COMBINED EFFECT OF ANGIOTENSIN RECEPTOR BLOCKER, AND ANTIOXIDANTS ON RECOVERABILITY OF RENAL FUNCTIONS AFTER RELIEF OF PARTIAL URETERAL OBSTRUCTION OF SOLITARY KIDNEY

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Abstract

Objectives: *To evaluate the effect of a combination of angiotensin receptor blocker losartan, and antioxidant Ferulic acid on the recovery of renal function and renal damage after relief of partial ureteral obstruction (PUO) of a solitary kidney.* ***Methods:*** *thirty-two male mongrel dogs were classified into three groups: sham (8), control (12) and study (12). Right nephrectomy was done and dogs in the study and control groups were subjected to 4 weeks of PUO. Serum creatinine, creatinine clearance (CrCl), and renographic clearance (RC) were measured at baseline, 4 weeks of obstruction and 8 weeks after relief of obstruction. Markers of lipid peroxidation (malondialdehyde MDA), superoxide dismutase (SOD), and reduced glutathione (GSH), and immunostaining of markers of apoptosis (caspase 3 and Bcl2), cell proliferation (ki67) and interstitial fibrosis in the kidney were evaluated at the end of experiment.* ***Results:*** *a combination of losartan and FA enhanced the recovery of serum creatinine, CrCl and RC by an extra 30%, 30%, and 47% of the basal values at 8 weeks, after relief of 4 weeks obstruction, respectively. Also, this combination caused significant decrease in MDA, and significant increase in GSH and SOD. Moreover, this combination significantly reduced the interstitial fibrosis, and caspase 3 expression, and significantly increased the expression of Bcl2 and ki67 in kidney tissues at* 8^{th} *week after relief of obstruction.* ***Conclusion:*** *combination of losartan and FA enhances the recoverability of renal function and minimizes*

the renal damage through reduction of oxidative stress, tubular apoptosis and the interstitial fibrosis in the solitary kidney after relief of PUO.

Introduction

Obstructive uropathy is the eventual outcome of many urological disorders such as urinary stone and ureteric stricture. Ureteric obstruction (UO) is a common clinical finding in a solitary kidney[1]. The pathophysiology of renal damage in UO is complex and it was reported that the renal damage continues even after relief of UO2,[3]. Within the first week of induction of UO, a network of inflammatory, vasoactive and apoptotic processes results in the appearance of signs of tubular atrophy and features of tubulointerstitial fibrosis[4]. Ureteral obstruction triggers tubular cell death by apoptosis and necrosis[5], interstitial inflammatory infiltration[6], and progressive fibrosis with loss of renal parenchyma, myofibroblast activation and extracellular matrix deposition[7,8]. All these changes contribute to renal parenchymal damage leading to sustained decrease in renal function.

Reactive oxygen species (ROS) play an important role in pathophysiology of obstructive uropathy[9]. They may have an important role in the tubulointerstitial inflammation associated with obstructive nephropathy [10], due to the tubular injury caused by mechanical disturbance, which leads to a proinflammatory state and tubulointerstitial fibrosis[11]. After release of the obstruction, ROS12 causes overexpression of fibrogenic cytokines and chemoattractants[11]. It was reported that reduction of endogenous antioxidants (such as catalase) during obstructive uropathy aggravates renal apoptosis, while the administration of exogenous antioxidants attenuates tubular apoptosis[13,14]. Also, chronic UO increases the renal expression of tumor necrosis factor [alpha] (TNF-alpha), FAS ligand, and caspase activity[15]. In a recently study from our lab, we found that ferulic acid (FA), powerful antioxidant, enhanced the recovery of renal functions when given after relief of obstruction[16]. This was done increasing the activity of Bcl

2 (proapoptotic protein) and decreasing the activity of caspase 316.

Angiotensin II, a potent vasoconstrictor, mediates its biological action through its interaction with one of two receptors: angiotensin II type 1 (AT_1) and AT_2[17]. AT_1 receptors are more abundant and appear to be more biologically significant than AT_2 receptors in mammalian kidneys[18,19]. In prolonged UUO, a significant rise is noted in both AT_1 receptor expression and renal angiotensin II content[20]. Angiotensin II and AT_1 receptors have been linked to many of the pathophysiologic processes involved in renal obstruction, including alterations in renal hemodynamics, fibrosis, and apoptosis[21,22]. Angiotensin II up-regulates the expression of several profibrotic cytokines and transcription factors, including TGF-β1, TNF-α, and NF-κB [23,24]. Moreover, the blockade of AT_1 receptor inhibition has been correlated with a reduction in collagen expression, macrophage recruitment, and renal tubulointerstitial fibrosis[24]. Also, in a recent study by our lab, we reported that losartan (AT_1 receptor blocker) enhanced the recovery of renal functions in a canine model of chronic partial ureteral obstruction [25].

