

Original Article

Role of Ketotifen in Methotrexate-induced Nephrotoxicity in Rats

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A B S T R A C T

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Methotrexate (MTX) is a chemotherapeutic agent that interrupts folate metabolism by inhibition of dihydrofolate reductase; a required precursor for co-factors share in macromolecule biosynthesis. MTX is complicated by marked nephrotoxicity especially in high doses so that in current study the possible protective effect of an anti-inflammatory drug (ketotifen) on MTX-induced nephrotoxicity and the mechanisms involved were investigated. Two doses of ketotifen (1 and 10mg/kg) were given orally to rats for 14 days, in the presence or absence of nephrotoxicity induced by a single intraperitoneal (ip) injection of MTX (20 mg/kg) at day 11th of the experiment. Serum urea, creatinine, renal reduced glutathione (GSH), malondialdehyde (MDA), total nitrites (NO_x), catalase, superoxide dismutase (SOD) activity, caspase-3, tumor necrosis factor alpha (TNFα), nuclear factor kappa B (NFκB) immunoexpressions, and renal histopathology were measured. Results showed that ketotifen succeeded in reversing MTX-induced nephrotoxicity to levels insignificant from control.

Key Words: Methotrexate; Ketotifen; Nephrotoxicity; Antioxidant; Anti-inflammatory.

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1. INTRODUCTION

Methotrexate (MTX) is one of the most successful drugs in cancer chemotherapy. It has been used in treatment of various types of cancer, ectopic pregnancy and autoimmune disorders such as rheumatoid arthritis. It is an antiproliferative chemotherapeutic that disturbs folate metabolism by inhibition of dihydrofolate reductase a required precursor for co-factors involved in macromolecule biosynthesis (AL-Nailey, 2010; Stika., 2012).

The only effective medical intervention for ectopic pregnancy was surgery until medical management was introduced in the 1980s. MTX has been used for medical therapy for ectopic pregnancies. Its mechanism of action, competitive inhibition of folate-dependent steps in nucleic acid synthesis, effectively kills the rapidly dividing ectopic trophoblast. MTX was used in ectopic pregnancies in the 1960s to aide surgical removal of the placenta from its abdominal implantation sites. In the 1980s, MTX was used in multidose regimens with intervening folinic acid rescue (Stika, 2012; Skubisz and Tong, 2012).

The efficacy of MTX is limited by severe side effects and toxic conditions; nephrotoxicity is one of the major side effects of MTX and can cause acute renal failure in high doses. There is a great interest in expanding the clinical usefulness of MTX by developing new agents in order to reduce its nephrotoxicity (Morsy et al., 2013). There is a major role of inflammation and mast cells stimulation in MTX induced nephropathy which was

enhanced by MTX induced free radicals (Khurana et al., 2011; Theoharides et al., 2012). Several antioxidants were tried to protect from MTX induced nephrotoxicity as curcumin (Morsy et al., 2013) and caffeic acid phenethyl ester (Akyol et al., 2014). Ketotifen has an anti-inflammatory, histamine receptor antagonist effects, prevents calcium entry, and inhibits the release of leukotrienes (El-Haggar et al., 2015). Ketotifen has anti-inflammatory and anti-oxidant properties so it was reported that ketotifen had protective effect in different models of nephrotoxicity (Tong et al., 2016; Reena, 2016).

In present study the possible protective effect of another anti-oxidant and anti-inflammatory (ketotifen) on MTX nephrotoxicity were evaluated.

2. MATERIALS AND METHODS

2.1. Chemicals

Ketotifen fumarate powder was from APG Co.USA. MTX vial was from Menapharm co., Egypt. The polyclonal rabbit/antirat caspase-3, tumor necrosis factor alpha (TNFα) and nuclear factor kappa B (NFκB) antibody were from Lab Vision, USA. In addition, biotinylated goat antirabbit secondary antibody was from (Transduction Laboratories, USA), urea, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase kits were from Biodiagnostic, Egypt and creatinine from Humen, Germany.

2.2. Animals and experimental design

Adult male Wistar albino rats weighing about 450–550 g were purchased from the Animal Research Centre, Giza, Egypt. Rats were kept in cages in standard housing conditions and were left to acclimatize for one week. Animals were supplied with laboratory chow and tap water. This study was conducted in accordance with ethical standards and approved by committee of faculty of medicine, Minia University, Egypt.

Ketotifen powder was dissolved in water.

Rats were randomly divided into 6 groups (n = 6 each); group I: received vehicle distilled water for 14 days and ip saline at day 11; group II was treated with low dose of ketotifen (1mg/kg/d orally) for 14 days and ip saline at day 11; group III was treated with high dose of ketotifen (10 mg/kg/d orally) and ip saline at day 11; group IV was treated with vehicle for 14 days and MTX (20 mg/kg) at day 11; group V was treated with a low dose of ketotifen (1 mg/kg/d orally) for 14 days and ip injection of MTX (20 mg/kg) at day 11; group VI was treated with a high dose of ketotifen (10 mg/kg/d orally) for 14 days plus ip injection of MTX (20mg/kg) at day 11. The dose of MTX and ketotifen were based on previous studies (Asvadi et al., 2011; Fitzgerald et al., 2013; El-Haggar et al., 2015).

