EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 3
63-70
Year: 2019

EVALUATION OF THE POSSIBLE RENOPROTECTIVE EFFECT AND ANTIOXIDANT ACTIVITY OF MIRABEGRON ON GENTAMICIN-INDUCED NEPHROTOXICITY IN RATS

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Article Received on 23/12/2018 **Article Revised on 14/01/2019** **Article Accepted on 05/02/2019**

ABSTRACT

Introduction: Mirabegron is a novel β_3-adrenergic receptor agonist that recently approved for the management of overactive urinary bladder disease. Reactive oxygen species (ROS) have a major role in the pathogenesis of gentamicin nephrotoxicity. The aim of the present study was to investigate the renoprotective effect of mirabegron alone and in combination with N-acetylcysteine (NAC) in cases of nephrotoxicity induced by gentamicin in rats. **Materials and Methods:** 40 *Wistar* rats were divided into 5 groups. Group 1; treated with NaCl 0.2 ml i.p. Group 2; received 100 mg/kg of gentamicin i.p. for 8 days for induction of nephrotoxicity. Group 3; treated with gentamicin + NAC (500 mg/kg i.p.for 8 days). Group 4; treated with gentamicin + mirabegron (10 mg/kg orally for 8 days). Group 5; treated with gentamicin + NAC + mirabegron. After 8 days, blood samples were used for assessment of renal function. Serum nitric oxide, renal malondialdehyde (MDA) and glutathione (GSH) levels were measured. **Results:** Gentamicin caused a significant elevation of serum creatinine, urea, uric acid and nitric oxide with significant elevation of kidney MDA with reduction of GSH. Treatment with both of NAC and mirabegron each alone or in combination with each other caused restoration of renal function parameters and caused significant decrease in serum nitric oxide and MAD and increase in GSH. These changes were more marked with combination of NAC and mirabegron. **Conclusion:** Mirabegron has a modest antioxidant activity which may be responsible for its protective effect against nephrotoxicity induced by gentamicin.

KEYWORDS: Mirabegron, Gentamicin, Nephrotoxicity - N-acetylcysteine, Oxidative stress.

INTRODUCTION

Mirabegron is the first β_3-adrenargic agonist that approved to treat symptoms of hyperactive urinary bladder disease. It shows high affinity for β_3-adrenoceptors and very low affinity for β_1 and $\beta 2$-adrenoceptors.[1] It causes increase in cAMP concentrations in the urinary bladder tissue of rat and causes relaxation of the bladder.[2] B_3-adrenergic receptor is a new member of β-adrenergic receptors. It has an important role in the regulation of lipolysis in fat tissues.[3] Now, it may participate in physiology of the urinary system[4] and cardiovascular system.[5] The function of this receptor in renal tissue is still unknown. In mice, there is evidence that mRNA of β_3-receptor is expressed by the renal artery.[6] DNA analysis in rats indicated that β_3-receptors are found in the renal medulla.[7] Studies in humans demonstrated that thiazide diuretic action may be mediate in part through β_3-adrenergic receptors.[6] β_3-receptor is relatively resistant to desensitization caused by prolonged exposure to agonists.[8] β_3-agonist relatively has few side effects because of the scanty of tissues that contain this receptor in comparison with $\beta_1$and β_2-receptors.[5] In human, β_3-adrenergic receptor is the main type of receptors in the urinary bladder smooth muscular layer, but in rat urinary bladder, β_2 and β_3-receptors are the main types of receptors.[9]

N-acetylcysteine (NAC) is an exogenous antioxidant drug that has a protective effect against injury induced by oxidative stress. The mechanism underlying this protection may be related to its ability to inactivate the free radicals, increase generation of reduced glutathione or due to its effect on nitric oxide production.[10] NAC may cause vasodilation of small blood vessels of the kidney.[11]

Gentamicin is an antibacterial agent that is very useful in management of sepsis caused by gram negative bacteria but their usefulness is limited due to severe nephrotoxicity that has been seen in up to 30% of people who receive the drugs.[12] The production of excessive free radicals or the deficiency of the protective antioxidant mechanism may be responsible for the

gentamicin-induced kidney toxicity.[13] Gentamicin-induced nephrotoxicity increases many proinflammatory cytokines.[14]

There is a report that suggested that, CL 316,243 which is highly specific β3-adrenoceptor agonist had an antiobesity effect in mice with obesity induced by using of monosodium glutamate.[15] There is also a study that indicated that β3-adrenoceptor agonist that contains aryloxypropanolamine moiety may have antioxidant activity in vitro.[16] Since mirabegron is a novel β_3-adrenoceptor agonist that lack adequate pharmacological characters, so the present research is designed to evaluate the renoprotective and antioxidant activity of mirabegron alone and in combination with N-acetylcysteine in kidney toxicity caused by gentamicin in adult rat.