On the basis of the observations that reactive oxygen species and AngII are imperative in initiating and promoting renal damage, apoptosis, and fibrosis even after relief of obstruction, we hypothesized that a combined therapy with exogenous antioxidant and AngII blockade may have additive or synergistic effects on recovery of renal functions after release of obstruction by simultaneously blocking the pathogenic actions of ROS and Ang II. In this study, we aimed to study the impact of a combination of losartan and ferulic acid on the recovery of renal functions after relief of UO in a solitary kidney, and its effect on apoptosis, fibrosis, and oxidative stress markers after UO relief.

Materials and Methods

Experimental animals and design :

Thirty two male mongrel dogs aged 2-3 years, weighing 18-25 kg were involved in this study. The dogs were randomly divided into 3

groups: i) Sham group: 8 dogs; right nephrectomy + left sham surgery + no medications, ii) Control group: 12 dogs; right nephrectomy + left partial ureteral obstruction (PUO) + no medications, and iii) Study (FA and Losartan) group: 12 dogs; right nephrectomy + PUO + FA. The dogs were housed in individual boxes under habitual conditions in a temperature controlled room (24°C). They received a balanced diet plus free access to water. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals. All protocols were approved by our local committee of Animal Care and Use Committee. Our institution approved the study and animal care standards were adhered to Institute for Laboratory Animal Research, National Research Council, Washington, DC: National Academy Press, no. 85-23, revised 1996.

Experimental model

Dogs were anaesthetized by thiopental sodium (10mg/kg) with endotracheal intubation and mechanical ventilation. Right nephrectomy was carried out. The model of unilateral left PUO was performed as described by Shokeir, [26]. The study and control groups were subjected to 4 weeks of left PUO. Then, were reopened and subjected to Lich-Grigoir ureterovesical re-implantation. All dogs of the control and study groups were sacrificed by the end of the 8th week after relief of obstruction.

Sham operated animals

The abdomen was entered with a midline incision and right nephrectomy was done. The bladder was opened, a 6 F ureteric catheter was inserted into the left ureteric orifice for 2 hours for collection of urine samples and a blood sample was taken from the left renal vein. The catheter was then removed and the bladder and wound were closed without induction of left PUO. Dogs of the sham group were subjected to sham surgery at basal condition, 4 and 8 weeks and sacrificed thereafter.

Ferulic acid and losartan treatment

Dogs of the study group were given FA (purchased from Sigma, USA) in the drinking water (or

milk or bone soup) at a dose 70 mg/Kg per day with the onset of relief of 4 -week obstruction and continued until sacrifice. Also, Losartan was given at a dose of 2 mg/kg once daily in drinking water (after overnight fasting of dogs) throughout the duration of the study.

Renal functions :

Blood and urine samples were collected from the corresponding kidney during surgery just before induction of obstruction, during relief of obstruction (at 4 weeks of obstruction) and at sacrifice of the dogs at the end of the 8th week after relief of obstruction. Two urine samples were collected, each for 2 hours from the corresponding ureter and blood samples obtained from the corresponding renal vein. Blood samples and the mean of two readings of urine samples were used for calculation of the creatinine clearance (CrCl) from the following equation[27]:

$$\text{CrCl (ml/min)} = \frac{\text{Urine creatinine (mg/dl)} \times \text{urine volume (ml/24hr)}}{\text{Serum creatinine (mg/dl)} \times 1440 \text{ (minutes)}}$$

Doppler ultrasonography and resistive index

Doppler ultrasonography (DUS) with measurement of renal resistive index (RI) of the corresponding kidney were carried out at basal condition before induction of obstruction, just before relief of obstruction (at 4th week of obstruction) and at 4 and 8 weeks after relief of obstruction.

Renograms :

Radioisotope renography with calculation of the split function of the corresponding kidney was performed. Renogram was carried out at basal condition before induction of obstruction, just before relief of obstruction (at 4th week of obstruction) and at 4 and 8 weeks after relief of obstruction as previously described[28].

