

Evaluation of The Protective Effect of Nebivolol against Gentamicin- Induced Nephrotoxicity in Rats

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ABSTRACT

This study was designed to evaluate the protective effect of nebivolol, beta-1 blocker, on the nephrotoxicity induced by gentamicin in male albino rats. The protective effect of silymarin, as natural antioxidant, and acetylcystein, as nephroprotective agent were compared. The results revealed that three weeks of nebivolol administration prior to gentamicin, protected the rats from the oxidative stress produced by gentamicin. Nebivolol lowered the marked increase in serum levels of creatinin and urea. It also raised the activities of superoxide dismutase (SOD), glutathione-s-transferase (GST), catalase (CAT) and decreased malondialdehyde (MDA) level in kidney homogenate. In addition, pretreatment with nebivolol, silymarin and acetylcystein, each separately, attenuated the elevation in the activities of lysosomal enzymes; acid phosphatase (ACP), β - N-acetyl- D-glucosaminidase (β -NAG) and β -galactosidase (β -GAL) ; that was induced by gentamicin treatment. Histopathological examination of kidney sections of the different groups was coincident with the obtained biochemical data. In conclusion, this study demonstrated that nebivolol is a potent nephroprotective against gentamicin-induced nephrotoxicity as compared with the protective effect of silymarin and N-acetylcystein. Therefore, it is worthy to use nebivolol as antihypertensive drug for hypertensive patients with probable nephritis. It has dual action as antihypertensive and nephroprotective and hence, it can be used instead of antioxidants to justify the blind use of antioxidant supplements by hypertensive patients for the protection of their kidneys.

Keywords: Nebivolol, gentamicin, antioxidant and marker lysosomal enzymes

Introduction

Recent studies have raised concern about the correlation between chronic kidney disease (CKD) and cardiovascular diseases. So, there is an evidence of increased risk of hypertension and cardiovascular morbidity and mortality in CKD patients (Briet *et al.*, 2013). In addition, there is a high prevalence of peripheral arterial disease (PAD) in individuals with kidney disease and both are important risk factors for cardiovascular events (Sarmiento *et al.*, 2013). Tsuda, (2013) suggested that CKD might have a close correlation with impaired rheological behavior of RBCs and microcirculatory disorders in hypertensive subjects.

Like carvedilol, nebivolol (Nbv1) belongs to the third generation of beta-1 blockers, which possess direct vasodilator properties in addition to their adrenergic blocking characteristics. Nbv1 substantially improves endothelial dysfunction via its strong stimulatory effects on the activity of the endothelial nitric oxide synthase and via its antioxidant properties (Münzel and Gori , 2009). So, nebivolol contributes to reverse evolution of oxidative changes in patients with chronic heart failure and hyperglycemia (Belenkov *et al.*, 2011). In addition, Nbv1 had protective effects on renal function on human beings who were subjected to iodinated contrast agent (Günebakmaz *et al.*, 2012).

Gentamicin(GM) derived from gram-positive bacteria called *Micromonospora purpurea* present in soil and water having potential in treating aerobic gram-negative bacteria. Accumulation of gentamicin in proximal renal tubules may cause nephrotoxicity which leads to brush border network damage (Whiting and Brown, 1996). The nephrotoxicity involves renal free radical production and accumulation, consumption of antioxidant defense mechanisms, glomerular congestion, and acute tubular necrosis, leading to diminished creatinine clearance and renal dysfunction (El Mouedden *et al.*, 2000). In the literature, there are increasing multifactorial mechanisms suggested as the leading cause of gentamicin nephrotoxicity. Oxidative stress, lysosomal apoptosis, necrosis and phospholipidosis have been suggested to play a pivotal role in gentamicin-induced nephrotoxicity (Laurent *et al.*, 1982).