2.3. Collection of the samples and storage.

After 3 days of MTX injection, each rat was weighed then sacrificed. Venous blood were collected from the jugular vein and centrifuged at 5000 rpm for 15min (JanetzkiT30 centrifuge, Germany).

Both kidneys were removed and weighed. One kidney was sectioned longitudinally then fixed in 10% formalin and embedded in paraffin for histopathological and immunohistochemical examinations. The remained part of the kidneys was snap frozen in liquid nitrogen and kept at -80°C. Renal tissue homogenate was prepared for biochemical analysis, kidneys were homogenized (Glas-Col homogenizer, USA) and a 20% w/v homogenate was prepared in ice-cold phosphate buffer (0.01M, pH 7.4). The kidney homogenate was centrifuged at 3000 rpm for 20 min and the supernatant was kept at -80°C till used.

2.4. Measurements.

2.4.1. Assessment of serum urea and creatinine

Urea (Vassault et al., 1999) and serum creatinine (Bartels et al., 1971) were determined using colorimetric diagnostic kits according to the manufacturer's instructions for detection of renal function and nephrotoxicity.

2.4.2. Assessment of renal GSH levels.

Assessment of renal tissue GSH, SOD and catalase enzyme levels were detected for evaluation of renal antioxidant defense mechanisms. A spectrophotometric

kit was used for GSH. Results were expressed as mmol/g tissue (Beutler et al., 1963).

2.4.3. Assessment of renal catalase levels.

Assessment of renal homogenate catalase enzyme activity was determined from the rate of decomposition of H₂O₂ as described by colorimetric kit. The results were expressed as unit/g tissue (Aebi, 1984).

2.4.4. Assessment of renal SOD levels.

The assessment of SOD levels was based on the ability of the enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye and results were expressed as unit/g tissue (Nishikimi et al., 1972).

2.4.5. Assessment of renal MDA Levels.

Kidney lipid peroxidation was determined as thiobarbituric acid reacting substance and is expressed as equivalents of MDA, using 1, 1, 3, 3-tetramethoxypropane as standard. Results were expressed as nmol/g tissue (Mihara and Uchiyama, 1983).

2.4.6. Assessment of NO_x Levels.

The assessment of renal oxidation end products of NO_x, nitrite and nitrate served as an index of NO_x production. This method was depended on Griess reaction. Results were expressed as nmol/g tissue (Sogut et al., 2003).

2.4.7. Histopathological and immunohistochemical measurements.

For histopathological assessment; renal tissue was fixed in 10% formalin, embedded in paraffin, sectioned by a microtome at 5µm thickness and stained with hematoxylin and eosin. Three slides from each animal group; each one with three sections was subjected to a scoring system used in assessing the histopathological changes using light microscopy (Olympus CX41).

Caspase-3, TNFα and NFκB immunolabeled cells were counted. 3 sections were examined and cells were counted in 3 adjacent non overlapping fields. Immunohistochemical staining was performed for caspase-3, TNFα and NFκB using polyclonal rabbit/antirat antibody according to previously published protocol (Shirai et al., 1985; Côté et al., 1993) respectively. Sections were deparaffinized, hydrated then washed in 0.1M phosphate buffer. These sections were treated with 0.01% trypsin for 10 min at 37°C then washed with phosphate buffer for 5 min. Endogenous peroxidases were quenched by treatment with 0.5% H₂O₂ in methanol and nonspecific binding was inhibited by normal goat serum diluted 1:50 in 0.1M phosphate buffer. Tissue was incubated in the primary antibody overnight at 4°C. Afterwards, tissue was washed and incubated in biotinylated goat antirabbit secondary

antibody (1:2000) for 30 min. Following 30 min incubation in vectastain ABC reagent, the substrate diaminobenzidine was added for 6 min which gives brown color at the immunoreactive sites.

2.4.8. Scoring of the histopathological changes

The renal tissues were examined in random microscopic areas by a histopathological scoring system. 40 high power and the numbers of changes were assessed by counting of 3 non overlapped fields for the same slide of each animal. Hematoxylin and eosin stained sections of the renal tissue were graded for interstitial damage (tubular dilatation or atrophy and interstitial expansion with edema, inflammatory infiltrate). The normal cortical tubulointerstitium scored -; mild tubulointerstitial damage affecting up to 25% of field scored +; moderate tubulointerstitial damage affecting 25-50% of an objective field scored ++; and severe tubulointerstitial damage exceeding 50% of the field scored +++ (Atessahin et al., 2006). The examiner was blinded to the treated groups and the randomly selected 3 cortical fields were scored for each animal and the mean score attributed to the animal.