Morphometric evaluation of renal interstitial fibrosis

Kidney tissues for the histological study were fixed in 10% formalin (pH 7.4) and embedded in paraffin. Sections (3 μm thick) were prepared, and stained with Hx & E and Masson's trichrome to evaluate the fibrosis of cortical interstitium. The sections were

observed on a Olymbus BX51 light microscopy. Pictures were obtained by a PC-driven digital camera (Olymbus E-620). The computer software (Cell* Olymbus Soft Imaging Solution GmbH) allowed the performance of morphometric analysis. Interstitial volume index was determined as described before[29] by superposing a grid containing 100 (10x10) sampling points on pictures of 10 non-overlapping fields (x 200) of Masson's trichrome stained sectons. The number of points overlaying interstitial space were counted and expressed as percentage of all points. Large arteries and glomeruli are excluded from the quantification.

Immunohistochemistry for assessment of apoptotic index, antiapoptotic activity and proliferative index

For immunohistochemistry, 3 µm thick sections were prepared on coated slides and deparaffinised. All sections were incubated for 30min with 0.3% hydrogen peroxide in methanol and microwave heated in 10mM citrate buffer, pH 6.0, for 10-20 min. Subsequently, an indirect immunoperoxidase technique was applied, using monoclonal antibodies for: Anti-caspase 3 (Abcam Cat.# ab79123) cytoplasmic staining with human tonsils as positive control. Anti-Bcl2 (Abcam Cat.# ab59348) cytoplasmic staining with human colon carcinoma tissue as positive control. Anti-K67 (Abcam Cat.# ab86373) nuclear staining with human lymph node as positive control. Indirect immunoperoxidase was performed using ImmunoPure Ultra-Sensitive ABC Peroxidase (Thermo Scientific Cat. # 32052) with (DAB) as chromogen.

Apoptotic index and antiapoptotic activity were assessed with a standard point counting method for the percentage of labelled tubular cells in each of the examined ten non-overlapping randomly selected X 400 fields of each slide. Labelling indices were expressed as the average scores of the[10] fields[30]. The proliferation index was defined as the percentage of the counted immunoreactive nuclei per at least 1000 tubular cells[31].

Estimation of oxidative and antioxidative parameters.

Kidney tissue was perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots. Then, kidney was weighed, minced, homogenized in 5 - 10 ml cold buffer (i.e. 50 mM potassium phosphate, pH 7.5. 1 mM EDTA). Homogenates were centrifuged at 10000 x g for 15 minutes at 4°C and the supernatant was kept at -80°C till used for analysis of lipid peroxides malondialdehyde, MDA), superoxide dismutase (SOD), and reduced glutathione (GSH). MDA, SOD, and GSH were measured by using colorimetric kit (Bio-Diagnostics, Dokki, Giza, Egypt) according to manufacturer's instructions.

Statistical analysis :

The data of dogs of the three groups were compared at different time points of assessment. Statistical analyses were carried out with the 2-tail student's t and ANOVA tests. A p value <0.05 was considered as significant.

Results

Changes in the serum creatinine :

The mean values of serum creatinine were comparable among dogs of the sham, control and combination groups at the basal conditions. The sham operated group showed stable serum creatinine level during the whole duration of the study (Fig. 1). By the end of the 4th week of obstruction, the mean values of serum creatinine of both the combination and control groups were significantly higher than the sham group ($P < 0.01$) (Fig. 1). There was no significant difference in the mean value of serum creatinine of the combination group (4.1 ± 0.8 mg/ dl) and the control group (3.5 ± 0.62 mg/ dl) at the end of the 4th week of obstruction (Fig. 1). The mean serum creatinine of the combination group (1.2 ± 0.3 mg/ dl) was significantly lower than that of the control group (2.1 ± 0.19 mg/ dl) by the end of the 8th week after relief of obstruction, ($P<0.01$) (Fig. 1). At 4 weeks of obstruction; there was no significant difference in the percentage decrease of serum creati-

nine in both combination and control groups. The ability of the kidney to regain its function at 8 weeks after relief of obstruction was significantly better in the combination group compared with the control one. Combination of losartan and Ferulic acid enhanced regain of the serum creatinine at 8-weeks after relief of obstruction by extra 30% in comparison to the control group (Table 1).

Changes in the Creatinine clearance

The mean values of creatinine clearance of the left kidney were comparable among dogs of the three groups at the basal conditions. The sham operated group showed stable creatinine clearance level of the left kidney during the whole duration of the study (Fig. 2). By the end of the 4th week of obstruction, the mean values of creatinine clearance of both the combination and control groups were significantly lower than the sham group. There was no significant difference in the mean value of creatinine clearance of the combination group (22.8 ± 2.6 ml/ min) and control group (25.6 ± 2.8 ml/ min) at the end of the 4th week of obstruction (P = 0.39) (Fig. 2). The mean creatinine clearance of the combination group (34.8 ± 3.5ml/ min) was significantly higher than that of the control group (31.5 ± 4.5 ml/ min) by the end of the 8th week after relief of obstruction, (P< 0.01) (Fig. 2). At 4 weeks of obstruction; there was no significant difference in the percentage decrease of creatinine clearance in both combination and control groups. The ability of the kidney to regain its function at 8 weeks after relief of obstruction was significantly better in the combination group compared with the control one. Combination of losartan and Ferulic acid enhanced regain of creatinine clearance at 8-weeks after relief of obstruction by extra 30% of the basal value in comparison to the control group (Table 1).

