rats. In this study, forty adult male albino rats were equally divided into four groups. Control group: rats were daily injected intraperitoneally with physiological saline (0.5 ml for each rat) for 5 days, HPE group: rats were subcutaneously injected with HPE at a dose level of 10.08 mg/Kg b.w/day for 2 weeks, MTX group: rats were intraperitoneally injected with MTX at a dose level of 5 mg/Kg b.w/day for 5 consecutive days, MTX and HPE group: rats were intraperitoneally injected with MTX (at the same dosage of MTX group) for 5 days and at the same time they were subcutaneously injected with HPE (at an exact dosage of HPE group), daily for 2 weeks. Twenty-four hours after the last dose for each treatment, rats were killed and blood samples were collected for determination of urea, creatinine, sodium (Na^+) and potassium (K^+) levels. Kidney tissues were taken for histological examination and immunohistochemical staining of both cysteine-aspartic protease-3 (caspase-3) and proliferating antigen Ki-67 (Ki-67) expressions.

Results: From the obtained data, MTX induced nephrotoxicity through a highly significant increase in urea, creatinine, Na^+ and K^+ levels compared with the control group. In addition to massive histological alterations, a highly significant increase in caspase-3 expression and a significant decrease in Ki-67 expression were observed. On the other hand, injection with HPE ameliorated urea, creatinine, Na^+ and K^+ levels comparing to MTX group. Moreover, HPE markedly improved the histological and immunohistochemical changes resulted from MTX treatment.

Conclusions: It is concluded that HPE ameliorated the nephrotoxicity induced by MTX.

Keywords: Methotrexate, Human placental extract, Nephrotoxicity, Oxidative stress, Caspase-3, Ki-67, Rats

Background

Chemotherapy is one of the most effective and important methods for cancer treatment. It is probably the best way to monitor cancers that do not respond to either surgery or radiation for several years. Large numbers of chemotherapeutic drugs are effective in treating human cancers. The most widely used chemotherapeutic drugs include methotrexate (MTX), cyclophosphamide and 5-fluorouracil (Sarder et al., 2015).

Methotrexate, a structural analogue of folic acid, is one of the most effective and extensively used drugs for treating many kinds of cancers and severe or resistant forms of autoimmune diseases (Koźmiński et al., 2020). Prolonged use of MTX causes many side effects on different organs including kidney, liver, lung, testis, bone marrow and brain (Asci et al., 2017).

Nephrotoxicity is a common adverse effect caused by MTX and can lead to acute or chronic kidney disease and increase health care spending. It has a high incidence of morbidity and mortality in 40–70% of kidney injuries and

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accounts for 66% of cases of kidney failure in the elderly population (Raza & Naureen, 2020).

Methotrexate-induced renal injury in rats was evidenced by significant increase in circulating kidney function markers and tumor necrosis factor alpha as well as significant increase in lipid peroxidation and nitric oxide levels with concomitant marked decline in the anti-oxidant defenses. It induced nephrotoxicity with possible mechanisms of attenuating oxidative stress and inflammation (Abd El-Twab et al., 2016).

Fouad and Al-Melhim (2018) and Aldossary (2019) documented that MTX induced kidney injury in rats by inducing oxidative/nitrosative stress, inflammation and apoptosis. Besides, Hassanein et al. (2019) reported that kidney of MTX-treated rats revealed severe vacuolization of the epithelial cells lining the renal tubules, severe congestion of intertubular blood capillaries, moderate congestion with hypercellularity of glomerular tuft associated with moderate focal renal hemorrhage, mild inflammatory cells infiltration and atrophy of glomerular tuft.

Kitamura et al. (2018) reported three cases of acute kidney injury resulted from MTX toxicity in patients with hematological malignancies. An abrupt increase in serum creatinine is a distinctive feature of high dose of MTX-induced acute kidney injury. The authors suggested that the major etiology of acute kidney injury was high dose of MTX administration in these three cases.

The placenta is a metabolically active interfacial organ that plays crucial roles in fetal nutrient delivery, gas exchange and waste removal reflecting dynamic maternal and fetal interactions during gestation (Saoi et al., 2020).

Human placenta has been used in clinical practice for over a century, and there is substantial experience in clinical applications of its extract for different indications. Aqueous extract of human placenta contains growth factors, cytokines, natural metabolic, anti-oxidants, amino acids, vitamins, trace elements and biomolecules. These contents, individually or in combination, show accelerated cellular metabolism, immunomodulatory and anti-inflammatory effects, cellular proliferation and stimulation of tissue regeneration processes (Joshi et al., 2020).

Human placental extract (HPE) has shown great promise as a therapeutic agent. Various studies investigated the impact of HPE on inflammation in different mammalian organs. It was documented that pre-treatment with HPE significantly decreased pro-inflammatory cytokines and oxidative stress induced by benzo[alpha] pyrene in rats (Park et al., 2010). Furthermore, treatment with HPE ameliorated the hepatic injury, fibrosis and oxidative stress in a mouse model of non-alcoholic steatohepatitis (Yamauchi et al., 2017). Moreover, HPE has an effective suppressive role on neuroinflammation induced by benzo[alpha]pyrene in the hippocampus of male rat pups through reduction of inflammatory markers (Parida et al., 2019). Human placental extract can promote cellular proliferation and tissue regeneration because it contains supraphysiological rates of anabolic factors (Gwam et al., 2019).

Serum urea, creatinine and electrolytes are renal biochemical markers that are perturbed with the advent of nephrotoxicity (Adikwu & Bokolo, 2018). The proliferating antigen Ki-67 (Ki-67) is a nuclear protein associated with cellular proliferation (Bullwinkel et al., 2006). In contrast, cysteine-aspartic protease-3 (caspase-3) is an important marker controls apoptosis which may be associated with many diseases (Wang et al., 2021).

Methods

Drugs

Methotrexate

Methotrexate was obtained in the form of vials manufactured by the Hikma Specialized Pharmaceuticals Company, Egypt. Each vial (2 ml) contains 50 mg MTX.

Human placental extract

Human placental extract is commercially marketed as Laennec. It was obtained in the form of ampoules, from Japan Bio Products Co. Ltd., Korea. Each ampoule (2 ml) contains 112 mg a water-soluble HPE.

Chemicals

All assay kits and chemicals used in the present study were of analytical grade and high purity. They were obtained from Chemical Kits Companies in Egypt.

Animal selection and care

Forty healthy adult (3 months old) male albino rats (*Rattus norvegicus*), weighing about 120 g, were used in the present study. They were obtained from Animal House Colony of the National Research Centre, Dokki, Giza, Egypt. Rats were housed in clean plastic cages and fed a standard commercial pellets diet, and water was provided ad libitum. They were acclimatized at a temperature of 24 ± 2 °C, a relative humidity of 60–70% and light set at a 12-h light–dark cycle, for 2 weeks before starting the experiment.