MATERIALS AND METHODS

1. Drugs and chemicals

Mirabegron was purchased as a commercial tablet (Betmiga® 50 mg) from Astellas Pharma Europe and dissolved in normal saline. N-acetylcysteine white power was purchased from Sigma-Aldrich, USA and dissolved in normal saline. Gentamicin obtained from Schering Plough as commercial ampules (Garamycin® 20 mg). Commercially available kits were used for assessment of renal parameters. Reduced glutathione, Ellman's reagent and malondialdehyde were purchased from Sigma Aldrich, United State of America.

2. Animals

Male *Wistar Albino* rat with a weight 160-210 g was chosen in the research. The animal's house of the University of Assiut was the source of the used rats. Rats consumed water and laboratory food *ad libitum.* Rats were kept inside laboratory for seven days adaptation before the start of research. All the animal experiments were conducted in accordance with the guide for the care and use of laboratory animals of the National Institutes of Health (NIH 1985). The research was approved by the ethics committee of the College of Medicine, University of Assiut (approval no: 17300266).

3. Induction of nephrotoxicity

Gentamicin 100 mg / Kg b.w. is injected intraperitoneally daily for eight days for induction of kidney toxicity.[17]

4. Animal grouping

Five groups of animals, eight rats in each one were used in the study. The first group injected i.p. with 0.2 ml of Na Cl and used as control group. The second one received i.p. injection of gentamicin. The third group injected with gentamicin + NAC (500 mg/kg i.p. for eight days). The fourth group injected with gentamicin + mirabegron (10 mg/kg orally using oral gavage needle for eight days). The fifth group injected with gentamicin + NAC (500 mg/kg i.p. for eight days) + mirabegron (10 mg/kg orally using oral gavage needle for eight days).
Depending on the range of doses of previous investigations, we selected the mirabegron and NAC doses.[18,19] On the eighth day, rats were decapitated after anesthetizing the animals by using of pentobarbital (50 mg / kg b.w.). Then collection of the samples of blood was done. Centrifugation of the blood for ten min at 3000 revolutions / min was done. Then storage of serum at -20 °C was performed for further assessment of nitric oxide and renal function parameters. Then, the right kidneys were removed and immediately frozen in the liquid nitrogen for the evaluation of renal oxidative stress. The left kidneys were removed and preserved in formalin 10% for hematoxylin and eosin staining and histopathological studies.

Later on, the right kidneys were weighed and homogenization in phosphate buffer was done. Then cold centrifugation for fifteen min at 10500 revolutions / min was performed. Then glutathione and MDA were measured in the kidney homogenate.

5. Renal function assessment

Using the kits which are commercially available, measurement of serum urea, uric acid and creatinine was done. These kidney parameters were used as indicators for kidney function and for assessment of kidney toxicity.

6. Evaluation of the renal oxidative stress

Determination of the renal reduced glutathione (GSH)
According to the method indicated by Boyne and Ellman,[20] the renal GSH levels were measured. Trichloroacetic acid 10 % was mixed with the renal homogenate. Then cold centrifugation at 5000 revolutions / min for ten min was performed. Disodium hydrogen phosphate buffer (pH 8.4) was mixed with the supernatant and 0.25 ml Ellman's reagent was added and then incubated for ten min. The absorbance of the color was measured spectrophotometrically at 412 nm.

Malondialdehyde (MDA) assay
According to the method described by Ohkawa and his group,[21] MDA level was measured in kidney homogenate. It was measured spectrophotometrically through colorimetric reaction with thiobarbituric acid. MDA level is a good marker for oxidative stress and lipid peroxidation.

