رقم البحث (24)

COMPARATIVE STUDIES OF SOME MEDICAL PLANTS, PDE5 INHIBITOR AND ANG-II RECEPTOR ANTAGONIST ON GENE EXPRESSION IN CISPLATIN RAT

BY

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ABSTRACT

Molecular studies applied to investigate the effects of tadalafil™ (Tad), losartan™ (Los), grape seed extract (G.S) and ginko biloba (G.B) in cisplatin (CDDP) treated rats. A total number of one hundred and eight healthy male albino rats randomized into six groups, eighteen rats in each. **Control group** received no treatment, **CDDP group** received a single dose of CDDP (4 mg/kg) intraperitoneal (I.P) per week for 4 weeks. The other groups in addition to CDDP, they injected intraperitoneal with 0.4 mg/kg BW Tad, **Tad group**; **G.B group** received 300 mg/kg BW G.B by stomach tube while **G.S group** received 200 mg/kg BW by stomach tube and **Los Group** injected intraperitoneal with 10 mg/kg BW los. At the end of 1st, 2nd and 4th weeks of treatment animals were sacrificed and tissue were collected from kidney and heart of each group and subjected to RNA extraction and RT-PCR. The results showed a significant increased ($P<0.0001$) in levels of mRNA genes expression of intercellular adhesion molecule-1(ICAM-1), tumor necrosis factor alpha (TNF-α), tumor necrosis factor receptor-1 (TNFR-1), hemeoxygenase 1(HO-1), desmin, nephrin and VEGF in CDDP group when compared with control rats. Four weeks after treatment, expression fold changes significantly decreased in all treated groups with variable degrees. Based on obtained data we concluded that, Los was the best to decrease the fold changes of nephrin, TNF-α, TNFR-1, VEGF and HO-1 genes expression, whereas, G.B extract was superior to reduce desmin and ICAM-1 in the kidney tissue.

Keywords: cisplatin, genes expression, tadalafil™, losartan ™.

INTRODUCTION

Cisplatin (CDDP) is a potential drug for solid tumors produces nephrotoxicity and disturbs endothelial function. CDDP induced nephrotoxicity is a multifactorial process as it activates several signaling process pathways that lead to tubular toxicity, inflammation, oxidative stress, and change in the renal circulation (**Demkow and Stelmaszczyk-Emmel, 2013; dos Santos et al., 2012**). Furthermore, animal experiments and clinical reports have also demonstrated that the vascular effects of CDDP could contribute to renal dysfunction (**Daher and Yeh, 2008**).

The mechanisms of CDDP-induced vascular toxicity might involve oxidative stress, leukocytes infiltration and proinflammatory state **(El-Naga, 2014**). Clinical studies also demonstrated that CDDP triggers a degenerative process of medium-sized vessel walls, thus causing occlusive vascular disease in the long term and development of hypertension (**Morlese et al., 2007**).

The present work was aimed to compare between the effects of Tadalafil, losartan™ and some medicinal plants; grape seed extract and ginko biloba on mRNA genes expression related to inflammation (tumor necrosis factor-alpha and its receptor-1 (TNF-α, TNFR-1), oxidative stress (hemoxygenase-1 (HO-1)), leukocytes adhesion (intracellular adhesion molecule (ICAM-1) and basement membrane molecules (desmin, and nephrin) in CDDP treated rats.

MATERIALS AND METHODS

2. 1. Animals

A total number of one hundred and eight healthy male albino rats initially weighting between 200 and 220 g were used in this study. The animals purchased from animal house in Helwan, and housed in Department of Physiology, Faculty of Veterinary medicine, Mansoura University Animals were left for one week to acclimatize the place. Rats were kept in cages in a rate of six rats per cage in a controlled environment, maintained under a 12 hours light:dark cycle, 24^{o}C (± 3^{o}C) and 50-70% humidity. Rats provided with standard diet and water ad-libitum.

2.2. Drugs:

2.2.1. Cisplatin (CDDP)

CDDP was purchased from Sigma Company (Sigma, St. Louis, Mo, USA): in the form of liquid 1mg/ml sterile concentrate and it was injected intraperitoneally (IP) in a dose rate 4 mg/Kg BW once a week for 4weeks **(Carozzi et al., 2009).**

2.2.2. Tadalafil (Tad)

Tadalafil (Tad) tablets was purchased from ELI LILLY (American global pharmaceutical company) and it was administrated (IP) in a dose of 0.4 mg/kg BW daily **(Ali et al., 2011)**.