Silymarin (SLM) is a mixture of flavonolignans from the fruits of *Silybum marianum*. It contains silybin, isosilybin, silydianin, and silychristin (Skottova and Krecman, 1998). It was approved as a safe herbal product for renal protection in high doses. It has antioxidant effects via increase of gene expression of antioxidant enzymes and a number of the most important protection mechanisms against free radicals damage as catalase (Karimi *et al.*, 2011). Silymarin has multiple beneficial actions, like: nephroprotective action, hepatoprotective

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action, regulation of biomembranes functions, anti-inflammatory and anti-carcinogenic activity (Radko and Cybulski, 2007).

N-acetylcysteine (NAC) is a thiol compound classically known as a mucolytic agent, which is a potent antioxidant that scavenges a wide variety of oxygen-derived-free-radicals and may be capable of preventing acute kidney injury (Brigouri *et al.*, 2011). Duong *et al.* (2005) studied the effect of NAC in preventing radiocontrast-induced nephropathy (RCIN). RCIN is associated with increased morbidity and mortality. They found that prophylaxis with NAC significantly reduces the risk for RCIN. Borisenok *et al.* (2012) proved that NAC administration decreases the number of necrotized proximal convoluted tubules of cortical nephrons and reduces their internal diameter, and increases the height of paving epitheliocytes.

The objective of this study was to investigate the protective effect of neбиволол against the gentamicin-induced nephrotoxicity, compared with the protective effects of silymarin, as natural antioxidant and NAC, a nephroprotective agent in male albino rats.

Materials and Methods

Animals

Adult male albino rats (170 - 200 g) were obtained from NODCAR animal house facility (Giza, Egypt). The animals were housed in groups of six per cage under controlled conditions of humidity(55±5%) and temperature (25±2 °C) and acclimatized to a 12 hr light/dark cycle. Animals were fed with commercially available standard laboratory chow. Animal experiments were conducted according to the guidelines of institutional animal ethical committee.

Drugs and Chemicals

- Nebivolol was purchased from Marcyryl Pharmaceutical Industries, Egypt.
- Silymarin and Acetylcysteine was purchased from SEDICO pharmaceuticals.
- *Gentamicin*: was purchased from MISR pharmaceutical company, Egypt.
- All other chemicals and solvents used were of highest purity and analytical grade.

Experiment Design

Rats were divided into eight groups, each consisting of six animals, and treated as shown in table (1):

Table 1: Schedule of treatment, doses, route of administration and durations.

Groups	1 Control	2 GM	3 NBVL	4 NBVL + GM	5 SLM	6 SLM + GM	7 NAC	8 NAC + GM
Dose	Food +water	80mg/kg	8 mg/kg	8mg/kg +80mg/kg	100 mg/kg	100 mg/kg +80mg/kg	150 mg/kg	150mg/ kg +80mg/kg
Route	Free access	i.p.	oral	Oral Nbv +i.p. GM	oral	Oral SLM +i.p. GM	oral	Oral NAC +i.p. GM
Dura- tion	30 days	Last 7 days	30 days	30 days Nbv + Last 7days GM	30 days	30 days SLM + Last 7 days GM	30 days	30 days NAC + Last 7 days GM

GM: *Gentamicin*, Nbv: *Nebivolol*, SLM: *Silymarin*, NAC: *N-Acetylcysteine*; n = 6

Methods:

(A)- Blood collection and handling

Blood samples were withdrawn from the retro-orbital vein of each animal. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 10 min. The separated sera were used for the estimation of serum levels of urea and creatinine.