7. Determination of serum nitric oxide

Nitric oxide (NO) oxidation causes release of stable products as nitrite. So measurement of serum nitrite is a good indicator for serum nitric oxide which is involved in several inflammatory conditions.[22] Serum nitrite can be determined using spectrophotometric method after adding Griess reagent.[23]

8. Histopathological examination

The samples of the kidney tissues were cut by a special microtome and staining was done by hematoxylin and eosin stain. Examination of the sections by the light digital microscopy was done. The damage in kidney tissue was scored as grade zero if there was normal renal

tissue, grade (+) for mild renal affection, grade (++) for moderate renal affection and grade (+++) for severe renal affection.[24]

9. Statistical analysis

Data were represented as the mean ± S.E. One way analysis of variance (ANOVA) was used to detect if there is statistical significant difference between the different groups. For multiple comparisons, Tukey's post hoc test was used. If P < 0.05, the results were considered as statistically significant differences. The analysis was done with Prism software (Graph-Pad Software, version 7).

RESULTS

1. Results of the renal function assessment

Effect of gentamicin, NAC and mirabegron on serum creatinine, urea and uric acid.

In the present study, severe nephrotoxicity appeared when the gentamicin (100 mg/kg i.p.) was used for eight days as it was evident from figures 1, 2 and 3 that showed a significant ($p< 0.05$) increase in the serum creatinine, serum urea, and serum uric acid in comparison to the saline-treated group. NAC (500 mg/kg i.p. for eight days**)** caused a significant ($p< 0.05$) nephroprotective effect as noted by the reduction of the levels of the serum urea, serum creatinine and serum uric acid in comparison with the gentamicin injected rats (figures 1, 2 and 3). Treatment with mirabegron (for 8 days) caused a significant ($p< 0.05$) nephroprotective effect against gentamicin-induced nephrotoxicity as noted by the reduction of the level of the elevated renal parameters in comparison with the gentamicin treated group but this nephroprotective effect was less marked than the nephroprotective effect induced by NAC and the reduction in serum creatinine was non-significant (figures 1, 2 and 3). The combination of NAC with mirabegron caused significant ($p< 0.05$) nephroprotective effect as noted by the reduction of the level of the elevated renal parameters in comparison with the gentamicin treated rats. However, this nephroprotective effect was higher than the nephroprotective effect induced by the NAC or mirabegron when used alone (figures 1, 2 and 3).

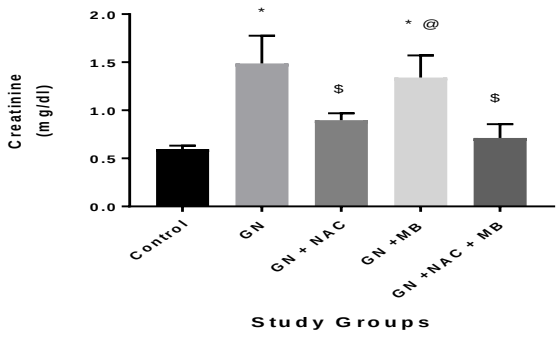

Fig. 1. Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the serum creatinine.

Group 1; received normal saline 0.2 ml i.p. Group 2; received GN i.p.100 mg/kg for 8 days. Group 3; treated with GN and NAC (500 mg/kg i.p. for 8 days). Group 4; treated with GN and MB (10 mg/kg orally for 8 days). Group 5; treated with GN and NAC (500 mg/kg i.p. for 8 days) with MB (10 mg/kg orally for 8 days).

Values are expressed as mean ± SEM. (n = 8 rats in each group)
*p < 0.05 when compared to normal control group.
$p < 0.05 when compared to GN treated group.
@p < 0.05 when compared to GN + NAC treated group.

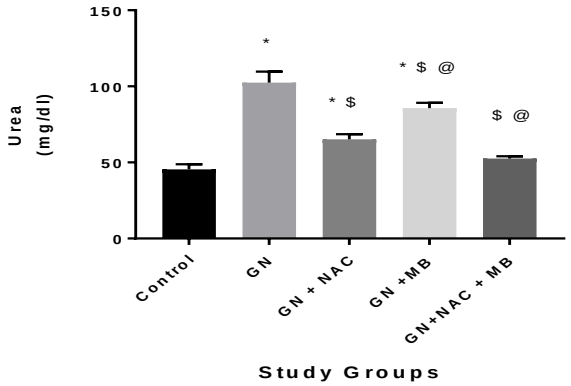

Fig. 2. Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the serum urea.

Values are expressed as mean ± SEM. (n = 8 rats in each group)
*p < 0.05 when compared to normal control group.
$p < 0.05 when compared to GN treated group.
@p < 0.05 when compared to GN + NAC treated group.