Changes in the renographic clearance :

The mean values of split renographic clearance of the left kidney were comparable among dogs of the three groups at the basal conditions. The sham operated group showed stable split renographic clearance level of the left kidney during the whole duration

of the study (Fig. 3). By the end of the 4th week of obstruction, the mean values of renographic clearance of both the combination and control groups were significantly lower than the sham group. There was no significant difference in the mean value of renographic clearance of the combination group (21.93 ± 3.6 ml/min) and the control group (23.8 ± 2.4ml/min) at the end of the 4th week of obstruction (P = 0.67) (Fig. 3). Significant higher values of the split renographic clearance in combination group compared with the control one was observed at 4 and 8 weeks after relief of obstruction (Fig. 3). The mean split renographic clearance in combination group (36.4 ± 1.7 ml/min) is significantly higher than the control group (28.2 ± 4.2 ml/min) at 8 weeks after relief of obstruction (P<0.01). At 4 weeks of obstruction; there was no significant difference in the percentage decrease of renographic clearance in both combination and control groups. The ability of the kidney to regain its function at 8 weeks after relief of obstruction was significantly better in the combination group compared with the control one. Combination of losartan and Ferulic acid enhanced regain of split renographic clearance at 8-weeks after relief of obstruction by extra 47 % of the basal value in comparison to the control group (Table 1).

Renal resistive index (RI)

There was no significant difference in the mean RI of the left kidney of the sham (0.45 ± 0.04); control (0.47 ± 0.03) and combination group (0.49 ± 0.06) groups at basal condition. The mean RI of the sham operated group remained stable during the whole study duration (Fig.4). After 4 weeks of obstruction, there was a significant rise in mean renal RI from a basal value of 0.47 ± 0.03 to 0.71 ± 0.02 in the control group and from a basal value of 0.48±0.04 to 0.7±0.05 in the combination group; (P values < 0.001 & < 0.001, respectively). By the end of the 4th week of obstruction, there was no significant difference in the mean RI of the combination group (0.7 ± 0.05) and the control one (0.71 ± 0.02). Marked drop of the RI to near basal values was observed at 4 weeks after relief of obstruction in both the control (0.48±0.02) and

the combination (0.47 ± 0.05) groups. Follow-up of RI at 8 weeks after relief of obstruction showed almost stable values similar to those of 4 weeks after relief of obstruction in both the control and combination groups (Fig. 4).

Morphometric evaluation of renal interstitial fibrosis

Compared with the sham-operated animals, the harvested kidneys of the control group exhibited a marked interstitial fibrosis mostly in perivascular and intertubular areas, indicated by a positive blue color in Masson's trichrome-stained sections. In contrast, harvested kidneys from animals treated with FA and losartan had significantly less sclerotic damage. The mean percentage of fibrosis in the animals treated with FA and losartan was significantly lower than the control group ($p< 0.05$) (table 2). A combination of FA and losartan reduced interstitial fibrosis by 49.23% in comparison with control. Figure 5-a represents one of the sham group, figure 5-b represents the control group, while figure 5-c represents one of the animals treated with FA and losartan.

Assessment of apoptotic indices, antiapoptotic activity and proliferation indices:

There was significantly lower tubular apoptosis, higher expression of Bcl2, and higher expression of Ki 67 in the kidneys harvested from animals treated with FA and losartan compared with the control one (table 2). A combination of FA and losartan reduced apoptosis by 58.86%, increased expression of Bcl2 by 191.64%, and increased expression of Ki 67 by 98.01% compared with control group (table 2). Figures 6-a & b, 7-a & b, and 8-a & b are examples of the tubular apoptosis indices, Bcl2, and Ki67 expression in the control and combination groups, respectively.

Assay of oxidants and antioxidants :

There was significantly lower MDA, higher GSH, and higher SOD levels in the combination group compared with the control one ($p<0.05$). A combination of losartan and FA reduced MDA by 57.27%, increased levels of GSH by 85.06%, and increased levels of SOD by 70.65% compared with the control group respectively (table 3).