(B)- Tissue sampling

After 30 days, the rats were sacrificed by decapitation. Then, the whole kidney tissues were excised. The kidney was divided into three portions. The first portion was homogenized in physiological saline to prepare 25% w/v (0.5 g/ 2ml) homogenate. The homogenate was centrifuged at 3000 rpm for 20 min and the supernatant

was used for the estimation of the activities of CAT, GST, SOD, and MDA level. The second part was used for the preparation of lysosomal fraction to estimate the activities of the three marker lysosomal enzymes. The third part was fixed in 10% formalin and preserved for histopathological examination according to the method of Banchroft *et al.* (1996)

(C)- Determination of biochemical parameters

1. Determination of urea, creatinine, glutathione-S-transferase, and superoxide dismutase : It was done using test reagent kits purchased from commercial sources.
2. Determination of catalase: It was determined according to method of Aebi, (1984).
3. Determination of lipid peroxidation levels: Lipid peroxides formation was determined in the kidney homogenate as Thiobarbituric acid reactive substances (TBARS). It was determined according to the method of Buege and Aust, (1987).
4. Determination of lysosomal marker enzymes activities: The activities of the three marker lysosomal hydrolases: ACP, β -GAL and β -NAG were measured according to the method described by Van Hoof and Hers, (1968) with a slight modification by Younan and Rosleff, (1974) and El Deib *et al.* (2011).

Statistical Analysis

In the present study, all values were presented as Mean \pm S.E.M (standard error of the mean) for six animals in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using the SPSS/10 software package for Windows. Post hoc testing was performed for inter group comparisons using the least significance difference (LSD) test; statistically significant values at $p < 0.05$ has been given respective symbols in the tables.

Results

Table (2) shows that the relative kidney weight (kidney/body weight ratio) is elevated in the GM treated group by 46.3% compared to control group. Pretreatment with Nbv1, SLM and NAC lowered this elevation by 25.7%, 22.5% and 24.5%, respectively.

Table 2: Effect of nebulivolol on the body weight and kidney weight of rats compared with silymarin and acetylcysteine.

Parameters	Body weight (g)	Kidney weight (g)	Kidney weight/ Body weight ratio %
Control	181.2 \pm 2.07	1.08 \pm 0.029	0.596 %
GM	172 \pm 2.92	1.50 \pm 0.038	0.872 % ↑ 46.3%
Nbv1	181.67 \pm 3.03	1.05 \pm 0.045	0.578 % ↓ 3 %
Nbv1 + GM	183.7 \pm 2.16	1.32 \pm 0.027	0.719 % ↑ 20.6%
SLM	187.5 \pm 2.19	1.00 \pm 0.029	0.533 % ↓ 10.6%
SLM + GM	180.17 \pm 3.38	1.33 \pm 0.025	0.738 % ↑ 23.8%
NAC	181.5 \pm 2.01	1.04 \pm 0.042	0.573 % ↓ 3.9%
NAC + GM	179.17 \pm 3.45	1.30 \pm 0.066	0.726 % ↑ 21.8%

Data are expressed as Mean \pm S.E, of six rats per group. Nbv1; Nebivolol, SLM; Silymarin, NAC; N-Acetylcysteine, GM; Gentamicin

Modulation of serum urea and creatinine levels:

Table (3) revealed the protective effect of nebulivolol on kidney function against the nephrotoxic effect of GM. GM elevated the serum urea level by 185.8% as compared to control. Nbv1 lowered this elevation by 67.5%. SLM and NAC decreased the nephrotoxic effect of GM by 75.8% and 106.5%, respectively. For serum creatinine level, SLM reduced the elevation produced by GM by 477%; acetylcysteine lowered that elevation

by 501% and NbvI proved its nephroprotective effect by 473% reduction in serum creatinine level when compared to GM -treated group.

Table 3: Effect of nebivolol on the kidney function parameters compared with silymarin and acetylcysteine in rat serum.