Experimental design

Rats were randomly divided into four experimental groups, each 10 rats, as follows:

Control group

Rats were daily injected intraperitoneally with physiological saline (0.9% NaCl), 0.5 ml for each rat, for 5 days.

Human placental extract group:
Rats were daily subcutaneously injected with HPE at a dose level of 10.08 mg/Kg b.w (equivalent to the therapeutic dose for human, Paget & Barnes, 1964) for 2 weeks.

Methotrexate group
Rats were intraperitoneally injected with MTX at a dose level of 5 mg/Kg b.w/day for 5 consecutive days (Li et al., 2016).

Methotrexate and HPE group
Rats were daily intraperitoneally injected with MTX (at the same dose of MTX group) for 5 days, and at the same time they were subcutaneously injected with HPE (at an exact dose of HPE group), daily for 2 weeks.

During the experimental period, rats of all groups were daily checked for clinical symptoms.

Samples collection
Twenty-four hours after the last dose, animals of all groups were killed. Blood samples were collected from heart in plain tubes and left to clot and then centrifuged for 10 min at 3000 rpm to obtain clear sera. Sera were stored at −20 °C for determination of urea, creatinine, sodium (Na^+) and potassium (K^+) levels.

Kidneys of rats of all the experimental groups were fixed in 10% neutral formalin for both the histological and immunohistochemical examinations.

Biochemical analysis
Kidney function tests
Urea and creatinine levels were determined by using the kits of Biodiagnostic Company (catalog numbers UR 21 10 and CR 12 51, respectively) according to Fawcett and Soctt (1960) and Bartles et al. (1972), respectively.

Sodium and K^+ levels were measured by using colorimetric method by the kits of Biomed Diagnostics Company (catalog numbers SOD100100 and POT100100, respectively) according to Henry et al. (1974).

Histological studies
The formalin-fixed kidney tissues of all the experimental animals were dehydrated, cleared and embedded in molten paraffin wax (melting point of 58 °C). Sections of 4 µm thickness were cut by a rotary microtome (Leitz 1512, Leitz, Wetzlar, Germany). The prepared sections were then stained with hematoxylin and eosin (Bancroft & Cooks, 1994), examined and photographed by a light microscope with digital camera (Olympus, Tokyo, Japan).

Immunohistochemical studies
Caspase-3 and Ki-67 expressions
Paraffin kidney sections of 4 µm thickness were used for immunohistochemical staining of both caspase-3 and Ki-67 expressions according to Hsu et al. (1981). The endogenous peroxidase activity was blocked by incubation using 3% H_2O_2 for 5 min. Sections were incubated at 37 °C with caspase-3 and Ki-67 monoclonal antibodies and then washed with phosphate buffer saline (PBS) for about 5 min. The monoclonal antibodies were then bound with biotinylated goat anti-mouse IgG antibody for 30 min. Sections were washed with PBS for 5 min, and then they were incubated at 37 °C with streptavidin-conjugated peroxidase about 30 min. A brown-colored reaction was developed by exposing sections to 3,3-diaminobenzidine tetrahydrochloride liquid about 5 min. The sections were washed with distilled water and stained with hematoxylin. The intensity of both caspase-3 and Ki-67 expression was quantitatively evaluated by using ImageJ software (Varghese et al., 2014).

Statistical analysis
All the present data are presented as mean ± SE ($n = 5$) and were analyzed using the one-way ANOVA. Statistical Program of Social Science (SPSS) software was used. Statistical significance between the experimental groups was assessed by least significant difference post hoc test, with set at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

Results
Clinical observations
Examination of rats during the experimental period showed that both the control and HPE-treated rats were healthy. They did not show any signs of illness and appeared to have normal activity with normal adequate food and water intake. Methotrexate-treated rats revealed general weakness, loss of appetite, disability for walking and severe diarrhea. On the other hand, MTX and HPE-treated rats appeared nearly normal.

Biochemical results
Serum kidney function tests: urea and creatinine
Data in Fig. 1(A & B) represented urea and creatinine levels in sera of animals of the different experimental groups.

There was an insignificant difference in the levels of both urea and creatinine between the control group and HPE-treated group. Methotrexate-treated rats showed a