Table (1): Percentage regain of renal function 8 weeks after relief of obstruction in the Combination and control groups

	Control group	Combination group	P value
Serum creatinine (mean ± SD, mg/dl)			
4 W obstruction	3.5±0.62	4.1±0.8	0.1
8W after relief of obstruction	2.1± 0.19	1.2 ± 0.3	< 0.01
Creatinine improvement	1.4	2.9	<0.01
% improvement from basal value	40	70	< 0.01
Creatinine Clearance (mean ± SD, ml/min)			
4 W obstruction	25.6±2.8	22.8±2.6	0.39
8W after relief of obstruction	31.5±4.5	34.8±3.5	< 0.01
Cr Cl improvement	5.9	12	< 0.01
% improvement from basal value	23	53	< 0.01
Renographic Clearance (mean ± SD, ml/mln)			
4 W obstruction	23.8±2.4	21.9 ±3.6	0.67
8W after relief of obstruction	28.2±4.2	36.3±1.7	< 0.01
Cr Cl improvement	4.4	14.4	<0.01
% improvement from basal value	18	65	< 0.01

Table (2): Score of interstitial fibrosis (%), Ki 67 expression, Bcl2 expression, and apoptosis (caspase 3) expression in different groups at the end of study

Group	% Interstitial fibrosis	Apoptosis (caspase +ve cells)	Antiapoptotic Bcl2	Ki 67
Sham group	1.17 ± 0.98	2.67 ± 0.81	13.83 ± 4.44	0.67 ± 0.81
Control group	26.0 ± 6.0[a]	12.76 ± 2.31[a]	6.46 ± 2.87[a]	5.54 ± 1.51[a]
Combined group	13.20 ± 3.39[ab]	5.25 ± 1.6[ab]	18.84 ± 3.45[ab]	10.97 ± 1.30[ab]
% change of the mean in combined group compared with control group	- 49.23	-58.86	+ 191.64	+ 98.01

All data are expressed as Mean ± SD. One way ANOVA test with posthoc Scheffe's test. a= significant with sham, and b= significant with control.

Table (3): Markers of oxidative stress (MDA) and antioxidants (GSH and SOD) in different groups at the end of study

Group	MDA mmol/gm tissue	GSH mg/gm tissue	SOD % inhibition
Sham group	1.56 ± 0 .46	177.02 ± 7.808	89.91 ± 4.82
Control group	11.61 ± 3.94[a]	111.24 ± 4.78[ab]	41.80 ± 7.18[a]
Combined group	4.96 ± 1.87[ab]	205.87 ± 47.29[bc]	71.33 ± 12.60[ab]
% change of the mean in combined group compared with control group	- 57.27	+ 85.06	+ 70.65

MDA= malondialdehyde, GSH= reduced glutathione, SOD (superoxide dismutase).All data are expressed as M±SD. One way ANOVA test with posthoc Scheffe's test. a= significant with sham, b= significant with control.

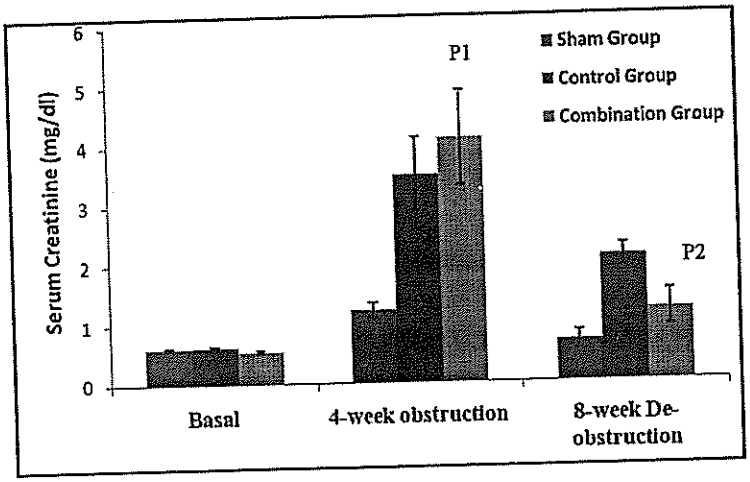

Fig. (1): Changes in the mean serum creatinine of the left kidney after 4 weeks of obstruction and 8 weeks after relief of obstruction in the study and combination groups and in the sham group at basal condition, 4 and 8 weeks of sham surgery (W=Week; Obst= obstruction; De-obst. = de-obstruction; P_1 = combination and control versus sham; P_2 = combination versus control).