Parameters	Urea (mg/ dl)	Creatinine (mg/ dl)
Control	25.29 ± 0.509	1.21± 0 .04
GM	72.28* ± 0.832 ↑ 185.8%	7.84* ± 0.06 ↑ 548%
NbvI	24.0 ± 0.253 ↓ 5.14%	1.15 ± 0.07 ↓ 4.96%
NbvI + GM	55.22* ± 0.322 ↑ 118.3%	2.12* ± 0.04 ↑ 75.2%
SLM	22.65 ± 0.415 ↓ 9.9%	1.17± 0.05 ↓ 3.3%
SLM + GM	53.12* ± 0.328 ↑ 110%	2.07* ± 0.03 ↑ 71%
NAC	24.1 ± 0.266 ↓ 4.7%	1.1 ± 0.04 ↓ 9.1%
NAC + GM	45.34* ± 0.427 ↑ 79.3%	1.77 ± 0.03 ↑ 46.3%

Data are expressed as Mean ± S.E, of six rats per group. * Significant difference from control group at $P < 0.05$. NbvI; Nebivolol, SLM; Silymarin, NAC; N-Acetylcysteine, GM; Gentamicin

The level of lipid peroxidation LPO in the kidney of GM treated group increased significantly by 4.4 folds when compared to control group as revealed from table (4). Pretreatment with nebivolol lowered the magnitude of lipid peroxidation caused by GM by 2.8 folds. Combination of SLM and NAC with GM showed that SLM and acetylcystiene reduced the LPO caused by GM treatment by 2.9 & 2.5 folds respectively.

Table 4: Effect of nebivolol on activities of CAT, GST, SOD and MDA compared with Silymarin and acetylcysteine in rat kidney tissue.

Parameters	CAT (U/gm tissue)	GST (U /gm tissue)	SOD (U/gm tissue)	MDA (nmole/mg protein)
Control	32.2 ± 1.55	1.95 ± 0.1	6.27 ± 0.12	1.73 ± 0.04
GM	6.31* ± 0.68 ↓ 80.4%	1.27* ± 0.1 ↓ 34.9%	1.23* ± 0.078 ↓ 80.38%	7.67* ± 0.20 ↑ 343.4%
NbvI	36.3 ± 3.34 ↑ 12.7%	1.99 ± 0.04 ↑ 2.05%	6.35 ± 0.18 ↑ 1.28%	1.69 ± 0.11 ↓ 2.31%
NbvI + GM	16.2* ± 1.22 ↓ 49.7%	1.81* ± 0.10 ↓ 7.2%	4.27* ± 0.14 ↓ 31.9%	2.81* ± 0.19 ↑ 62.4%
SLM	34.8 ± 2.13 ↑ 8.07%	1.98 ± 0.04 ↑ 1.54%	6.39 ± 0.13 ↑ 1.91%	1.70 ± 0.08 ↓ 1.73%
SLM + GM	21.2* ± 1.08 ↓ 34.2%	1.82 ± 0.11 ↓ 6.7%	4.85* ± 0.26 ↓ 22.6%	2.66* ± 0.15 ↑ 53.8%
NAC	35.6 ± 2.7 ↑ 10.6%	1.99 ± 0.05 ↑ 2.05%	6.31 ± 0.19 ↑ 0.64%	1.65 ± 0.10 ↓ 4.62%
NAC + GM	22.3* ± 1.10 ↓ 30.7%	1.88 ± 0.08 ↓ 3.6%	4.93* ± 0.22 ↓ 21.4%	3.37* ± 0.21 ↑ 94.8%

Data are expressed as Mean ± S.E, of six rats per group. * Significant difference from control group at $P < 0.05$. NbvI; Nebivolol, SLM; Silymarin, NAC; Acetylcysteine, GM; Gentamicin

Also, table (4) shows that the activity of catalase in kidney homogenate of rats treated with GM showed a significant decrease by 80.4% as compared to control. Prophylaxis by Nbv1, SLM and NAC prevented this drop in catalase activity by 30.7%, 46.2% and 49.7% ,respectively. Also, table (4) shows that the activity of CAT in kidney homogenate of rats treated with GM showed a significant decrease by 80.4% as compared to control. Prophylaxis by Nbv1, SLM and NAC prevented this drop in catalase activity by 30.7%, 34.2% and 49.7% ,respectively.