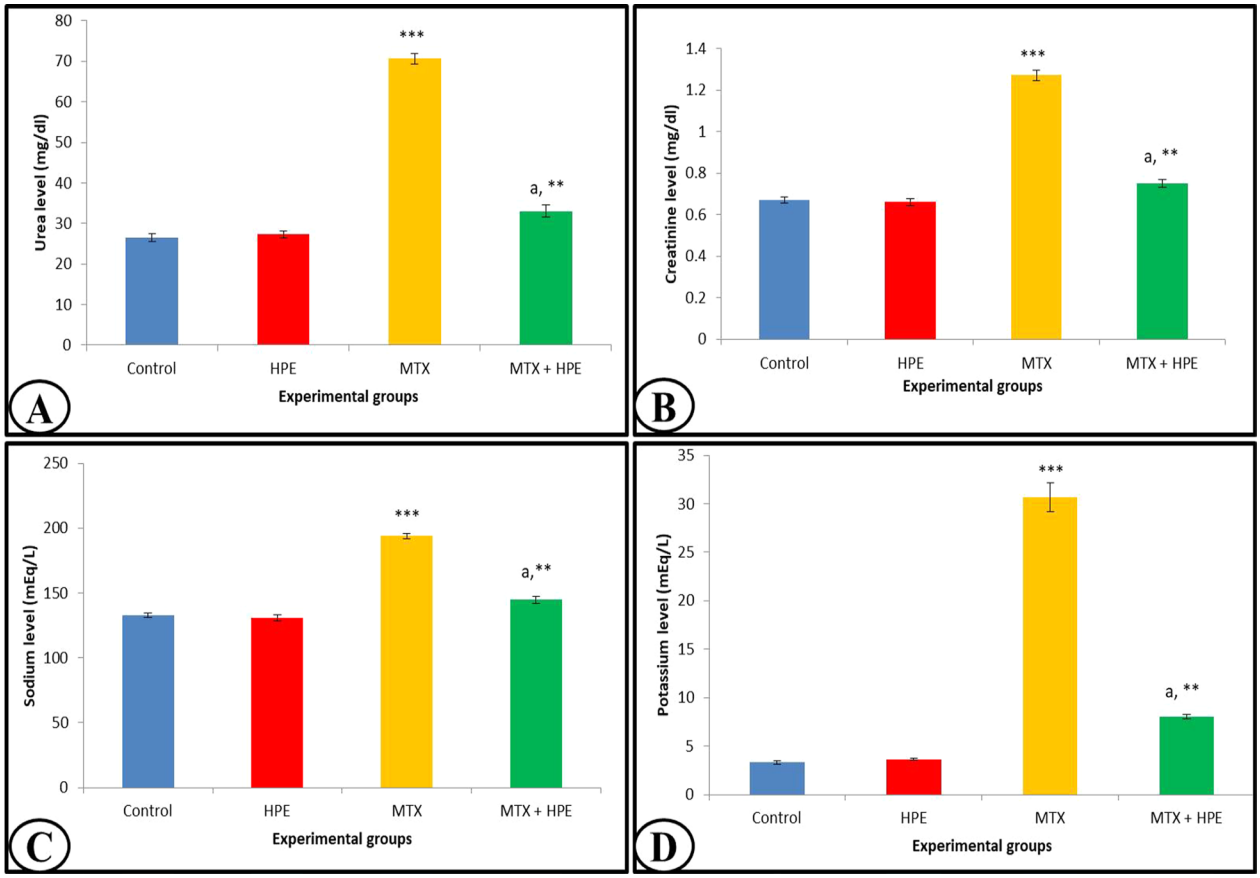

Fig. 1 The levels of (**A**) urea, (**B**) creatinine, (**C**) sodium and (**D**) potassium in sera of animals of the different experimental groups. (***): highly significant at $p < 0.001$ compared with the control group. (**): significant at $p < 0.01$ compared with the control group. (a): highly significant at $p < 0.001$ compared with MTX group

highly significant increase ($p < 0.001$) in the levels of both urea and creatinine compared with the control group.

When rats were treated with MTX and HPE, the levels of both urea and creatinine showed a highly significant decrease ($p < 0.001$) when compared with MTX group, although a significant increase ($p < 0.01$) was still being recorded compared with the control group.

Serum electrolytes: sodium and potassium

Sodium and potassium levels are represented in Fig. 1(C & D). Human placental extract-treated rats showed insignificant difference in both Na^+ and K^+ levels as compared with the control group. Treating rats with MTX resulted in a highly significant increase ($p < 0.001$) in both Na^+ and K^+ levels when compared with the control animals.

Animals treated with MTX and HPE showed a highly significant decrease ($p < 0.001$) in both Na^+ and K^+ levels, compared with MTX-treated rats. However, a significant increase ($p < 0.01$) was still being recorded compared with the control group.

Histological observations

Control group

Light microscopic examinations of hematoxylin- and eosin-stained kidney sections of control rats revealed normal structure of both the outer cortex and inner medulla. The kidney is covered by a smooth fibrous capsule. The cortical parts of the nephron are Malpighian or renal corpuscle, proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) (Fig. 2A & B). Malpighian corpuscle is composed of a capillary tuft (glomerulus) and parietal epithelium (simple squamous epithelium) forming Bowman's capsule. A narrow space presents between the glomerulus and Bowman's capsule; Bowman's space or urinary space (Fig. 2B). The morphometric measurements of the glomerular diameters and Bowman's space widths are represented in Fig. 3(A & B).

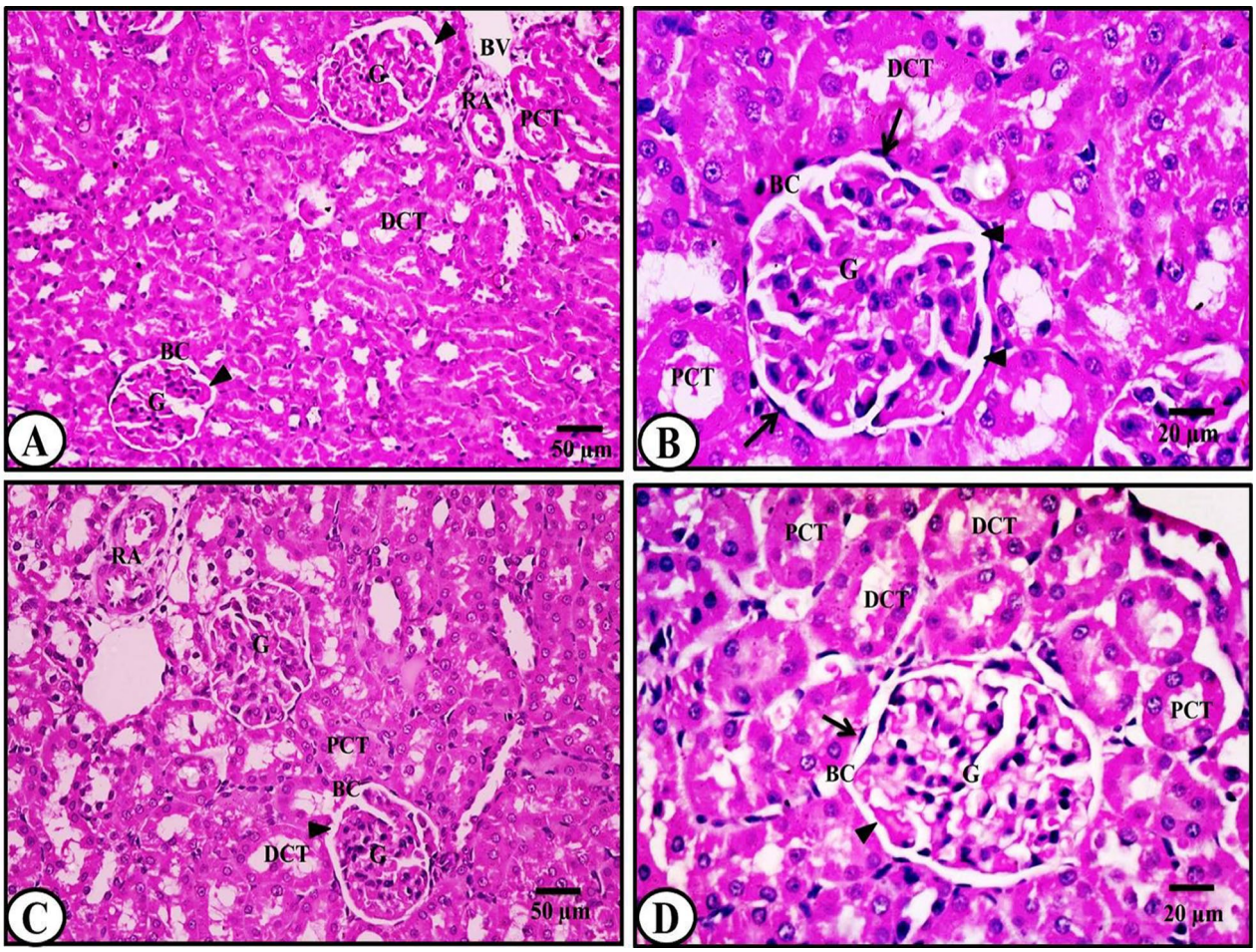

Fig. 2 Photomicrographs obtained from hematoxylin- and eosin-stained kidney sections of (**A**&**B**) control rats and (**C**& **D**) HPE-treated rats showing normal Malpighian corpuscles with Bowman's capsules (BC) composed of squamous epithelium (arrows), Bowman's spaces (arrowheads) and glomeruli (G), proximal (PCT) and distal (DCT) convoluted tubules lined with cuboidal epithelium, blood vessel (BV) and renal artery (RA)

Proximal convoluted tubules occupy most of the cortex. They are lined with a single layer of large cuboidal epithelial cells with apical brush borders of microvilli and narrow lumens. The epithelial cells have eosinophilic cytoplasm and central spherical nuclei (Fig. 2B).