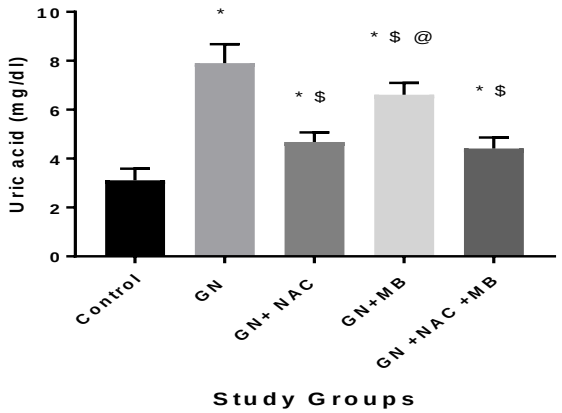

Fig. 3: Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the serum uric acid.

Values are expressed as mean ± SEM. (n = 8 rats in each group)
*p < 0.05 when compared to normal control group.
$p < 0.05 when compared to GN treated group.
@p < 0.05 when compared to GN + NAC treated group.

2. Results of the renal oxidative stress assessment

1. Effect of gentamicin, NAC and mirabegron on the reduced glutathione (GSH): A significant ($p<0.05$) reduction in GSH was caused by the administration of gentamicin for 8 days (figure 4). NAC caused a significant ($p<0.05$) elevation of the renal GSH in comparison with gentamicin treated group (figure 4). Treatment with 10 mg/kg of mirabegron caused also non-significant elevation of the renal GSH (figure 4). The combination of NAC with mirabegron caused a significant ($p< 0.05$) elevation in the GSH and this increase was more marked than the increase that caused by the NAC or mirabegron when used alone (figure 4).

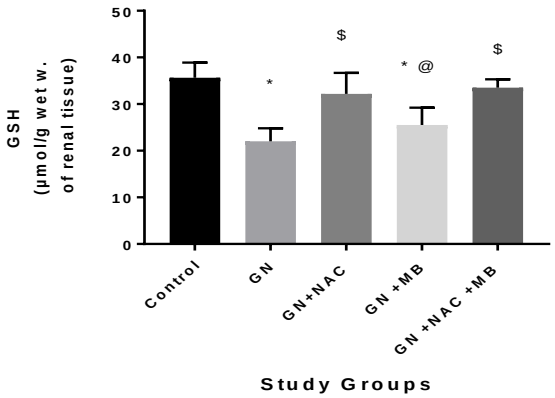

Fig. 4. Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the renal reduced glutathione (GSH).

Values are expressed as mean ± SEM. (n = 8 rats in each group)

*p < 0.05 when compared to normal control group.

$p < 0.05 when compared to GN treated group.

@p < 0.05 when compared to GN + NAC treated group.

2. Effect of gentamicin, NAC and mirabegron on the renal malondialdehyde (MDA): 100 mg/kg of gentamicin intraperitoneally when used for eight days resulted in a significant ($p< 0.05$) elevation of MDA (figure 5). 500 mg/kg of NAC resulted in a significant ($p< 0.05$) decrease in the MDA as compared to the gentamicin treated group (figure 5). Treatment with mirabegron caused also a non-significant reduction in the MDA in comparison with the gentamicin treated rats (figure 5). The combination of NAC with mirabegron resulted in a significant ($p< 0.05$) reduction of renal MDA as compared to the gentamicin treated rats (figure 5).

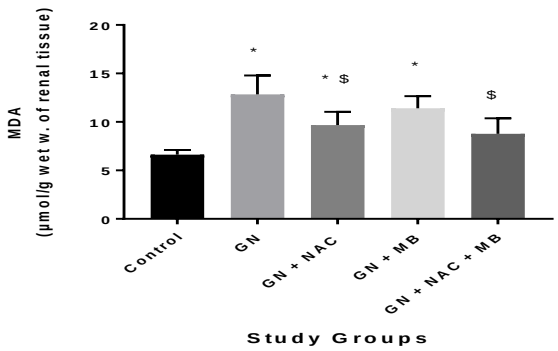

Fig. 5 Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the renal malondialdehyde (MDA).

Values are expressed as mean ± SEM. (n = 8 rats in each group)

*p < 0.05 when compared to normal control group.

$p < 0.05 when compared to GN treated group.