In addition, the activity of GST in kidney homogenate of animals decreased by 34.9% for GM treated group. Nbv1 helped to decrease the disastrous effect of GM by 27.7%. In addition the well known anti-oxidant SLM reduced the oxidizing effect of GM by 28.2%. The determination of SOD activity level in rat kidney revealed that GM decreased its activity by 80.38% and that Nbv1 lowered this decrease in activity of SOD by 48.5%. Comparing the effect of Nbv1 to SLM and NAC, we found that they reduced the decrease in activity of SOD enzyme done by GM by 57.8% and 59%, respectively.

Effect of gentamicin and pretreatment with nebulivol, silymarin and acetylcystiene on the activity of rat kidney lysosomal enzymes

Table (5) showed that Acid phosphatase activity increased significantly by effect of GM on rat kidneys by 268.6% as compared to control. Treating rats with Nbv1 before GM administration lowered the damaging influence of GM by 192.3%. Pre-treatment with SLM and NAC reduced the disastrous effect of GM on ACP activity by 171.2% and 182.8%, respectively.

As can be seen from table (5), β -NAG activity increased by 256.9% for GM treated rats when compared to control group. Nbv1, SLM & NAC lowered this increase in activity of β -NAG by 160.6%, 159.4% and 187%. In addition, β -GAL activity was significantly increased by 223.5% in rats treated with GM. Nebivolol lowered that increase in activity of β -GAL by 119.3%. Pretreatment with SLM and NAC reduced the influence of GM on β -GAL activity by 144.3% and 143.2%, respectively.

Table 5: Effect of nebulivol on the enzymatic activity of rat kidney lysosomal enzymes compared with silymarin and acetylcystiene.

Parameters Groups	ACP (n mol /ml/hr)	β -NAG (n mol /ml/hr)	β - GAL (n mol /ml/hr)
Control	1109.2 \pm 10.7	802.0 \pm 16.6	426.7 \pm 12.69
GM	4087.6* \pm 7.1 ↑ 268.6%	2862.8* \pm 9.5 ↑ 256.9%	1380.41* \pm 21.49 ↑ 223.5%
Nbv1	1085.6 \pm 33.3 ↓ 2.12%	706.1 \pm 10.2 ↓ 11.9%	403.71 \pm 12.07 ↓ 5.4%
Nbv1 + GM	1954.7* \pm 11.6 ↑ 76.3%	1574.7* \pm 26.1 ↑ 96.3%	871.24* \pm 14.78 ↑ 104.2%
SLM	1052.27 \pm 9.7 ↓ 5.13%	720.4 \pm 18.96 ↓ 10.2%	398.1 \pm 8.97 ↓ 6.7%
SLM + GM	2188.7* \pm 14.3 ↑ 97.4%	1295.5* \pm 25.4 ↑ 61.5%	764.75* \pm 17.18 ↑ 79.2%
NAC	1044.6 \pm 34.6 ↓ 5.82%	774.3 \pm 16.5 ↓ 3.49%	386.27 \pm 10.79 ↓ 9.47%
NAC + GM	2059.79 * \pm 44.6 ↑ 85.8%	1362.9* \pm 29.2 ↑ 69.9%	769.2* \pm 22.85 ↑ 80.3%

Data are expressed as Mean \pm S.E, of six rats per group. * Significant difference from control group at $P < 0.05$. Nbv1; Nebivolol, SLM; Silymarin, NAC; Acetylcystiene, GM; Gentamicin

Microscopical examination of kidney tissues

In animals treated with GM, the microscopical examination showed different changes in tufts and tubules where, the epithelial cells of tubules are swollen and, and display hydrobic degeneration with pyknotic nuclei can be recognized. Atrophy of some glomerular tufts was observed together with edema and inflammatory cells in the interstitial tissues . On the other hand, the Nbv1-treated group showed that, the lesion