Distal convoluted tubules differ from PCT in that they have wider lumen and their lining cells are smaller and flatter with less eosinophilic cytoplasm, spherical apical nuclei and did not have brush borders (Fig. 2B). The morphometric measurements of both PCT and DCT dimensions (length and width) are represented in Fig. 3(C & D).

Human placental extract-treated group

When kidney sections of HPE-treated rats were examined; they showed a nearly normal histological structure as in the control rats (Fig. 2C & D).

The morphometric measurements showed an insignificant difference in the glomerular diameters, Bowman's space widths (Fig. 3A & B) and PCT and DCT dimensions (Fig. 3C & D), when compared to the control rats.

Methotrexate-treated group

Examination of kidney sections of MTX-treated rats showed obvious alterations and deteriorations in the renal cortex. The renal capsule appeared separated, in some places, from the renal cortex (Fig. 4A).

Regarding the renal corpuscles, shrinkage of glomerular capillary network and dilation of Bowman's spaces were observed (Fig. 4B). A highly significant decrease in the glomerular diameters ($p < 0.001$) and a highly significant increase ($p < 0.001$) in Bowman's space widths were recorded when compared to the control group (Fig. 3A & B).

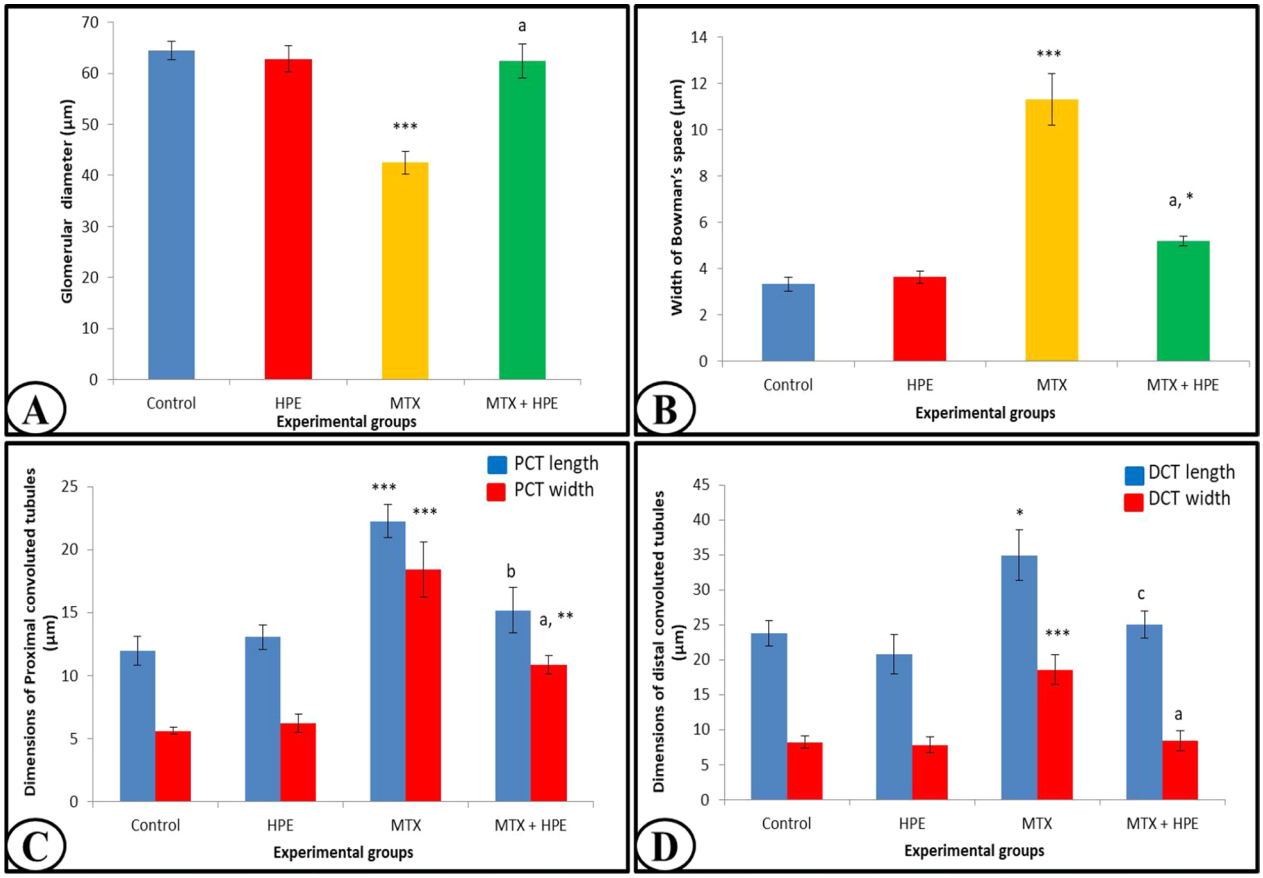

Fig. 3 Morphometric measurements of (**A**) glomerular diameters, (**B**) width of Bowman's spaces, (**C**) dimensions of proximal convoluted tubules and (**D**) dimensions of distal convoluted tubules of animals of the different experimental groups. (***): highly significant at $p < 0.001$ compared with the control group.(**): significant at $p < 0.01$ compared with the control group. (*): significant at $p < 0.05$ compared with the control group. (a): highly significant at $p < 0.001$ compared with MTX group. (b): significant at $p < 0.01$ compared with MTX group. (c): significant at $p < 0.05$ compared with MTX group

The majority of renal tubules (PCT and DCT) were highly injured with dilated lumens and lining epithelial cells contained vacuolated cytoplasm and pyknotic nuclei (Fig. 4C). Furthermore, morphometric measurements of the length and width (dimensions) of both PCT and DCT showed a highly significant increase at $p < 0.001$ for PCT and significant increase at $p < 0.05$ and highly significant increase at $p < 0.001$, respectively, for DCT as compared to the control rats (Fig. 3C & D).