2.5. Statistical Analysis.

Results were analyzed by one way ANOVA followed by Dunnett multiple comparison test. The values are expressed as means \pm SEM. Statistical analysis was done using GraphPad Prism software (version 5). The difference was considered significant when the calculated P value is less than 0.05.

3. RESULTS

3.1. Effect of ketotifen on serum urea and creatinine in MTX-treated rats.

Table 1 shows the results of the effect of ketotifen on serum creatinine and urea. Rats receiving a single dose of MTX (20 mg/kg, ip) showed a significant increase in serum creatinine and urea levels compared to control group. Both doses of ketotifen (1, 10 mg/kg/day) resulted in significant decrease in serum urea and creatinine compared to MTX treated rats.

3.2. Effect of ketotifen on renal MDA and NO_x levels in MTX-treated rats.

Renal MDA was evaluated as an indicator of kidney lipid peroxidation and nitrite/nitrate ratio as an indicator of renal NO_x levels (table.1). MTX (20 mg/kg) significantly increased renal MDA and NO_x levels compared to control. Administering both doses of

ketotifen plus MTX significantly decreased MDA and NO_x when compared to MTX treated rats.

3.3. Effect of ketotifen on renal GSH, SOD and catalase levels in MTX- treated rats.

Treatment with MTX (20 mg/kg) caused significant decrease in renal GSH, SOD and catalase levels compared with untreated control group (table 2). Concomitant treatment of MTX with ketotifen (1 mg/kg/day) or (10 mg/kg/day) significantly increased the levels of renal GSH, SOD and catalase.

3.4. Histopathological results (Fig 1):

Histopathological examination revealed that A, B, C are control, KLD and KHD groups respectively, had normal structure of renal glomeruli (G) and cortical tubules (T). On the other hand, D is MTX treated group represented dilated Bowman's space and marked damage of renal tubules manifested by tubular necrosis, exfoliated cells, marked vascular degeneration and cystic dilatation. E, F represents concomitant administration of KLD and KHD with MTX showed reversal of histopathological damage induced by MTX with regeneration of epithelial lining of cortical tubules and return of normal morphology to renal cortex. This improvement was significantly higher in KHD than KLD group

Scoring of the histopathological changes.

The severity of the morphological changes was assessed, MTX exposed group showed severe glomerular and tubular morphological changes at the light microscopic levels when compared with control group. These changes were suppressed by administration of both doses of ketotifen but the high dose showed marked improvement than the low dose (table 3).

3.5. Immune-histochemical results.

Caspase-3 (Fig 2), TNF α (Fig.3) and NF κ B (Fig. 4) immunohistochemical staining of rat kidney showed that administration of MTX caused significant increase in the immunoreactivity of them which were highly expressed in renal glomeruli and tubules both in the cytoplasm and in some nuclei. Administration of both doses of ketotifen concomitantly with MTX decreased the expression of them compared to MTX group alone. Administration of both doses of ketotifen alone and control groups showed no expression

Table (1): Effect of ketotifen (1 mg/kg/day) and (10 mg/kg/day) on serum creatinine, serum urea, kidney MDA and NO_x levels in MTX (20 mg/kg) induced nephrotoxicity.

Group	Creatinine (mg/dl)	Urea (mg/dl)	MDA(nmol/g tissue)	NO _x (nmol/g tissue)
Control	0.6590 ± 0.04	41.80± 1.61	24.19 ± 1.07	216.9 ± 10.65
KLD	0.7241 ± 0.06	42.02 ± 1.70	25.79 ± 0.917	241.5 ± 22.05
KHD	0.5977 ± 0.03	41.21 ± 2.18	24.22 ± 1.92	226.4 ± 14.60
MTX	1.295 ± 0.05 ^a	84.32 ± 4.39 ^a	34.44 ± 0.47 ^a	781.5 ± 75.52 ^a
MTX /KLD	1.07 ± 0.05 ^{ab}	64.31 ± 2.3 ^{ab}	28.93 ± 1.21 ^{ab}	440.5± 41.42 ^{ab}
MTX /KHD	0.88 ± 0.03 ^{ab}	54.80± 1.62 ^{ab}	26.79 ± 0.10 ^b	202.2± 4.26 ^b

Values are representation of 4-6 observations as means ± S.E.M. Results are considered significantly different when P < 0.05. ^a Significant from control, ^b significant from MTX group. KLD is ketotifen low dose group; KHD is ketotifen high dose group; MTX is methotrexate given group.

Table (2): Effect of ketotifen (1 mg/kg/day) and (10 mg/kg/day) on kidney GSH, catalase and SOD in MTX (20 mg/kg) induced nephrotoxicity.