2.2.3. Losartan™ (Los)

Losartan™ (Losatan Potassium 50 mg) purchased from Ameriyah Pharmaceuticals Industries (Alexandria, Egypt) and it administrated in a dose of 10 mg/kg B.W IP daily **(Deegan et al., 1995)**.

2.2.4. Grape seed proanthocyanidin extract (G.S):

Grape seed extract obtained from Pharco Pharmaceuticals Company (Alexandria, Egypt). The extract was administrated by stomach tube in a dose 200 mg/Kg B.W daily **(Yamakoshi et al., 2002).**

2.2.5. Ginkgo biloba (G.B)

G.B was obtained from Pharco Pharmaceuticals Company (Alexandria, Egypt) administrated by stomach tube in a dose 300 mg/Kg B.W daily **(Li et al., 2011)**.

2.3. Experimental procedure

For the experiment, rats were randomized into six groups (n = 6-8); **Control group** were administered with a single dose of 0.9% saline I.P and three times of distilled water by oral gavage; **CDDP group** received a single dose of CDDP (4 mg/kg) I.P per week for 4 weeks; **Tad group** received 0.4 mg/kg BW Tad I.P + 4mg/kg BW CDDP I.P daily for 4 weeks; **G.B group** received 300 mg/kg BW G.B by stomach tube+4mg/kg BW CDDP I.P daily for 4 weeks, **G.S group** received 200 mg/kg BW by stomach tube+ 4mg/kg BW + 4mg/kg BW CDDP I.P daily for 4 weeks and **Los group** received 10 mg/kg BW los I.P + 4mg/kg BW CDDP I.p daily for 4 weeks.

2.4. Sample collection

At the end of 1st, 2nd and 4th weeks of the experiment, six rats sacrificed from each group by decapitation. Heart and both kidneys quickly removed from all rats. Slices from heart and right kidneys washed with ice-cold normal saline and homogenates (10%, w/v) were prepared in PBS. Tissue homogenate centrifuged at 1500 xg for 10 min at 4°C. After removal of the cell debris, the supernatant was stored at −80°C for RT-PCR assay.

2.5. RNA Isolation and Real-Time PCR Measurements

TRIzol reagent (Invitrogen, Carlsbad, CA) used, according to the manufacturer's protocols, for isolation of total RNA from the samples. Total RNA purified further using the Qiagen RNeasy cleanup kit (Qiagen, Valencia, CA). The A260/A280 ratio was at least 1.8. The quality of RNA assessed by agarose gel electrophoresis. Real-time PCR was performed using the iScript One-Step RT-PCR Kit with SYBR Green (BioRad, Hercules, CA). Total RNA (100 ng) was used as a template. The primer sequences for the studied genes were as the following **Table (1).**

Table (1): The primer sequences for the studied genes

Gene	Forward (5----3)	Reverse (5----3)
nephrin	CGGAGAAGACTGAGGCGCCTT	TCACACCAGATGTCCCCTCAG
desmin	TCAAGGGCACCAACGACT	GGTCTGGATCGGAAGGTTGAT
TNF-a	GCATGATCCGCGACGTGGAA	AGATCCATGCCGTTGGCCAG
TNFR-1	CCGGGCCACCTGGTCCG	CAAGTAGGTTCCTTTGTG
ICAM-1	AGATCACATTCACGGTGCTG	CTTCAGAGGCAGGAAACAGG
HO-1	GGAAAGCAGTCATGGTCAGTCA	CCCTTCCTGTGTCTTCCTTTGT
VEGF	GATCATGCGGATCAAACCTCACC	CCTCCGGACCCAAAGTGCTC
β-actin	CATGGATGACGATATCGCT	CATGAGGTAGTCTGTCAGGT

TNF-a = Tumor necrosis factor alpha, ICAM-1 = intercellular adhesion molecule-1, TNFR-1 = Tumor necrosis factor receptor 1, HO-1 = hemeoxygenase 1

Real time–PCR reactions applied according to package insert directions. Amplification was done in a Rotor-Gene 3000 real-time thermal cycler (Corbett Research, Sydney, Australia). Reactions were incubated at 50°C for 10 min followed by 95°C for 5 min to achieve first-strand synthesis. PCR was cycled for 40 cycle, 95°C for 10 s, 55°C for 30 s, and 72°C for 1 min. Reaction was completed at 72°C for 10 min. The standard curve was obtained using serial dilutions of RNA from 2-month-old animals.