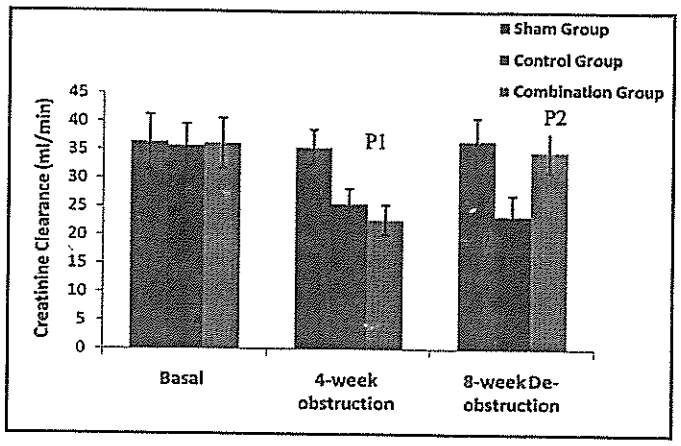

Fig. (2): Changes in the mean creatinine clearance of the left kidney after 4 weeks of obstruction and 8 weeks after relief of obstruction in the combination and control groups and in the sham group at basal condition, 4 and 8 weeks of sham surgery (W=Week; Obst. = obstruction; De-obst. =de-obstruction; P_1 = combination versus control versus sham; P_2 = combination versus control).

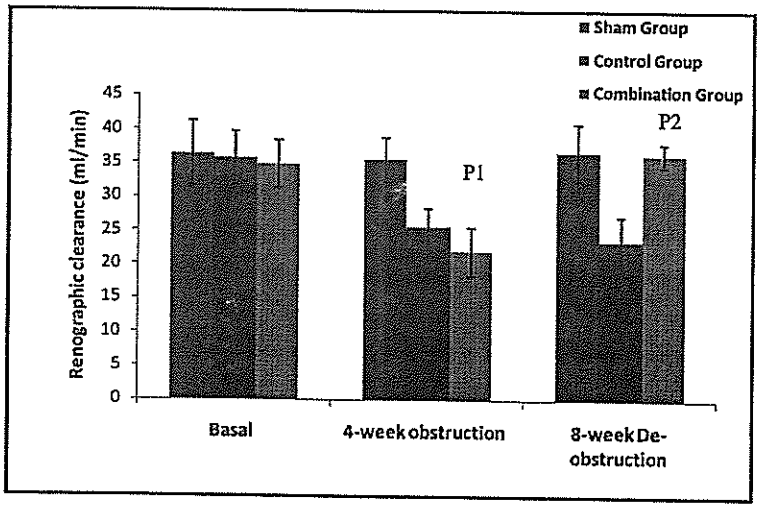

Fig. (3): Changes in the split renographic clearance of the left kidney after 4 weeks of obstruction and 4 and 8 weeks after relief of obstruction in the combination and control groups and in the sham group at basal condition, 4 and 8 weeks of sham surgery (W=Week; Obst. = obstruction; De-obst. =de-obstruction; P_1 = combination versus control versus sham; P_2 = combination versus control).

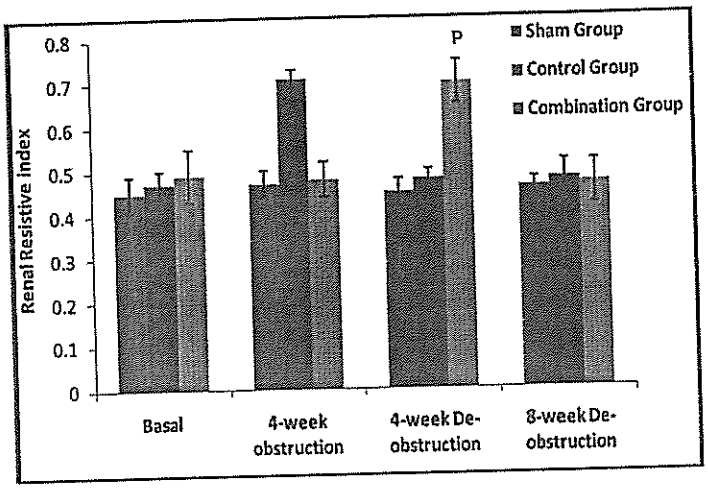

Fig. (4): Changes in the renal RI of the left kidney after 4 weeks of obstruction and 4 and 8 weeks after relief of obstruction in the combination and control groups and in the sham group at basal condition, 4 and 8 weeks of sham surgery (W=Week; Obst. = obstruction; De-obst. =de-obstruction; P = combination and control versus sham).