In addition, hemorrhage, congestion of renal arteries and dilation of blood vessels were observed (Fig. 4B). Moreover, large areas of fibers, as a sign of fibrosis and leukocytic infiltrations, were also prominent in these specimens (Fig. 4D).

Methotrexate and HPE-treated group

Histological examination of kidney sections of rats treated with MTX and HPE showed a marked degree of improvement. The renal capsules appeared nearly normal. Most of Malpighian corpuscles appeared with their normal structure; Bowman's capsules, normal glomeruli and normal Bowman's spaces (Fig. 4E & F). Morphometrically, a highly significant increase ($p < 0.001$) in the glomerular diameters and a highly significant decrease ($p < 0.001$) in the Bowman's space widths were recorded, as compared to MTX-treated rats. Insignificant difference in the glomerular diameters was recorded, but significant increase ($p < 0.05$) in Bowman's space widths was still being recorded as compared to the control group (Fig. 3A & B).

Moreover, the lining cells of both PCT and DCT restored their normal structure and the tubular lumens appeared nearly normal (Fig. 4F). Furthermore, the morphometric measurements of PCT and DCT recorded a significant decrease at $p < 0.01$ and $p < 0.05$ in their length, respectively and highly significant decrease at $p < 0.001$ in their width, as compared to

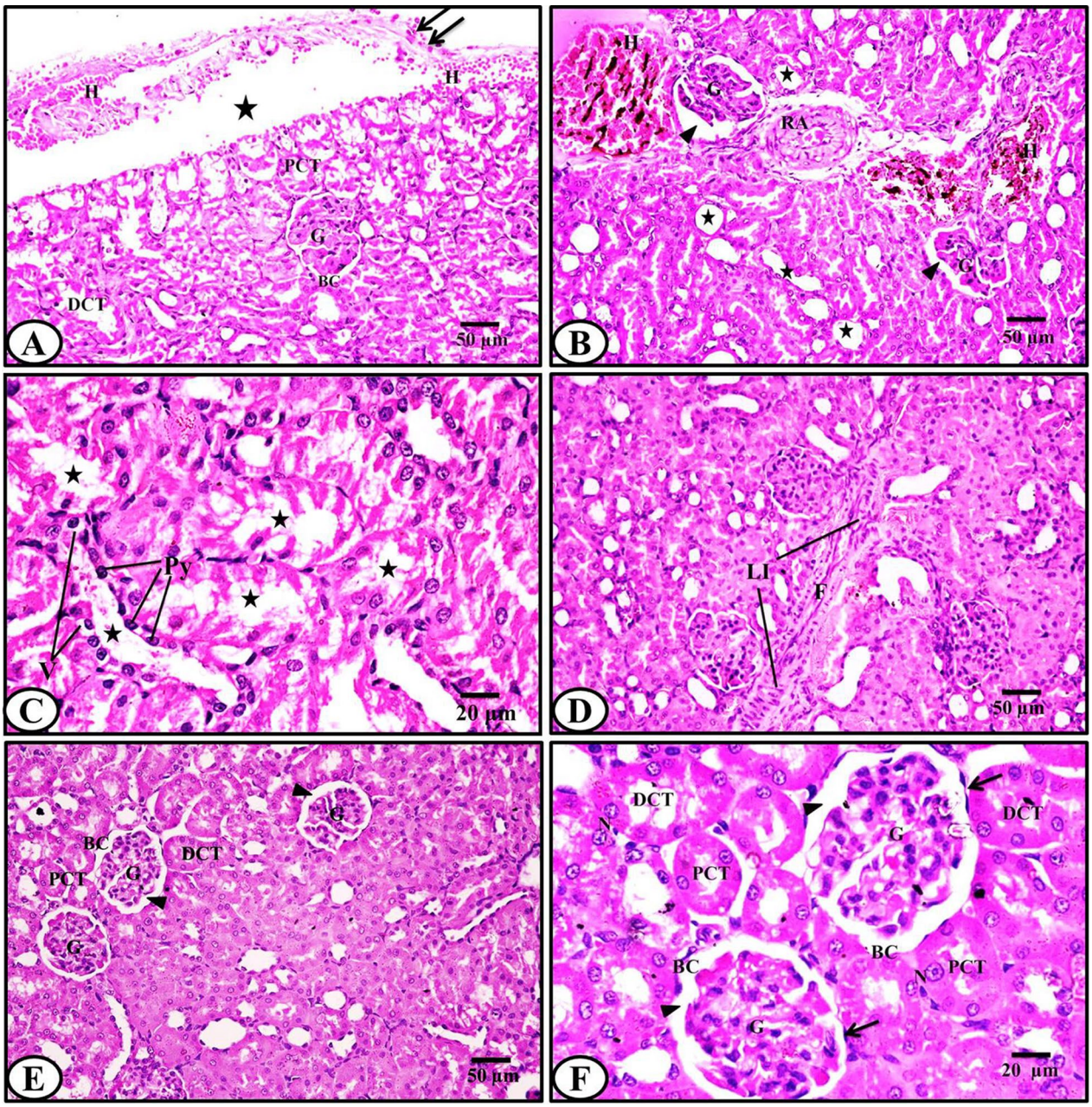

Fig. 4 Photomicrographs from hematoxylin- and eosin-stained kidney sections of (**A**–**D**) MTX-treated rats showing separation of the capsule (double arrow) from the renal cortex by a wide space (star) containing hemorrhage (H), Malpighian corpuscles with damaged glomeruli (G), Bowman's capsule (BC) and dilated Bowman's spaces (arrowheads), proximal (PCT) and distal (DCT) convoluted tubules with dilated lumens (stars) and lining epithelial cells with vacuolated cytoplasm (V) and pyknotic nuclei (Py), congested renal artery (RA), leukocytic infiltration (LI) and fibers (F) and **E&F**) MTX and HPE-treated rats showing nearly normal Malpighian corpuscles with Bowman's capsule (BC) with squamous epithelium (arrows), Bowman's spaces (arrowheads) and glomeruli (G) and proximal (PCT) and distal (DCT) convoluted tubules (DCT)

MTX-treated animals. On the contrary, the data were still recording a significant increase only in PCT width ($p < 0.01$) as compared to the control rats (Fig. 3C & D).

Immunohistochemical observations

Expression of caspase-3

In kidney sections of both control rats and those treated with HPE, caspase-3 was expressed as brown color in few nuclei of the tubular epithelial cells (Fig. 5A & B).