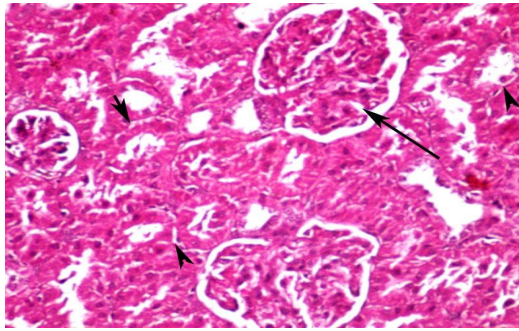


Fig 1: A Photomicrograph of specimen of normal kidney (control) showing intact glomerular tuft and renal tubule with vesicular nuclei. H& E, X: 200.

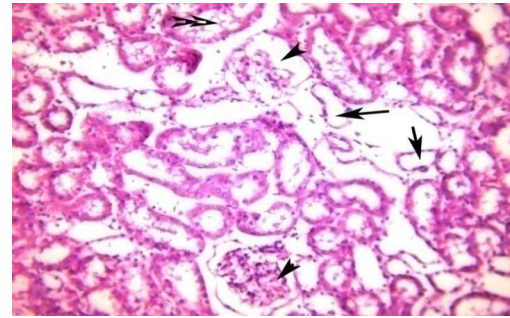


Fig 2: A Photomicrograph of specimen of kidney of GM-treated rat showing atrophied glomerular tuft and renal tubule. H& E, X: 200.

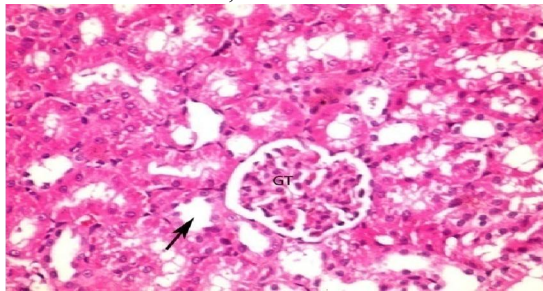


Fig. 3: A Photomicrograph of renal tissue of NbvI treated animals showing intact glomerular tuft. H& E, X: 200.

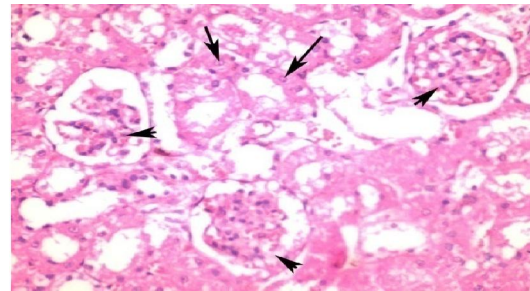


Fig. 4: A Photomicrograph of renal tissue treated with NbvI and GM showing moderate improvement. H&E, X: 400.

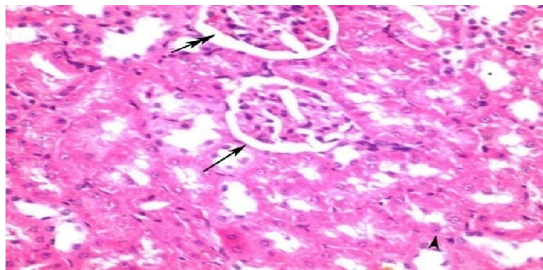


Fig. 5: A Photomicrograph of renal tissue of NAC-treated animals. showing normal tuft. H& E, X: 200.

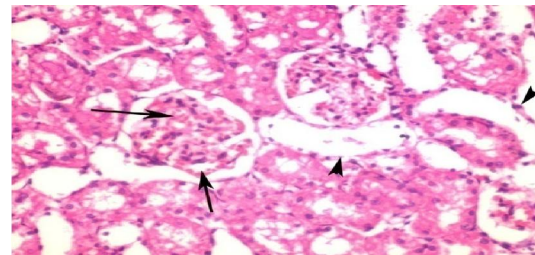


Fig. 6: A Photomicrograph of renal tissue treated with NAC and GM rat showing some atrophied tubule. H& E, X: 400.