2.6. Statistical analysis

Data were analyzed by two way (ANOVA) using the general liner model procedure of SAS ***(SAS Institute, 2004)***. The mean values were significant at $P<0.05$.

RESULTS

3.1. Quantification of genes

The fold changes of nephrin, desmin, TNF-α, TNFR-**1,** VEGF, HO-1, ICAM-1 gene expression were significantly (P<0.0001) up regulated in CDDP group compared to control group. After four weeks of treatment, nephrin, TNF-α and desmin were significantly decreased in all treated groups with variable degrees. Nephrin, TNF-α, TNFR-1, VEGF, HO-1became the lowest after treatment with Los, whereas, desmin and ICAM-1 were the lowest after treatment with G.B extract (**Table 2&3**).

DISCUSSION

Several studies demonstrated that CDDP-based chemotherapy induces renal abnormalities and signs of cardiovascular damage (**Daher and Yeh, 2008; Demkow and Stelmaszczyk-Emmel, 2013; Saleh et al., 2014)**. Multiple cellular mechanisms have been suggested underlie these abnormalities. These include oxidative stress-induced tubular epithelial cell toxicity, reduced NO-induced vasoconstriction in the renal microvasculature, and proinflammatory effects (**Yu et al., 2008**). Our study the protective roles of Tad, Los, G.S extract and G.B against CDDP induced vascular and renal alterations at the molecular level in rat model. Nephrin is a homologous molecule expressed in the podocyte slit diaphragms that are essential for normal glomerular ultrafiltration. It was demonstrated that an increased mRNA levels nephrin is associated with the initial stages of the loss of the permeability barrier in nephropathy (**Aaltonen et al., 2001**). Importantly, cytokine-induced upregulation of nephrin expression was also confirmed in primary human podocytes (**Huwiler et al., 2003**). Desmin is cytoskeletal proteins distribution in normal and diseased glomeruli; and the increase of desmin in glomerular epithelial cells was found associated with glomerular epithelial damage (**Zou et al., 2006**). HO-1 is a microsomal enzyme that catalyzes the initial and rate-limiting reaction in heme catabolism to biliverdin, bilirubin and CO, which are efficient peroxyl radical scavengers that possess potent antioxidant properties (**Abraham et al., 2007**). HO-1 has been found to exert an important physiological role in mediating cytoprotection related to the end-products of heme degradation (**Colin- Gonzalez et al., 2013**). Therefore it is considered that HO-1 gene expression is extremely sensitive to up regulation by oxidative stress in a variety of mammalian tissues (**Elmarakby et al., 2012**). The physiological role of TNF- α is based on its direct engaging with TNF-receptors

especially TNFR-1 on renal epithelial cells (**Yu et al., 2011**). **White et al. (2012)** also confirmed that TNFR1 pro-apoptotic signaling induces NF-kB activation. In addition, **Geering et al. (2011)** demonstrated that proapoptotic pathways after TNFR1 stimulation are initiated by p38 and PI3K, but not by caspase-8. Furthermore, it was found that loss of TNFR-1 could be a mechanism to limit inflammation in response to apoptotic cell death **(Madge et al., 1999).** Our results indicated that the fold changes of nephrin, desmin, TNF-α, TNFR-1, HO-1, ICAM-1 and VEGF gene expression were significantly ($P<0.0001$) increased in CDDP group compared to control group. After four weeks of treatment, nephrin, TNF-α and desmin expression fold changes were significantly decreased in all treated groups with variable degrees. Nephrin, TNF-α, TNFR-1, VEGF and HO-1became the lowest after treatment with Los, whereas, desmin and ICAM-1 were the lowest after treatment with G.B extract. In addition, several studies demonstrated that TNF-α is implicated in the pathogenesis of CDDP induced renal cell injury **(Ramesh and Reeves, 2006**). Moreover, TNF-α was found to stimulate the inflammatory response *in vivo* leading to exacerbating CDDP nephrotoxicity (**Uehara et al., 2011).** Thus the inhibition of TNF-α activity and deficiency of TNF-α has demonstrated protection from CDDP toxicity (**Benedetti et al., 2013**). Recent researches suggested that CDDP-induced inflammation and oxidative stress may also affects cardiovascular system (**Demkow and Stelmaszczyk- Emmel, 2013; Sekijima et al., 2011**). **Chirino and Pedraza-Chaverri (2009)** suggested that endothelial dysfunction is related to oxidative stress. The earlier studies documented the impact of CDDP on proliferation of endothelial cells *in vitro* and *in vivo* (**Morlese et al., 2007**). In addition, **Daher and Yeh (2008**) suggested that chemotherapy-induced vascular toxicity occurred due to cumulative effects on endothelium leading to endothelial dysfunction. **Saleh et al., (2014)** confirmed these data by staining VEGF in the kidneys, heart and aorta from CDDP group only. CDDP may induce vascular damage through induction of endothelial cells adhesion molecules and endothelial growth factors (**Yu et al., 2008**). Moreover, **Endemann and Schiffrin (2004)** reported that ROS raised adhesion molecules including ICAM-1. In addition, **Yu et al. (2008**) reported that CDDP injection increased the expression level of ICAM-1 through a nuclear factor kappa-B (TNF-β) dependent pathway, which in turn promotes leukocyte-endothelium interactions leading to endothelial cell apoptosis and thus arterial damage.