Group	GSH(mmol/g tissue)	Catalase (unit/g tissue)	SOD (unit/g tissue)
Control	7.60 ± 0.63	71.60 ± 1.45	822.9 ± 10.22
KLD	6.26± 0.32	70.67 ± 3.34	835.9 ± 11.05
KHD	6.33 ± 0.46	75.04± 3.26	837.2± 8.42
MTX	2.78 ± 0.14 ^a	23.86± 1.4 ^a	699.2 ± 13.64 ^a
MTX /KLD	6.83 ± 0.54 ^b	70.74 ± 5.74 ^b	809.9 ± 10.22 ^b
MTX /KHD	7.6 ± 0.59 ^b	82.73± 6.07 ^b	839.8 ± 2.26 ^b

Values are representation of 4-6 observations as means ± S.E.M. Results are considered significantly different when P < 0.05. ^a Significant from control, ^b significant from MTX group. KLD is ketotifen low dose group; KHD is ketotifen high dose group; MTX is methotrexate given group.

Table (3): Scoring of morphological changes observed in control and experimental groups by light microscope (n=6):

Groups	Tubular degeneration or necrosis	Tubular dilatation	Dilated Bowman' space	Protein casts
Control	-	-	-	-
KLD	-	-	-	-
KHD	-	-	-	-
MTX	+++	+++	+++	+++
MTX/KLD	++	++	+	+
MTX/KHD	+	+	+	-

Animal groups tested are control untreated group, animals treated with ketotifen (1 mg/kg/day, KLD) and ketotifen (10 mg/kg/day, KHD) respectively, animals treated with methotrexate (MTX, 20 mg/kg), or with MTX together with low or high doses of ketotifen (MTX/KLD or MTX/KHD), respectively.

Normal (-), mild (+), moderate (++) and severe (+++).

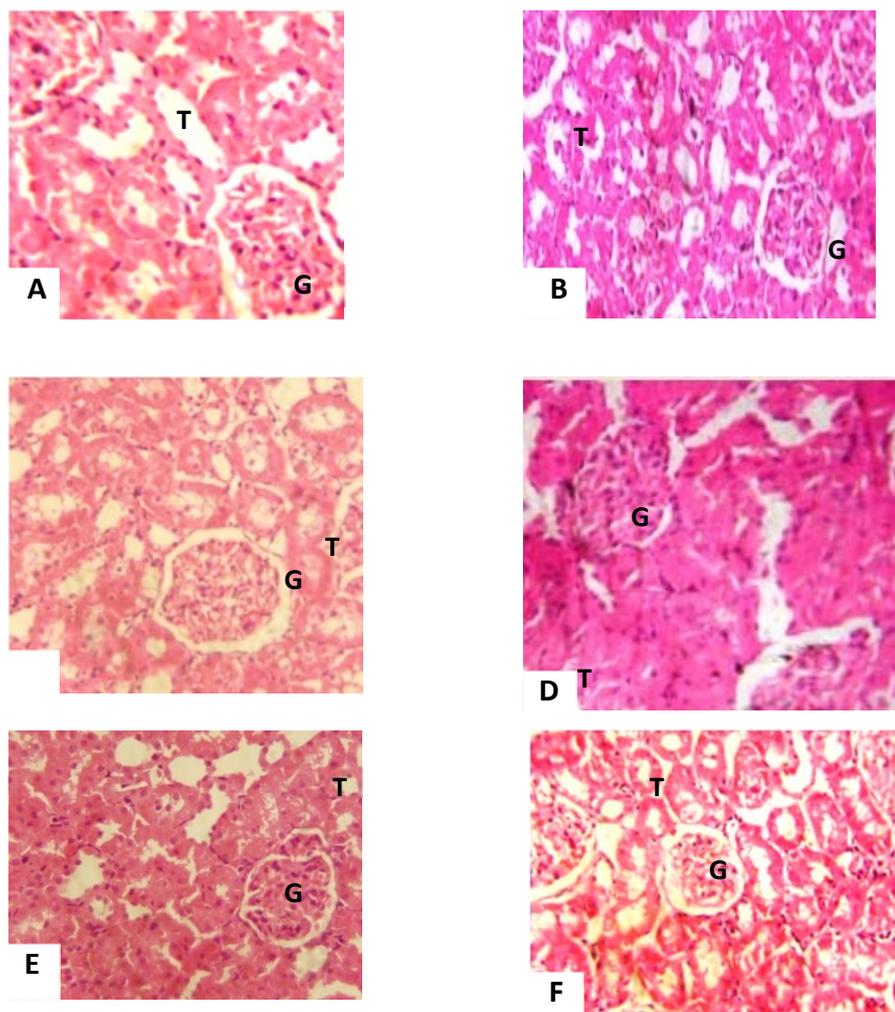


Fig.1: Effect of ketotifen on renal histopathological changes in MTX induced nephropathy.

A, B and C are the histopathological study of the rat renal cortical tissue of control, KLD and KHD groups; showed normal architecture; numerous renal corpuscles, proximal and distal convoluted tubules. **D** is MTX treated group; showed dilated Bowman's space and marked damage of renal tubules manifested by tubular necrosis, exfoliated cells, marked vacuolar degeneration and cystic dilatation. **E** is the MTX/ KLD group showed reversal of histopathological damage induced by MTX with regeneration of epithelial lining of cortical tubules (T) and return of normal morphology to renal cortex. **F** is the MTX/KHD high dose of ketotifen had more obvious decrease in the morphological changes caused by MTX exposure.