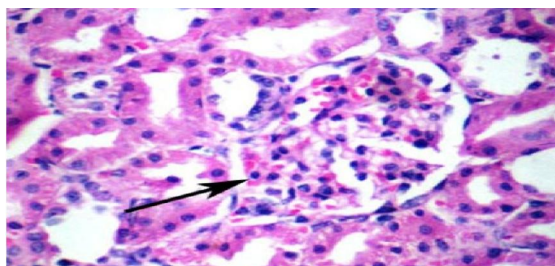


Fig. 7: A Photomicrograph of specimen of renal tissue treated with SLM showing normal glomerular tuft. H& E, X: 200.

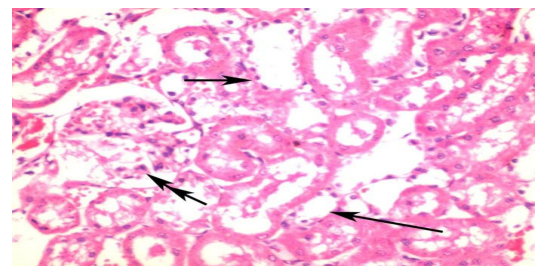


Fig. 8: A Photomicrograph of specimen of kidney of rat treated with SLM and GM showing mild improvement. H& E, X: 400.

in these group as well as the control group. The renal tissues of animals which treated with Nbv1 and GM showed that, 50% of the animals demonstrated mild to moderate improvement in glomerular tufts. Some of The renal tubules and tufts restored their normal appearance. The specimen of kidney of NAC-treated rats and group which took SLM only showed almost unaffected tubules, and seen as well as normal kidney tissues. Animals treated with NAC and GM and the animal treated with SLM and GM showed, atrophied renal tubules in 50% of animals with aggregates of inflammatory cells in the interstitial tissues. Also, there are some remaining cells lining the tubules having vesicular nuclei and eosinophilic cytoplasm. Some tufts were obliterated, Bowman spaces and basophilic Mesangial cells have been detected.

Discussion

Aminoglycoside antibiotics, as GM, are widely used in the clinical practice for the treatment of gram-negative infections. However, acute renal failure (ARF) is a major complication of GM treatment that limits its use (Maldonado *et al.*, 2003). The underlying mechanisms by which GM causes nephrotoxicity are not well understood. There may be several mechanisms of GM-induced nephrotoxicity -have been shown both in vitro and in vivo studies (Balakumar *et al.*, 2010). Reactive oxygen species (ROS) are considered to be one of the important mediators of GM-induced cell injury (Banday *et al.*, 2008). Priuska and schacht (1995) demonstrated that GM is an iron chelator, enhancing iron-mediated lipid peroxidation . Previous researchers have been reported that the stimulation of apoptosis is a significant cytotoxic mechanism of gentamicin in renal proximal tubular cells and mesangial cells (Servais *et al.*, 2006). Additional studies demonstrated that substances with antioxidant properties protect against kidney damage induced by GM (Nitescu *et al.*, 2006). Bledsoe *et al.* (2006) suggested that mechanisms not related to oxidative damage are involved in the nephroprotection provided by NAC.

In the present study, we demonstrated that the administration of NAC, SLM and Nbv1 are able to ameliorate the renal damage. Bledsoe *et al.* (2006) suggested that mechanisms not related to oxidative damage are involved in the nephroprotection provided by NAC.

This study revealed that GM induced increase in kidney homogenate MDA level and decrease in the activities of SOD, CAT and GST. GM caused progressive damage to cell membranes of kidney tissues by labilization of lysosomal membranes, leading to a significant elevation in the activities of lysosomal acid hydrolases.