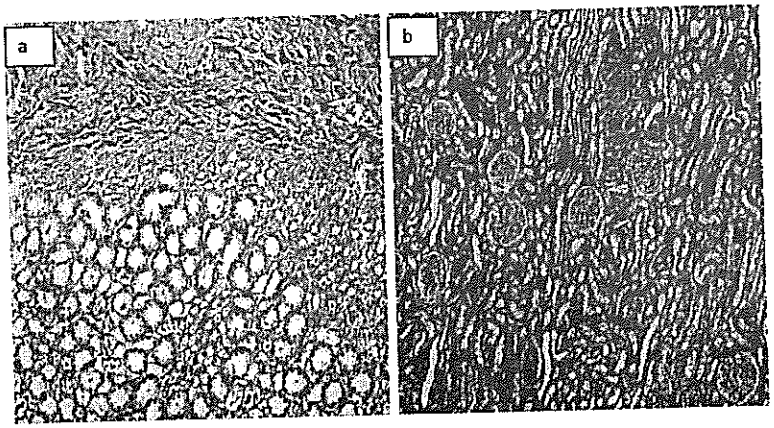

Fig. (5): The cross section of the harvested kidney from animals of different groups showed minimal interstitial fibrosis mostly in perivascular and intertubular areas, indicated by a positive blue color in Masson's trichrome-stained sections. Interstitial fibrosis is 45% in the control group (a), and 11% in the combination group (Masson trichrome stain X 100)

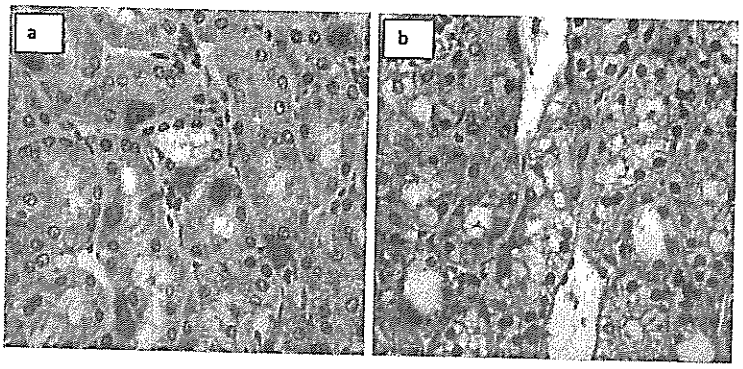

Fig. (6): The cross section of the harvested kidney from control and combination group-treated animals. Immunostaining of caspase 3 labeling index is 10% in the control group (a), and 5% in the combination group (Immunoperoxidase DAP X 400)

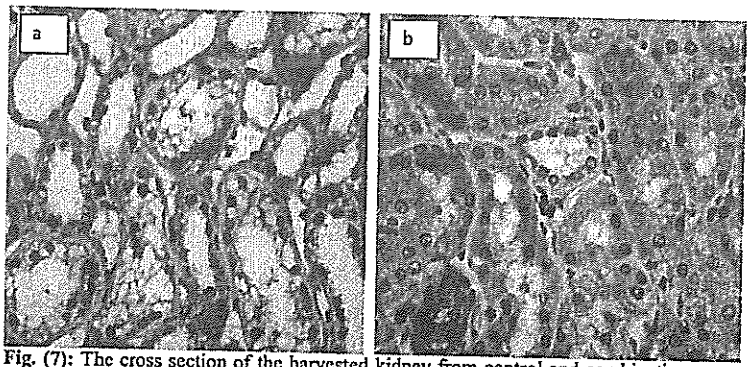

Fig. (7): The cross section of the harvested kidney from control and combination group-treated animals. Immunostaining of Bcl2 labeling index is 4% in the control group (a), and 24% in the combination group (Immunoperoxidase DAP X 400)

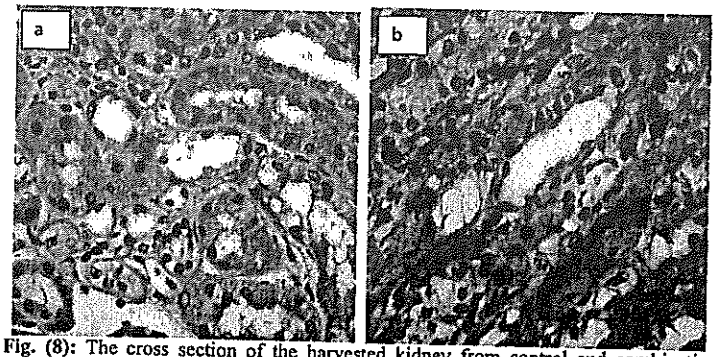

Fig. (8): The cross section of the harvested kidney from control and combination group-treated animals. Immunostaining of Ki 67 labeling index is 7% in the control group (a), and 14% in the combination group (Immunoperoxidase DAP X 400)