CONCLUSION

According to our results, Los (when administered at 0.4 mg/kg/BW IP daily), G.B extract (when administered at 300 mg/Kg BW by stomach tube) and Tad (when administered at 0.4 mg/kg BW IP) for a period not less than 4 weeks can reversed the increased leukocytes adhesion, oxidative stress and inflammation induced by CDDP. Based on obtained data we concluded that, Los was the best to decrease the fold changes of nephrin, TNF-α, TNFR-1, VEGF and HO-1 genes expression, whereas, G.B extract was superior to reduce desmin and ICAM-1 in the kidney tissue.

Table (1): Effect of different treatment on fold change of nephrin, desmin, TNF-α, TNFR-1 in control and experimental rats:

Group	1st Week	2nd Week	4th Week	F-test	
				F-value	P-value
Nephrin					
Control	1.00^{Ba}	1.00^{Ba}	1.00^{Ba}		
CDDP	39.40 ± 3.29^{Ab}	76.96 ± 12.45^{Aa}	84.066 ± 7.11^{Aa}	7.98	0.0204
Tad	2.03 ± 0.25^{Ba}	1.73 ± 0.36^{Ba}	2.500 ± 0.33^{Ba}	1.42	0.3129
Los	1.56 ± 0.23^{Ba}	1.43 ± 0.14^{Bb}	0.966 ± 0.11^{Bab}	3.31	0.1075
G.B	1.200 ± 0.05^{Ba}	1.33 ± 0.23^{Ba}	1.700 ± 0.45^{Ba}	0.73	0.5191
G.S	1.80 ± 0.28^{Ba}	3.13 ± 0.93^{Ba}	2.36 ± 0.36^{Ba}	1.30	0.3393
F value	130.66	36.22	133.18		
LSD	4.17	15.731	8.9791		
Desmin					
Control	1.00^{Ba}	1.00^{Ba}	1.00^{Ba}		
CDDP	32.56 ± 4.69^{Ab}	59.93 ± 2.52^{Aa}	65.100 ± 13.25^{Aab}	4.51	0.0638
Tad	2.36 ± 0.31^{Ba}	1.766 ± 0.22^{Ba}	2.366 ± 0.38^{Ba}	1.24	0.3539
Los	1.73 ± 0.25^{Ba}	2.86 ± 0.37^{Ba}	1.833 ± 0.44^{Ba}	2.83	0.1359
G.B	1.20 ± 0.22^{Ba}	1.20 ± 0.24^{Ba}	1.533 ± 0.09^{Ba}	0.97	0.4312
G.S	1.70 ± 0.10^{Ba}	3.43 ± 0.34^{Ba}	2.633 ± 0.97^{Ba}	2.04	0.2114
F value	43.62	501.98	22.63		
LSD	5.902	3.2523	16.722		
TNF-α					
Control	1.00^{Ba}	1.00^{Ba}	1.00^{Ba}		
CDDP	83.26 ± 14.33^{Ac}	710.70 ± 110.97^{Ab}	2616.0 ± 240.78^{Aa}	74.04	0.0001
Tad	2.33 ± 0.31^{Bb}	29.733 ± 6.11^{Ba}	27.33 ± 1.52^{Ba}	17.49	0.0031
Los	0.200 ± 0.01^{Bb}	2.700 ± 0.21^{Ba}	0.43 ± 0.03^{Bb}	128.72	0.0001
G.B	0.36 ± 0.02^{Bb}	4.966 ± 1.13^{Ba}	1.26 ± 0.30^{Bb}	13.17	0.0064
G.S	4.466 ± 0.96^{Bb}	1.766 ± 0.27^{Bb}	59.00 ± 6.83^{Ba}	65.72	0.0001
F value	32.30	40.02	116.40		
LSD	18.08	139.82	303		