Pretreatment with Nbv1, having antioxidant activity, ameliorated the case by increasing NO levels. Hence, the rats treated with Nbv1 showed an increase in the activities of SOD, CAT and GST in kidney homogenate, similar to that obtained in SLM and NAC treated groups. Nbv1, SLM and NAC produced stabilization of the lysosomal membranes, thus, a significant lowering in lysosomal enzyme activities appeared.

Renoprotective effect of Silymarin was studied mainly in rats. SLM preserved the lipid composition of membrane in gentamicin- induced renal toxicity by its membrane stabilizing activity (Abdel-Raheem *et al.* 2009). SLM, in addition to its free radical scavenging properties, also increases the activity of the antioxidant enzymes, such as SOD and glutathione reductase and inhibits lipid peroxidation (Soto *et al.*, 2003). SLM treatment resulted in statistically significant amelioration in the histological alterations and reduced the number of TUNEL-positive cells as compared with the methotrexate- treated rats (Dabak and Kocaman, 2015). On the other hand, the oral administration of SLM (100 mg/kg) significantly protected against the bromobenzene induced nephrotoxicity and renal mitochondrial dysfunction in rats (Vedi *et al.*, 2014).

N-acetylcysteine (NAC) is a thiol compound classically known as a mucolytic agent, which is a potent antioxidant that is capable of preventing acute kidney injury(Briguori *et al.*, 2011). Ramesh *et al.* (2006) conclude that prophylactic administration of NAC along with hydration diminishes the incidence of deterioration of renal function induced by contrast agents in patients with renal insufficiency during coronary angiographic procedures.

Deniz *et al.* (2012) showed us that NAC may be used in the cases of severe burns, not only for its effects on wound healing but also for its systemic effects. Also, Borisenok *et al.* (2012) showed that NAC (once per day for 10 days) significantly decreases manifestations of the GM nephrotoxicity.

Natasha *et al.* (2012) showed that there were reductions in nitrotyrosine and hydrogen peroxide with Nbv1 (suggesting a reduction in NADPH dependent superoxide production). In fact, Nbv1 was effective in affording functional and structural protection in this model.

Our results, showing the inhibition of tissue lipid peroxidation along with replenishment of GSH content by Nbv1 is beneficial in maintaining the oxidant -anti oxidant balance. Results of Akgullu *et al.* (2015) are in compliance with our results which proved that Nbv1 treatment significantly decreased high MDA levels in the brain, heart and liver tissues. Pires *et al.* (2007) found that Nbv1 was more efficient than atenolol as nephroprotective in renal damage induced by renal mass reduction in rats.

Furthermore, recent evidence suggests that β -NAG should more specifically be considered a marker of lysosomal activity, and negative findings in proximal tubular cell (PTC) injury indicate reductions in protein

reabsorption and degradation (Habibi *et al.*, 2011). In addition, Hayden *et al.* (2010) found that Nbv1 decreased urinary β -NAG, NADPH oxidase activity and 3-nitrotyrosine levels in PTC. These observations support the notion that Nbv1 may improve PTC reabsorption of albumin in association with the attenuation of oxidative stress and fibrosis in this rat model.

Histopathological examination of kidney sections of the different groups is coincident with the obtained biochemical data in that Nbv1 could effectively attenuate GM-induced nephrotoxicity in rats. The renal tissues of the animals treated with Nbv1 and GM showed moderate improvement in glomerular tufts. Also, many epithelial cells lining tubules with vesicular nucleolus.

Late several studies have indicated that blind use of antioxidants may have deleterious effects on human health. So, inappropriate use of antioxidant supplement will put people at risk (Rani and Yadav, 2014).

In conclusion, this study demonstrated the potential nephroprotective effect of nebevivolol against gentamicin-induced nephrotoxicity compared with the protective effect of silymarin and N-acetylcystein in rats. Hence, it is worthy to use nebevivolol as antihypertensive drug for hypertensive patients with probable nephritis. Also, it can be used to justify the unlimited and blind use of antioxidant supplements by patients suffering from hypertension accompanied by nephropathy.

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