@p < 0.05 when compared to GN + NAC treated group.

3. Results of the serum nitrite assessment

Gentamicin caused a significant ($p< 0.05$) increase in serum nitrite (figure 6). 500 mg/kg of NAC intraperitoneally resulted in significant ($p< 0.05$) decrease in the nitrite level in comparison with the rats injected with gentamicin (figure 6). Treatment with mirabegron caused also a significant ($p< 0.05$) reduction of the serum nitrite but this reduction was less marked than the reduction that was induced by NAC (figure 6). The combination of NAC with mirabegron caused a significant ($p< 0.05$) reduction of the serum nitrite (figure 6).

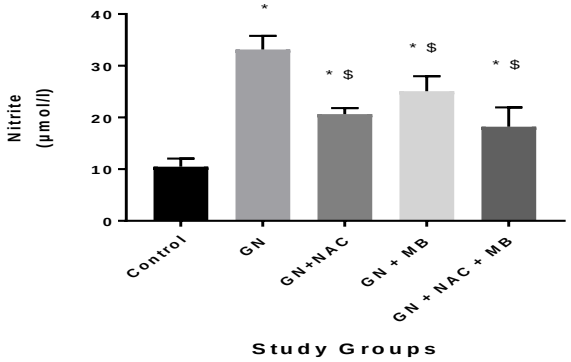

Fig. 6 Effect of Gentamicin (GN), N-acetylcysteine (NAC) and Mirabegron (MB) on the serum nitrite.

Values are expressed as mean ± SEM. (n = 8 rats in each group)

*p < 0.05 when compared to normal control group.

$p < 0.05 when compared to GN treated group.

@p < 0.05 when compared to GN + NAC treated group.

4. Histopathological results

Table 1 and figures 7-A to 7-E summarized the renal pathological changes caused by the different treatment.

Table. 1: Histopathological feature for the effect of gentamicin, NAC and mirabegron on rat kidney

Group	Vascular congestion	Inflammatory cell infiltration	Tubular necrosis	Intratubular hyaline casts
Control	-	-	-	-
Gentamicin	+++	+++	++	++
Gentamicin + NAC	+	+	-	+
Gentamicin + Mirabegron	++	++	+	+
Gentamicin + Mirabegron + NAC	+	+	-	+

Scoring was done as follows:
None (-), mild renal affection (+), moderate renal affection (++), and severe renal affection (+++).

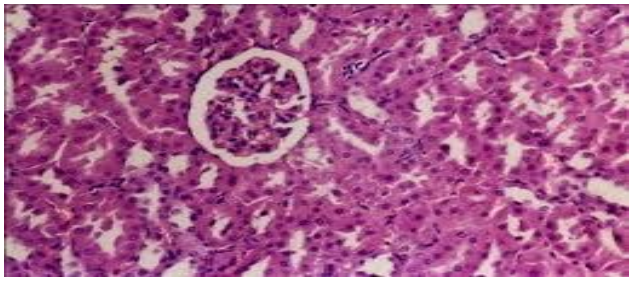

Figure. (7-A): Normal kidney of the control rat group showing the normal structure of renal parenchyma with intact glomerular tuft and renal tubule with vesicular nuclei.(H &E x 200).

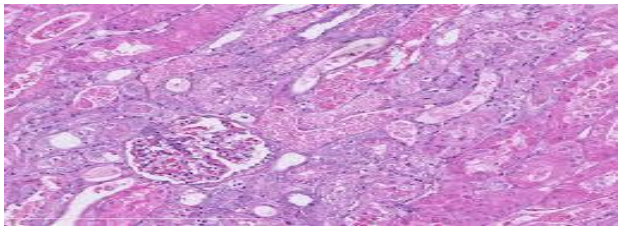

Figure (7-B): Kidney of rats treated with gentamicin showing marked thickening and vaculation in the wall of blood vessel as well as perivascular infiltration by leucocytes and atrophied glomerular tuft and renal tubule (H &E x 200).

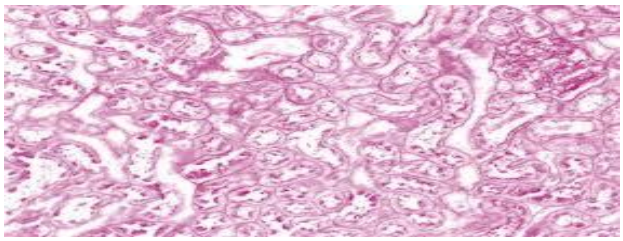

Figure (7-C): Kidney of rats treated with gentamicin + NAC showing moderate improvement of glomerular tuft and renal tubule (H &E x 200).