- Values are mean ±S.E
- Values with the different Capital letters in the same column are significantly different at ($P< 0.05$).
- Values with the different Small letters in the same raw are significantly different at ($P< 0.05$).

Table (2): Effect of different treatments on fold change of TNFR-1, VEGF in control and experimental rats:

Group	1st Week	2nd Week	4th Week	F-test	
				F-value	P-value
TNFR-1					
Control	1.00^{Ba}	1.00^{Ba}	1.00^{Ba}		
CDDP	300.3 ± 20.4^{Ac}	1418.2 ± 113.4^{Ab}	5312.7 ± 317.3^{Aa}	182.26	0.0001
Tad	1.066 ± 0.03^{Bb}	4.933 ± 0.49^{Ba}	2.30 ± 0.57^{Bb}	20.69	0.0020
Los	0.118 ± 0.01^{Bb}	1.400 ± 0.37^{Ba}	0.100 ± 0.01^{Bb}	13.70	0.0058
G.B	2.166 ± 0.14^{Ba}	0.333 ± 0.04^{Bb}	0.166 ± 0.02^{Bb}	158.33	0.0001
G.S	2.43 ± 0.62^{Bb}	0.466 ± 0.05^{Bc}	3.83 ± 0.10^{Ba}	20.43	0.0021
F value	215.66	155.97	280.17		
LSD	25.645	142.65	399.16		
VEGF					
Control	1.00^{Ca}	1.00^{Ca}	1.00^{Ba}		
CDDP	10.800 ± 1.15^{Aa}	10.96 ± 0.66^{Aa}	12.63 ± 2.68^{Aa}	0.34	0.7225
Tad	1.93 ± 0.14^{BCab}	2.766 ± 0.37^{Ba}	1.700 ± 0.11^{Bb}	5.37	0.0460
Los	1.83 ± 0.14^{BCa}	2.133 ± 0.26^{BCa}	1.633 ± 0.20^{Ba}	1.46	0.3040
G.B	2.56 ± 0.20^{Ba}	2.233 ± 0.14^{BCa}	2.633 ± 0.20^{Ba}	1.33	0.3318
G.S	2.06 ± 0.26^{BCa}	3.000 ± 0.57^{Ba}	3.200 ± 0.46^{Ba}	1.79	0.2462
F value	54.64	78.92	15.44		
LSD	1.5326	1.2607	3.4488		
HO-1					
Control	1.00^{Da}	1.00^{Ca}	1.00^{Ca}		
CDDP	12.33 ± 1.35^{Aa}	13.20 ± 1.27^{Aa}	14.700 ± 0.63^{Aa}	1.11	0.3875
Tad	2.00 ± 0.11^{CDa}	2.300 ± 0.17^{BCb}	3.66 ± 0.54^{Bb}	6.87	0.0280
Los	2.00 ± 0.28^{CDa}	2.300 ± 0.23^{BCa}	2.733 ± 0.31^{Ba}	1.71	0.2576
G.B	3.300 ± 0.23^{BCa}	3.033 ± 0.31^{Ba}	2.800 ± 0.11^{Ba}	1.12	0.3863
G.S	4.033 ± 0.26^{Ba}	2.60 ± 0.23^{BCa}	3.700 ± 0.69^{Ba}	2.81	0.1378
F value	50.63	66.31	113.83		
LSD	1.805	1.7115	1.4337		
ICAM-1					
Control	1.00^{Ba}	1.00^{Ba}	1.00^{Ba}		
CDDP	121.03 ± 25.13^{Ab}	228.200 ± 26.08^{Aa}	253.66 ± 26.53^{Aa}	7.38	0.0242
Tad	4.13 ± 0.12^{Bab}	3.033 ± 0.42^{Ba}	4.500 ± 0.54^{Bb}	3.57	0.0950
Los	2.93 ± 0.30^{Ba}	3.166 ± 0.15^{Ba}	3.400 ± 0.61^{Ba}	0.34	0.7239
G.B	2.20 ± 0.30^{Ba}	2.300 ± 0.11^{Ba}	2.800 ± 0.51^{Ba}	2.27	0.1847
G.S	3.500 ± 0.41^{Bb}	6.533 ± 0.78^{Bb}	3.833 ± 0.99^{Ba}	4.72	0.0587
F value	22.17	74.43	89.01		
LSD	31.605	32.814	33.41		

- Values are mean ±S.E
- Values with the different Capital letters in the same column are significantly different at ($P< 0.05$).
- Values with the different Small letters in the same raw are significantly different at ($P< 0.05$).