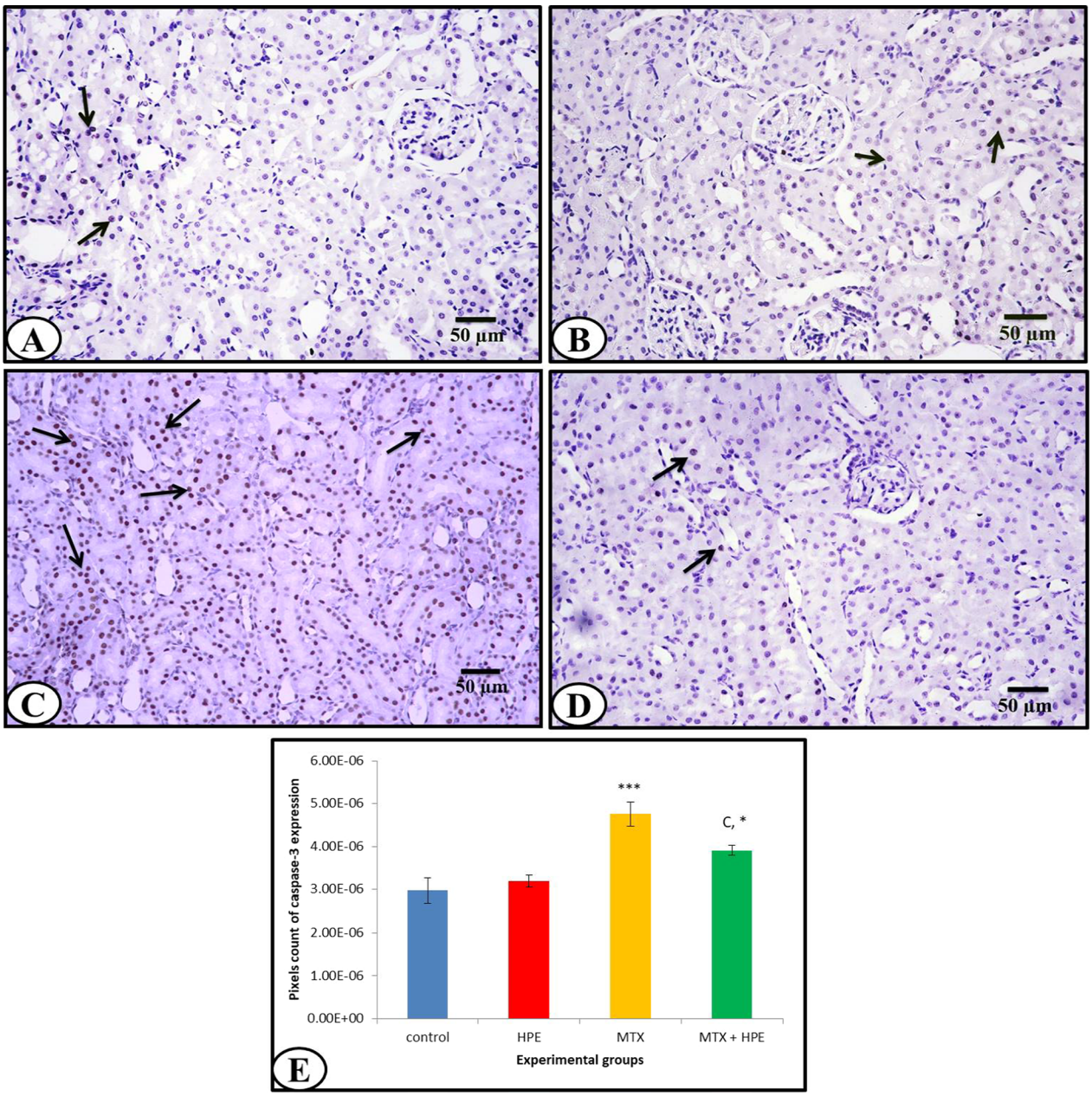

Fig. 5 (**A**–**D**) photomicrographs from kidney sections showing caspase-3 expression in (**A**&**B**) control and HPE-treated rats, respectively showing caspase-3 expression as brown color in few nuclei (arrows), (**C**) MTX-treated rat showing caspase-3 expression in large number of nuclei (arrows) and (**D**) MTX and HPE-treated rat showing caspase-3 expression in few nuclei (arrows), (caspase-3 immunostain, counterstained with hematoxylin). (**E**) Caspase-3 expression in the different experimental groups. (***): highly significant at $p < 0.001$ compared with the control group. (*): significant at $p < 0.05$ compared with the control group. (c): significant at $p < 0.05$ compared with MTX group

By using ImageJ software, caspase-3 expression in HPE-treated rats showed insignificant difference when compared with the control rats (Fig. 5E).

When kidney sections of MTX-treated rats were examined, caspase-3 was expressed in large number of nuclei (Fig. 5C). Highly significant increase ($p < 0.001$) in caspase-3 expression was recorded as compared with the control group (Fig. 5E).