Fig. 2. A:

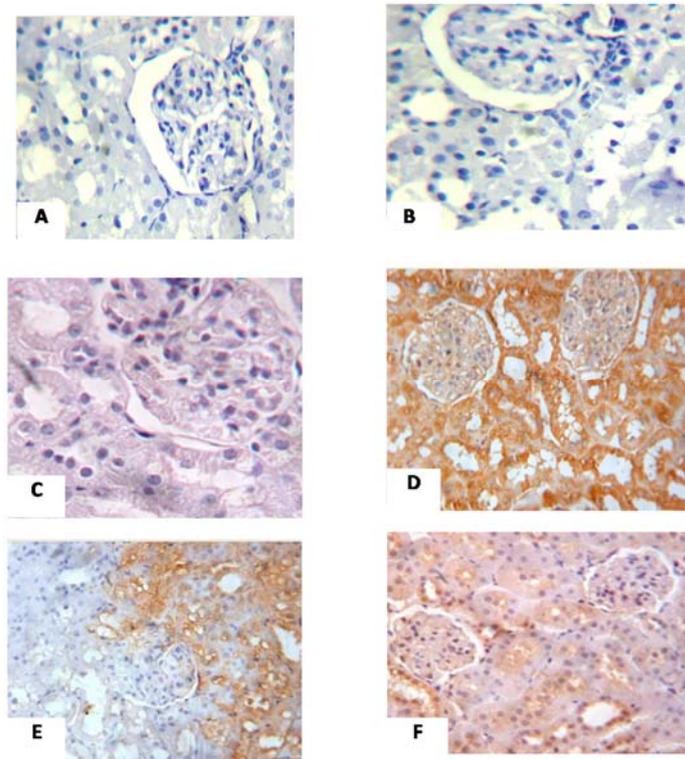


Fig. 2. B:

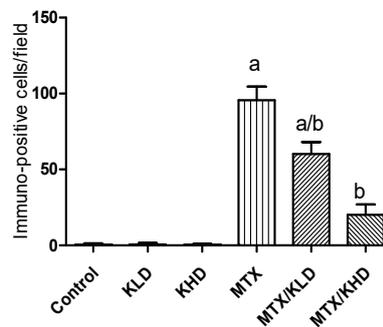


Fig.2.A: Effect of ketotifen on caspase-3 immunohistochemistry in MTX induced nephropathy.

A, B and C are the caspase-3 immunohistochemical staining of rat kidney respectively showed that control, KLD and KHD treated groups showed negative immune expression of caspase-3. D is the MTX treated group which caused marked increase in the immunoreactivity of Caspase-3 which highly expressed in renal glomeruli and tubules both cytoplasmically and in some nuclei.

E, F are KLD, KHD with MTX respectively decreased the expression of caspase-3 compared to MTX treated group but KHD showed less immunohistochemical expression than KLD.

Fig.2.B: Effect of ketotifen on caspase-3 immunohistochemistry in MTX induced nephropathy scoring system.

Caspase-3 analysis showed that administration of MTX caused significant increase in the immunoreactivity of Caspase-3 which highly expressed in renal glomeruli and tubules both cytoplasmic and in some nuclei compared to control group. Administration of KLD and KHD concomitantly with MTX significantly decreased the expression of Caspase-3 compared to MTX group but KHD showed less immunohistochemical expression than KLD. Administration of KLD, KHD alone and control groups showed no immunoreexpression.

Fig 3.A:

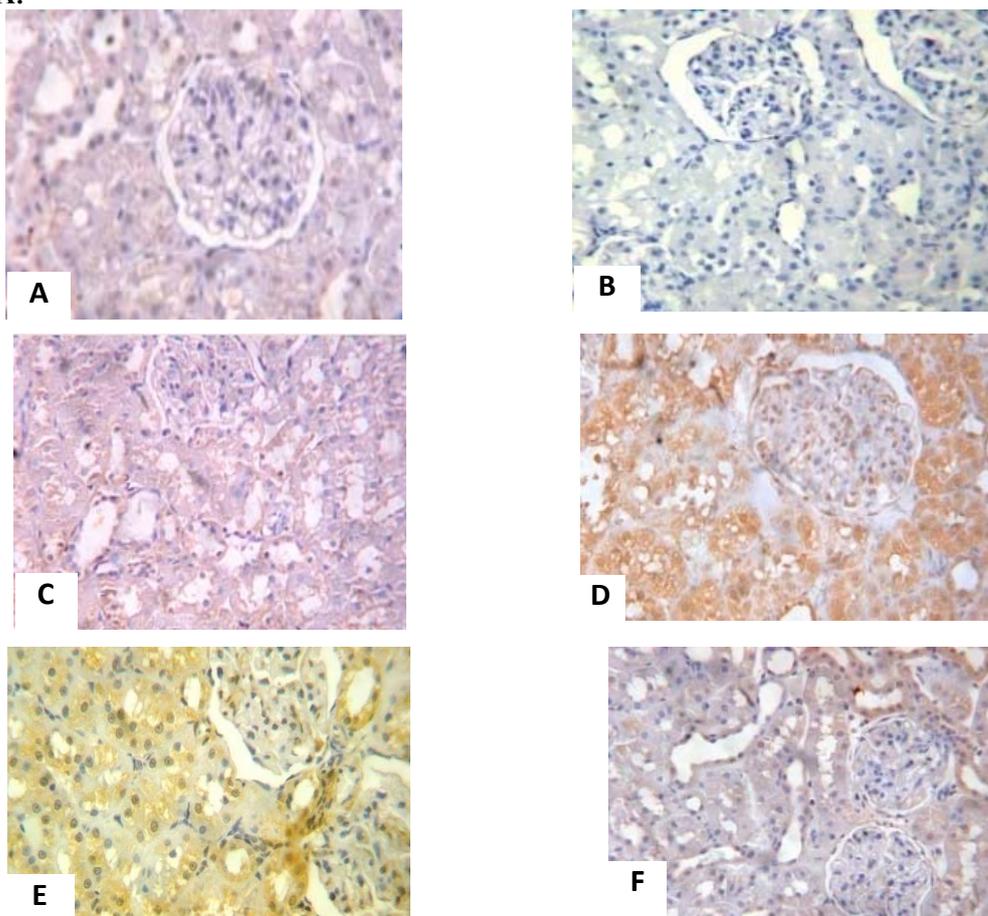


Fig 3.B:

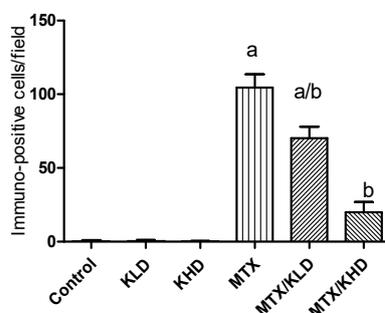


Fig.3.A: Effect of ketotifen on renal TNF α immunoeexpression in MTX treated group.

A, B and C are the TNF α immunohistochemical staining of rat kidney respectively showed that control, KLD and KHD treated groups had no immune expression of TNF α . D is the MTX treated group which had marked increase in the immunoreactivity of TNF α which highly expressed in renal glomeruli and tubules both cytoplasmically and in some nuclei. E, F are KLD, KHD with MTX respectively decreased the expression of TNF α compared to MTX group but KHD showed less immunohistochemical expression than KLD.

Fig.3.B: scoring of TNF α immunoeexpression.

TNF α scoring showed that administration of MTX caused significant increase in the immunoreactivity of TNF α which highly expressed in renal glomeruli and tubules both cytoplasmically and nuclear compared to control group. Administration of KLD and KHD concomitantly with MTX significantly decreased the expression of TNF α compared to MTX group but KHD showed less immunohistochemical expression than KLD. Administration of KLD, KHD alone and control groups showed no immunoeexpression.

Fig 4. A:

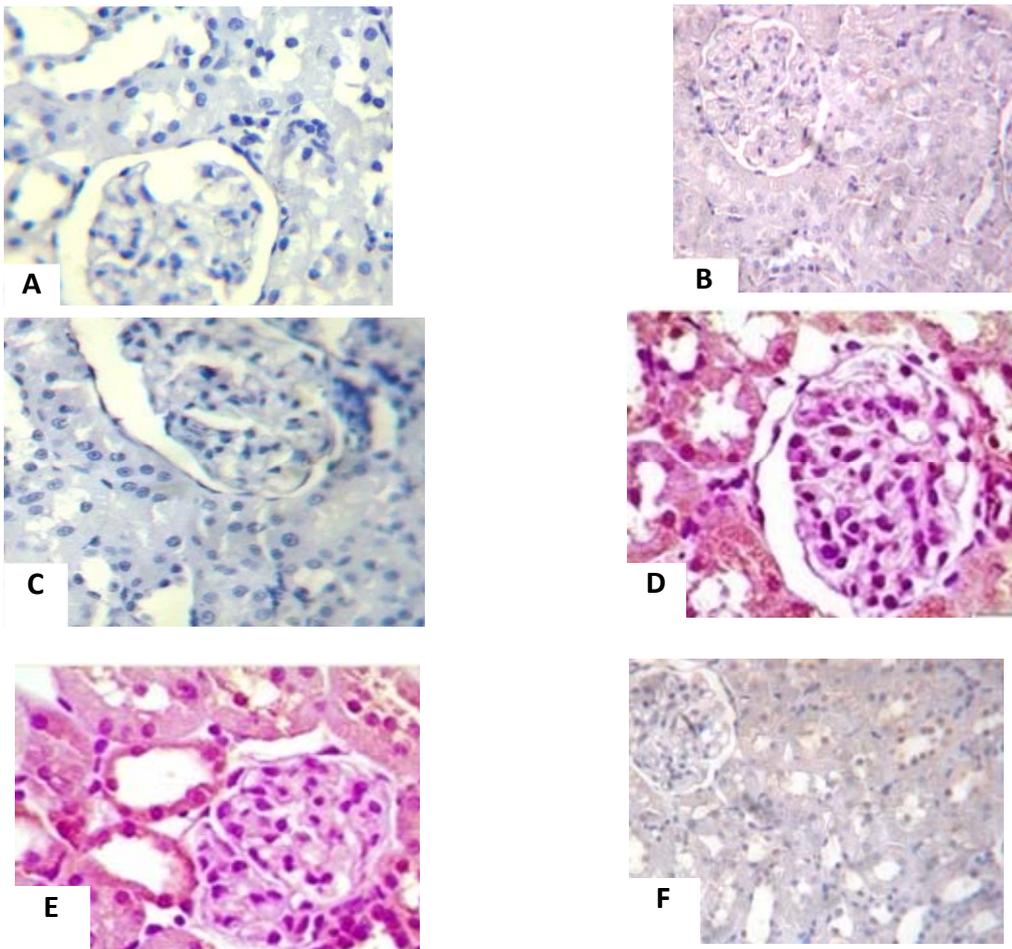


Fig 4.B:

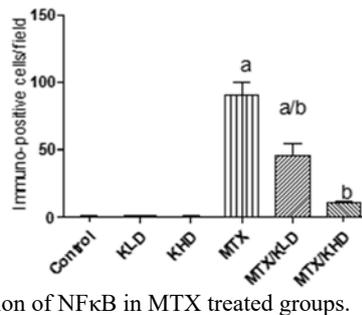


Fig. 4.A: Effect of ketotifen on renal expression of NFκB in MTX treated groups.

A, B and C are the NFκB immunohistochemical staining of rat kidney respectively showed that control, KLD and KHD treated groups showed no immune expression of NFκB. **D** is the MTX treated group which caused marked increase in the immunoreactivity of NFκB which highly expressed in renal glomeruli and tubules both cytoplasmically and in some nuclei. **E, F** are KLD, KHD with MTX respectively decreased the expression of NFκB compared to MTX group but this effect was more prominent with KHD.

Fig. 4.B: Effect of ketotifen on renal expression of NFκB scoring in ketotifen treated group.

NFκB scoring showed that administration of MTX caused significant increase in the immunoreactivity of NFκB which highly expressed in renal glomeruli and tubules both cytoplasmically and in some nuclei compared to control group. Administration of KLD and KHD concomitantly with MTX significantly decreased the expression of NFκB compared to MTX group but KHD showed less immunohistochemical expression than KLD. Administration of KLD, KHD alone and control groups showed no expression.

Values are represented as means \pm S.E.M of number of immune-positive cells for caspase-3, TNF α and NFκB. 3 animals of each group per field, 3 fields/animal. Significant difference is reported when $P < 0.05$. ^a Significant difference compared with control, ^b Significant difference compared with MTX group.

4. DISCUSSION

MTX is one of the most effective chemotherapeutic agents used as a curative drug against various types of cancer, autoimmune disorders and ectopic pregnancy mainly depending on its anti-metabolic effect against folate biosynthesis. However; the accumulation of MTX can lead to renal damage (AL-Nailey, 2010; Stika, 2012).

MTX-related renal injury is by direct tubular toxicity; MTX induces the formation of oxygen radicals in the kidney with subsequent cellular injury (Rahiem et al., 2013). Several antioxidants and anti-inflammatories tried to protect from MTX nephrotoxicity as mirtazapine (Uzkeser et al., 2012), alpha lipoic acid and pentoxifylline (Armagan et al., 2015) and silymarin (Dabak and Kocaman, 2015). All the above mentioned studies could protect from MTX nephrotoxicity.

Reactive oxygen species released by MTX enhance mast cells stimulation which has a major role in drug induced nephropathy (Khurana et al., 2011; Theoharides et al., 2012). The possible protective effect of an anti-inflammatory (ketotifen) on MTX induced nephropathy was studied especially following its success to protect from diabetic nephropathy (Khurana et al., 2011) and IgA induced nephropathy (Young et al., 2009). In current study the induction of MTX nephrotoxicity by single dose of MTX (20mg/kg) showed significant increase of serum urea and creatinine compared to control group and toxic histopathological changes in form of dilated Bowman's space with extensive tubular necrosis, exfoliated cells observed in the renal tubules and interstitial edema were detected. These findings are in agreement with (Asvadi et al., 2011; Ibrahim et al., 2014; Armagan et al., 2015). Administration of ketotifen in low dose (1mg/kg) and high dose (10mg/kg) protected the kidney tissue from MTX induced nephropathy with more improvement by using the high dose than low dose. This was proved by significant decrease of serum urea and creatinine compared to MTX treated group and normalization of the histopathological changes induced by MTX. Results are in agreement with Khurana et al., (2011) who detected that diabetic nephropathy is one of the major microvascular complications of diabetes. Mast cells are found infrequently in normal kidney tissue but mast cell infiltration is a prominent and early feature following renal injury. Excessive production of reactive radicals results in oxidative damage. However, in the tissues antioxidant defensive mechanisms evolve against these toxic oxygen radicals. When these anti-oxidant defense mechanisms fail, severe damage occurs in the tissues. SOD has a significant shielding role against oxidative injury induced by ROS. It transforms superoxide ion (O_2^-) to hydrogen peroxide (H_2O_2) which is later acted upon by catalase and glutathione peroxidase (Rashid et al., 2013). The results showed that the activities of SOD and catalase significantly decreased