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الملخص العربي

دراسات مقارنة لبعض النباتات الطبية ومثبطات الفسفودايستراز من النوع الخامس ومستقبلات الأنجيوتنسين من النوع الثاني على التعبير الجيني في الفئران المصابة بالسيسبلاتين

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تهدف هذه الدراسة الجزيئية إلى تقيم نشاط مستخلصي الجنكوبيلوبا وبذور العنب بالإضافة الى عقاري اللوسارتان والتادالافيل في الفئران بواسطة الحقن بمادة السيسبلاتين.وقد أجريت هذه التجربة على عدد ١٠٨ من الفئران قسمت إلى ستة مجموعات رئيسية (١٨ الفئران لكل منها) وكانت الاولى مجموعة السيسبلاتين وتم حقن الفئران داخل الغشاء البروتوني بجرعة واحدة ٤ ملج / كج من وزن الجسم في الاسبوع لمده اربعه اسابيع، و تركت دون علاج. ومجموعة اللوسارتان: وفيها تلقت الفئران سيسبلاتين (٤ ملج / كج من وزن الجسم) بالإضافة لجرعة يومية مكونة من ١٠ ملج / كج من وزن الجسم بعقار اللوسارتان داخل الغشاء البريتونى. ومجموعة التادالافيل وفيها تلقت الفئران سيسبلاتين (٤ ملج / كج من وزن الجسم) بالإضافة لجرعة يومية مكونة من ٠,٤ ملج/ كج من وزن الجسم بعقار التادالافيل داخل الغشاء البريتونى. ومجموعة مستخلص بذور العنب: تلقت الفئران سيسبلاتين (٤ ملج/ كج من وزن الجسم) بالإضافة لجرعة يومية من مستخلص بذور العنب (٢٠٠ ملج / كج من وزن الجسم) عن طريق أنبوب المعدة. ومجموعة مستخلص الجنكوبيلوبا: تلقت الفئران سيسبلاتين (٤ ملج / كج من وزن الجسم) بالإضافة لجرعة يومية من مستخلص نبات الجنكوبيلوبا (٣٠٠ ملج / كج من وزن الجسم) عن طريق أنبوب المعدة. واخيرا المجموعة الضابطة: وهي مجموعة فئران تلقت محلول ملح باستخدام أنبوب المعدة. وقد اوضحت النتائج ان حقن الفئران بالسيسبلاتين ادي الى تغيرات في التعبير الجيني لجينات nephrin وdesmin المرتبطة بالمرحلة الأولى من مراحل الفشل الكلوي. هذه التغيرات صاحبها زيادة في مستوي الجينات الالتهابية α- TNF و1- TNFR وكذلك الجينات المرتبطة بالإجهاد التأكسدي HO-1 كما صحب هذه الاختلافات تغيرات في التعبير عن الجينات ذات الصلة بوظائف الخلايا البطانية VEGF و1- ICAM. وقد لوحظ انخفاض ملحوظ احصائيا في التعبير الجيني بعد أربعة أسابيع من المعالجة فى جميع المجموعات المعالجة بدرجات متفاوتة .واستنادا إلى النتائج وجد أن اللوسارتان كان أفضل العلاجات في التعبير الجيني لجينات nephrin، α- TNF، 1- TNFR، VEGFوHO-1 في حين كان جنكوبيلوبا كان متفوق في التعبير الجيني لجينات desminو 1- ICAMفي أنسجة الكلى. أخيرا تشير نتائجنا أن العلاج بعقار اللوسارتان كان الأكثر فعالية عن باقي العلاجات التي تم اختبارها في هذه الدراسة للتخفيف من آثار السيسبلاتين يليه مستخلص الجنكوبيلوبا، التادالافيل وأخيرا مستخلص بذور العنب.