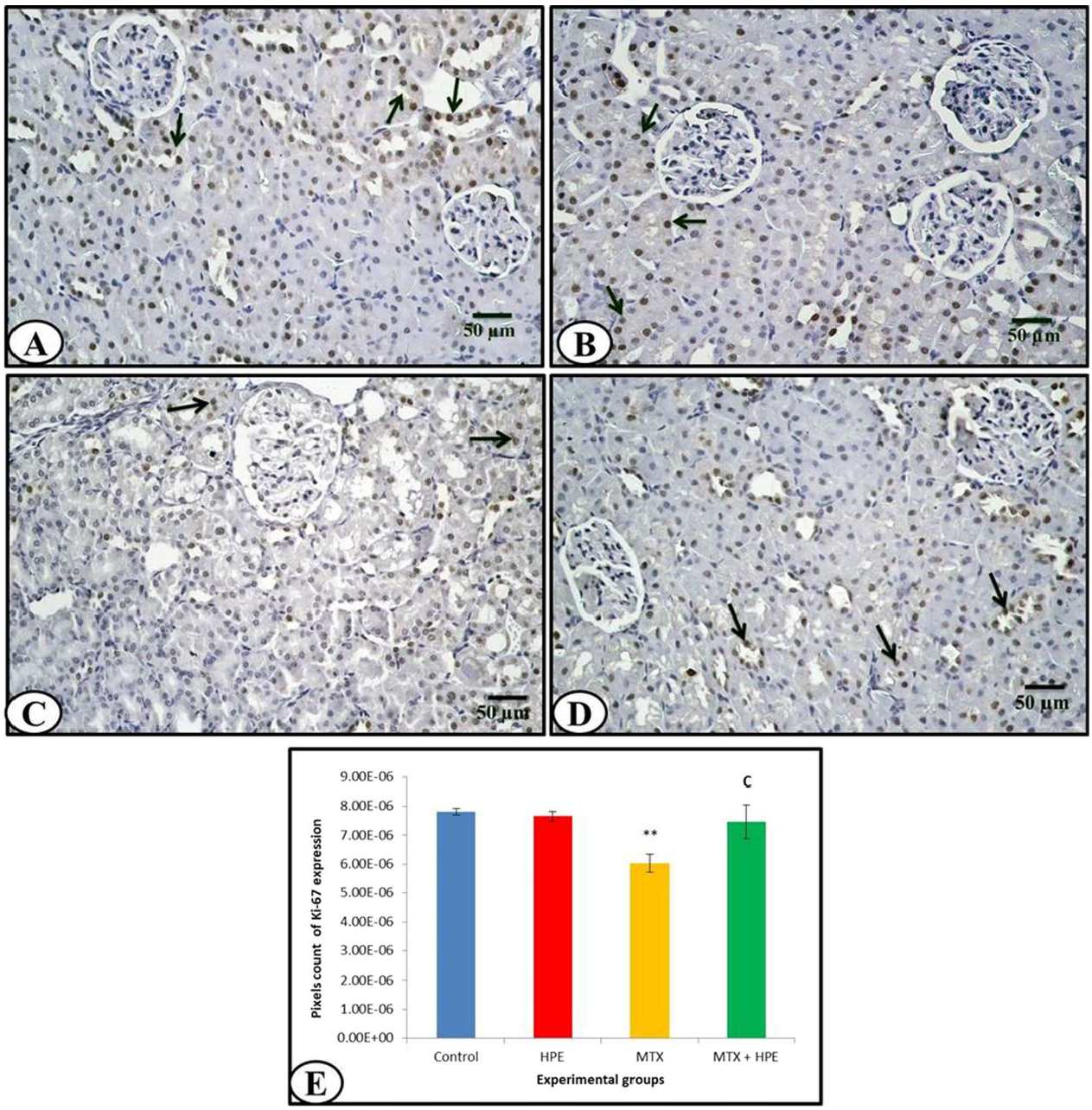

Fig. 6 (**A**–**D**) photomicrographs from kidney sections showing Ki-67 expression in (**A**&**B**) control and HPE-treated rats, respectively, showing Ki-67 expression as brown color in large number of nuclei (arrows), (**C**) MTX-treated rat showing Ki-67 expression in few nuclei and (**D**) MTX and HPE-treated rat showing Ki-67 expression in large number of nuclei (arrows), (Ki-67 immunostain, counterstained with hematoxylin). (**E**) Ki-67 expression in the different experimental groups. (**): significant at $p < 0.01$ compared with the control group. (c): significant at $p < 0.05$ compared with MTX group

Examination of kidney sections of animals treated with both MTX and HPE showed caspase-3 expression in few nuclei (Fig. 5D) with a significant decrease at $p < 0.05$ when compared with MTX group, but it was still recording a significant increase at $p < 0.05$ when compared with the control group (Fig. 5D & E).

Expression of Ki-67

In kidney sections of both the control and HPE-treated rats, Ki-67 was expressed as brown color in the nuclei of large number of the tubular epithelial cells (Fig. 6A & B).

Kidney sections of MTX-treated rats showed positive expression of Ki-67 in few nuclei (Fig. 6C). The decreased

expression was significant at $p < 0.01$ when compared with the control group (Fig. 6E).

On the other hand, kidney sections of animals treated with MTX and HPE showed Ki-67 expression in large number of the nuclei (Fig. 6D). This increase was significant at $p < 0.05$ when compared with MTX group and it showed insignificant difference when compared with the control group (Fig. 6E).

Discussion

In our study, MTX-induced nephrotoxicity through biochemical, histological and immunohistochemical alterations.

In the current study, there was a highly significant increase in both urea and creatinine levels in MTX-treated animals as compared with the control rats. This increase may be due to MTX or its metabolites toxicity leading to tubular injury. The toxicological mechanism of most chemotherapeutic drugs is production of reactive oxygen species (ROS) which plays a significant role in the MTX-induced nephrotoxicity (Jahovic et al., 2003). Recently, Elmansy et al. (2021) attributed the increased urea and creatinine levels, in MTX-treated rats, to the excessive production of ROS in the renal tissues, while Elsawy et al. (2021) attributed their increase to MTX precipitation and its direct toxic effects.

Serum urea and creatinine are recognized as biomarkers for determining renal functions and chronic kidney diseases. The progression of kidney damage is characterized by an increase in urea and creatinine levels whose evaluation in serum helps to assess glomerular filtration (Nisha et al., 2017).

Our results were supported by Rizk et al. (2018) who recorded a significant increase in both urea and creatinine levels after injection with MTX single dose (20 mg/Kg b.w) in rats.

In the present study, a highly significant increase in both Na^+ and K^+ levels was recorded in sera of rats treated with MTX as compared with the control group which may be due to kidney cellular damage induced by MTX treatment. Dhondup and Qian (2017) reported that Na^+ and K^+ disorders predictably occur with progressive loss of kidney function. Dehydration which produced in cases of diarrhea or vomiting increased Na^+ level (Yousafzai et al., 2011). Potassium disorders are common in patients with kidney diseases, particularly with tubular disorders and low glomerular filtration rate (Clase et al., 2020). The significant increase in both Na^+ and K^+ levels in MTX-intoxicated rats refers to impairment in the functional capacity of the kidney (Adikwu & Bokolo, 2018).

In the present study, light microscopic examination of renal cortex of MTX-treated rats revealed atrophied glomeruli, dilated Bowman's spaces and degenerated tubules with dilated lumens and lining epithelial cells with vacuolated cytoplasm and pyknotic nuclei. In addition, the morphometric measurements recorded highly significant decrease in the glomerular diameters, highly significant increase in Bowman's space widths, PCT dimensions (length and width) and DCT width as well as significant increase in DCT length. Inflammation, fibrosis, congestion and dilation of blood vessels and hemorrhage were also observed. These histopathological observations were confirmed with the biochemical results (increased urea and creatinine levels) which were obtained in the present study.

The decreased glomerular diameters and increased Bowman's space widths and tubular dimensions may be attributed to reduction in glomerular filtration rate after MTX treatment which may lead to kidney lesions. Balowria et al. (2019) reported that MTX precipitates in the renal tubules and directly induces tubular injury and a transient decline in glomerular filtration rate. Nephrotoxic drugs lead to damage of the renal tubules through stimulation of oxidative stress (Geetha et al., 2015). In harmony with our results, Jalili et al. (2020) recorded a significant decrease in the glomerular diameters and an increment in the tubular diameters in MTX-treated rats.

The observed inflammatory cells infiltration in the renal tissue of MTX-treated rats may be due to the tubular injury which was observed in the present study. The proximal tubular injury can trigger inflammation, fibrosis and distal tubular injury (Takaori et al., 2016). In agreement with our results, Çetin et al. (2017) reported that MTX increased the myeloperoxidase activity, a heme peroxidase enzyme found in neutrophil primary granules and monocyte lysosomes that lead to tissue damage in acute and chronic inflammation, pointing to an accumulation of inflammatory cells in the kidney tissue of rats.