in MTX-treated group compared to control rats. The accumulation of these highly reactive free radicals leads to reduce the activity of SOD and catalase which in turn results in damaging effects. These results are in agreement with (Akyol et al., 2014; Armagan et al., 2015). Another antioxidant is GSH which plays roles in the prevention of potential damage of free radicals, inactivation of toxic substances, DNA synthesis, repair of damaged DNA fragments and other metabolic functions (Uzkese et al., 2012). Results showed significant decrease of GSH in MTX treated group compared to control group and that in agreement with Uzkese et al., (2012); Çağlar et al., (2013). Measurements showed that ketotifen plus MTX increased SOD, GSH and catalase levels significantly and that is in agreement with Khurana et al., (2011); Zimiao et al., (2013). It is known that MTX produces ROS and thus causes lipid peroxidation by affecting the lipid components of the cell membrane and increases MDA level. An increased level of MDA, which is one of the end-products of lipid peroxidation represents an increase in free-oxygen radicals in the tissues and causes further damage in the cells (Armagan et al., 2015). Significant increase of MDA level in MTX treated group was found in current study compared to control group and in agreement with Akyol et al., (2014); Abdel-Raheem and Khedr, (2014). Coadministration of ketotifen decreased MDA level significantly compared to MTX treated group and that is in agreement with Khurana et al., (2011); Zimiao et al., (2013). Another oxidative stress parameter is NO, also known as endothelial-derived relaxing factor which is released into circulation during hypoxia, endotoxin or cellular damage stress. Over increase in the intracellular concentrations of NO triggers toxic events results in cell death. There are studies suggesting that increased production of NO plays a role in renal damage (Uzkese et al., 2012). Results showed increasing NO_x level in MTX treated group compared to control group and that in agreement with Morsy et al., (2013); Akyol et al., (2014). Coadministration of ketotifen decreased NO_x levels significantly compared to MTX treated group and that is in agreement with Anoush and Khani, (2015) who detected that ketotifen is able to decrease pro-inflammatory mediators including NO. Dabak and Kocaman (2015) showed that MTX injection exhibited dilated Bowman's space, inflammatory cell infiltration, vascular congestion and swelling of renal tubular cells. Apoptotic cell death was also markedly increased in renal tubules after MTX administration. ROS released during MTX administration increase pro-inflammatory cytokines and this leads to activation of caspase which mediates inflammatory response and apoptosis. This was proved by the present study which showed increased caspase-3 expression in MTX treated group compared to control group and the same histopathological changes also that is in agreement with AL-Nailey, (2010); Çağlar et al., (2013). Oxidative stress is known to stimulate transcription factors; including NFκB. Inflammatory

mediators including TNF α and COX-2 which play important roles in the pathogenesis of MTX nephrotoxicity (Morsy et al., 2013). In current experiment, there is marked increase in TNF α and NF κ B in MTX treated group compared to control group and that is in accordance with Ibrahim et al., (2014). Coadministration of ketotifen decreased caspase-3, TNF α and NF κ B levels significantly compared to MTX treated group and that is consistent with Young et al., (2009) who detected that cytokines play a major role in the pathogenesis of IgA nephropathy. Ketotifen could protect the experimental animal from IgA induced nephropathy by stabilizing the mast cells and decreasing the secretion of cytokines. In addition, Ramos et al., (2010) reported that ketotifen restrained serum TNF levels, improved survival rate and prevented apoptosis and caspase-3 activation in sepsis. Current experiment showed that ketotifen decreased caspase-3 and TNF α expression compared to MTX treated group. In addition; these results are in agreement with Zi-miao et al., (2013) who investigated the effect of ketotifen on oxidative stress of pancreatic beta cells in streptozotocin-induced type 2 diabetic rats. Results showed that Ketotifen 0.09 mg·kg⁻¹ could significantly decrease TNF α levels and reduce MDA content, increase the activities of SOD and improve the microscope observation of beta cells.

5. CONCLUSION

Ketotifen (1 mg/kg/day) or (10 mg/kg/day) protected against methotrexate-induced nephrotoxicity in rats most probably due to its antioxidant, mast cell stabilizing effect and anti-inflammatory activities.

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