Discussion

Many factors may play an active role in the progression of renal damage in obstructive uropathy after relief of obstruction. In this regard, it is not surprising to find out that targeting hyperactive RAS alone as in current clinical therapy may only have limited efficacy in preventing the progressive loss of renal function in UO. In, the present study we examined the hypothesis that a combined therapy by simultaneously targeting multiple pathogenic pathways may be more effective in preventing the progression of chronic renal fibrosis and damage in UO in solitary kidney. In the present study, we found that a combination of losartan and ferulic acid enhanced regain of the serum creatinine, creatinine clearance, and renographic clearance at 8-weeks after relief of obstruction by extra 30%, 30%, and 47% respectively in comparison to the control group. These findings indicate this combination enhanced the recovery of renal function after relief of UO. In a recent study from our lab we found that FA alone enhanced regain of serum creatinine, creatinine clearance, and renographic clearance by extra 22%, 26%, and 33.7% respectively. Also, Soliman et al[25] found that losartan enhanced regain of creatinine clearance, and renographic clearance by extra 26%, and 26%, respectively. These findings indicate that this combination do not provide more protection than each drug alone in enhancing the recovery of renal functions after relief of UO in solitary kidney.

Both oxidative stress and hyperactive renin angiotensin system are involved in pathogenesis of renal damage caused by UO. Also, many studies reported that they are involved in progression of renal damage after relief of obstruction[2,16]. The renal damage involves tubular cell apoptosis and necrosis[5], interstitial inflammatory infiltration[6], and progressive fibrosis with deposition of extracellular matrix[7,8]. So, the next step of our study was to investigate the impact of the combination on renal fibrosis induced by UO. In the present study, we found that a combination of losartan and ferulic acid improved renal fibrosis by 49.23%. This is more than FA alone because FA

alone improved renal fibrosis by 34.81%[16]. These findings suggest that addition of AT1 receptor blocker to FA might stop or slow the process of fibrosis. In agreement with our results, Young et al.,[12] examined a combination of AT1 receptor blocker, losartan, and hepatocyte growth factor (HGF) in protection against renal damage caused by UO. They found that AT1 blocker synergistically inhibits renal smooth muscle actin (SMA) expression (marker of renal fibrosis) and myofibroblast activation and attenuates renal interstitial fibrosis in obstructive nephropathy in mice.

In a previous study by our group, we reported that tubular cell apoptosis (as indicated by significant elevation of caspase3 and significant lowering of antiapoptotic protein Bcl-2) was still evident after 8 weeks after UO release. Moreover, tubular cell proliferation (as indicated by significant elevation of ki67) was significantly increased in obstructed kidney when compared to sham group16. In the present study we found that a combination of losartan and FA significantly reduced apoptosis by 58.86%, increased expression of Bcl2 by 191.64%, and increased expression of Ki 67 by 98.01% compared with control group. This effect is better than FA alone as reported by our previous study[16].

As mentioned in the background, oxidative stress plays an important role in pathogenesis of tubulo-interstitial inflammation during obstructive nephropathy[32], and after relief of obstruction[12]. Manucha et al.[33] and Sugiyama et al.,[34] demonstrated an increase in the concentration of reactive oxygen species (ROS) in obstructed kidney, together with decreased activities of the major protective antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The downregulation of antioxidant enzymes CAT, GSH-Px, and SOD from tubular cells from the obstructed kidney increases the vulnerability of the kidney to oxidative damage[35]. The results of our study are in consistence with the findings of the previous studies. In the present study, a combination of losartan and FA reduced MDA

by 57.27%, increased levels of GSH by 85.06%, and increased levels of SOD by 70.65% compared with the control group respectively. When we compare these findings with the results of our previous study[16], we can say that addition of losartan to FA don't offer more protective effect on oxidative stress in UO than FA alone. These findings suggest that this combination might slow the progress of fibrosis by a mechanism other than inhibition of oxidative stress. This may include inhibition of the release of transforming growth factor β (TGF-β). Both losartan and FA might act synergistically to inhibit the expression of TGF-β. However, in our study this was not measured, so this point is one of the limitations of our study and it will considered on a next studies.

In conclusion, a combination of FA and losartan did not enhance the recovery of renal functions in UO of a solitary kidney model of dogs. However, this combination slows the progress of fibrosis and apoptosis of renal tubular cells. Further studies are recommended to understand the underlying mechanisms of this combination in inhibition of renal fibrosis.

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