The observed cytoplasmic vacuolization, in our study, may be related to the increased sodium level that also was recorded in the present study. Accumulation of sodium in the cells leads to an increase in water content (Strayer & Rubin, 2008).

In the present study, bundles of fibers were noticed in the kidney of MTX-treated rats. In response to different types of tissue injury, formation and accumulation of the extracellular matrix fibrosis are essential for the normal healing, closely associated with inflammation and tissue regeneration that may occur during and after the inflammatory response (Djudjaj & Boor, 2019). The pathological deposition of collagen is a hallmark of kidney fibrosis, mainly interstitial and perivascular fibrosis (Baues et al., 2020). The excessive deposition of fibers leads to organ dysfunction and can be associated with high morbidity and mortality. During this process,

abundance of cell types (such as epithelial, endothelial and inflammatory cells) intervenes at different levels and recruit mesenchymal cells (such as fibroblasts and myofibroblasts) leading to fibrosis (Panizo et al., 2021). Toxin-induced nephropathy triggers the rapid production of pro-inflammatory mediators and the prolonged inflammation allows the injured kidneys to develop interstitial fibrosis (Li et al., 2021).

In agreement with our observations, Şener et al. (2006) observed a significant increase in collagen content in kidney of MTX-treated rats and considered it as a free-radical-induced.

In the present study, a highly significant increase in caspase-3 expression in the nuclei and a significant decrease in Ki-67 expression in the nuclei were observed in MTX-treated rats as compared to the control animals. These results may be due to inhibition of DNA synthesis induced by MTX leading to apoptosis and decreased cellular proliferation. Methotrexate inhibits proliferation of malignant cells primarily by inhibiting the de novo synthesis of purines and pyrimidines (Saka & Aouacheri, 2017).

Methotrexate induces apoptosis through oxidative stress by increasing caspase-3 levels (Elango et al., 2014). Reactive oxygen species released during MTX administration increase pro-inflammatory cytokines leading to activation of caspase which mediates inflammatory response and apoptosis (Refaie et al., 2017). During apoptosis progression, active caspase-3 is translocated in association with a substrate-like protein(s) from the cytoplasm into the nucleus (Kamada et al., 2005). Faleiro and Lazebnik (2000) found that caspase-9 (which is activated earlier than caspase-3) directly or indirectly inactivates nuclear transport and increases the diffusion limit of the nuclear pores which consequently allowing caspase-3 to enter or leave the nucleus.

Methotrexate and its active metabolites compete with folic acid for binding with the folate binding site of dihydrofolate reductase. This enzyme is responsible for reduction of folic acid to tetrahydrofolic acid which is essential for DNA synthesis and cellular replication, so competitive inhibition of this enzyme leads to blockage of tetrahydrofolate synthesis, depletion of nucleotide precursors and inhibition of DNA, RNA and protein synthesis (Widemann & Adamson, 2006).

Similarly, Refaie et al. (2017) and Elsawy et al. (2021) observed a significant increase in caspase-3 expression and Çaglar et al. (2013) reported reduction of Ki-67 expression in kidney of rats treated with MTX.

In the current study, treatment of MTX-treated animals with HPE led to a highly significant decrease in urea, creatinine, Na^+ and K^+ levels as compared to MTX-treated group. This improvement is considered as an indicator for improving the kidney tissue and may be due to the anti-oxidant effect of HPE. Placental extracts have been used to treat various chronic diseases due to their anti-oxidant effects (Xu et al., 2018). Moreover, it was reported that HPE reduced elevated liver enzymes which are considered as markers for hepatic injury (Bak et al., 2018).

In the current study, HPE improved the histopathological changes induced by MTX in kidney of rats which may be due to anti-oxidant, anti-inflammatory and cellular regeneration ability of HPE. Mitchell et al. (2016) reported that the administered placental extracts are readily absorbed by binding to specific receptors present on the surface of targeted cells followed by stimulating inactive or damaged cells, tissues and organs in the body, thus providing tissue repair and regeneration. Human placental extract contains growth factors, hormones, proteins, glycosaminoglycans, nucleic acids, polydeoxyribonucleotides, antibodies and other nutrients which may explain its capacity to stimulate tissue regeneration and cell proliferation (Samiei et al., 2016). Furthermore, Lee et al. (2019) confirmed that HPE contains numerous growth factors and cytokines that are associated with liver regeneration. Human placental extract can also upregulate expression of anti-apoptotic factors Bcl-2 and Bcl-xL in liver of mice (Yamauchi et al., 2017). In addition, Rozanova et al. (2012) reported that HPE possess anti-oxidant activity due to its high concentration of bioactive substances mainly proteins. The main amino acids in HPE are leucine (0.12%), arginine (0.08%), alanine (0.08%), phenylalanine (0.08%), serine (0.07%), threonine (0.06%), valine (0.04%), tyrosine (0. 03%), methionine (0.03%) and lysine (0.1%) (Park et al., 2018).

In the present study, HPE significantly increased Ki-67 expression and significantly decreased caspase-3 expression in kidney of MTX-treated animals as compared with animals treated with MTX only. This may be attributed to HPE content of growth factors and cytokines that have proliferative and anti-apoptotic effects. Human placental extract can stimulate tissue regeneration and cellular proliferation through increased Ki-67 expression causing hepatocytes proliferation and liver regeneration (Lee et al., 2019).

Kwon et al. (2019) reported that HPE significantly inhibited apoptosis by lowering caspase-3 activity on skin flap in rats. Human placental extract attenuated cellular senescence and cell apoptosis via reduction of ROS both in vitro and in vivo (Gwam et al., 2019). Pyroglutamic acid purified from HPE potentially stimulated DNA synthesis in rat primary hepatocytes (Inoue et al., 2015). Recently, Nensat et al. (2021) reported that

porcine placental extract decreased cleaved caspase-3 level through inhibition of ROS overproduction.

Conclusions

It is clear that MTX caused nephrotoxicity in albino rats. On the other hand, HPE ameliorated the elevated biochemical parameters and improved most of the histopathological and immunohistochemical changes induced by MTX.

Abbreviations

MTX: Methotrexate; HPE: Human placental extract; Na^+: Sodium; K^+: Potassium; Caspase-3: Cysteine-aspartic protease-3; ROS: Reactive oxygen species.

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MH and EM contributed to experimental design and supervised the practical experiments and revised the manuscript. KY supervised the practical experiments. GY carried out the practical experiments, analyzed the obtained data and wrote the first draft of the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

Data generated during this study are included in this published article. Please contact the corresponding author for data.

Declarations

Ethics approval and consent to participate

All experiments were done in compliance with the guide for care and use of laboratory animals approved by Faculty of Science, Menoufia University, Egypt (Approval No. FHI719), and according to the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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