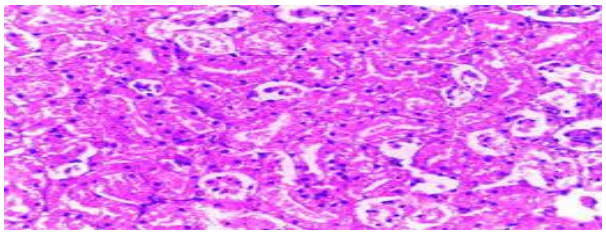

Figure (7-D): Kidney of rats treated with gentamicin + Mirabegron showing mild improvement of glomerular tuft and renal tubule (H &E x 200).

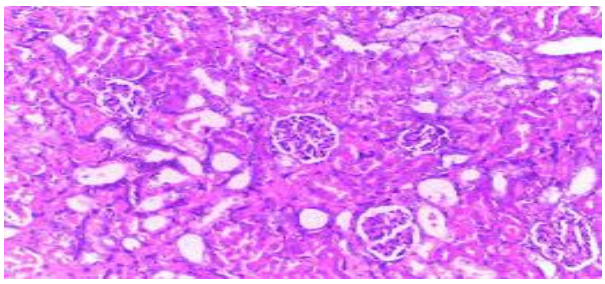

Figure (7-E): Kidney of rats treated with gentamicin +NAC + Mirabegron showing marked improvementof glomerular tuft and renal tubule (H & E x 200).

DISCUSSION

Severe elevation in renal function parameters was seen in this study after the use of gentamicin for eight days in rats. These results suggested the occurrence of kidney toxicity with renal dysfunction in the rats treated with gentamicin. Increase in the serum urea, creatinine and uric acid are signs for kidney dysfunction.[25] The gentamicin-induced renal functional abnormalities were associated with high renal oxidative stress as demonstrated with the marked reduction of the renal glutathione. Moreover, a marked elevation of the renal lipid peroxidation as demonstrated with the elevation in renal MAD was seen in the gentamicin-injected rats. These results were in agreement with the results of other investigators who demonstrated that the oxidative stress was the major factor responsible for renal toxicity of gentamicin.[26] Gentamicin toxicity was associated with an increase in the levels of MDA which is an indicator for the increase in ROS production.[27] Gentamicin increased serum nitrite which is a stable end product of nitric. This finding is in accordance with many studies that demonstrated that gentamicin may induce nitric oxide synthase enzyme causing an increase in the production of NO that elevates the levels of peroxy nitrite that may share in kidney toxicity caused by gentamicin.[26] Nitric oxide (NO) is needed for the normal renal function but its excessive production may be responsible for the damage of renal tissues.[28]

Many studies in the experimental animals indicated that gentamicin can cause kidney toxicity through changes in the structure and function of apical membrane.[29] Gentamicin can increase the generation of free nitrogen and oxygen radicals which may contribute in kidney damage through lipid membrane peroxidation,

denaturalizing effect on proteins and damaging effect on nucleic acids.[30] Antioxidant agents and antioxidant enzymes (as catalase and dismutase) can protect against kidney toxicity caused by high doses of gentamicin by converting these free radical into less toxic metabolites.[31]

These results indicated that the administration of NAC with gentamicin caused a statistically significant reduction in the elevated renal parameters. This is an indicator that NAC has a nephroprotective effect against gentamicin-induced nephrotoxicity. The results also demonstrated a decrease in renal MDA levels with NAC. Further, renal GSH level was also restored with the NAC. This is an indicator that NAC has a renal antioxidant activity which may be responsible for protection against kidney toxicity caused by gentamicin. NAC caused also reduction of the serum nitrite. This is an indicator for the reduction in the formation of the toxic peroxy nitrite which may have a role in gentamicin-induced nephrotoxicity.

As mentioned before, the nephrotoxicity induced by gentamicin is mainly due to the elevation of the levels of free radicals; NAC may cause a nephroprotection through its antioxidant activity. There were previous reports which indicated that NAC caused a restoration and regeneration of glutathione inside the cells.[32] It also caused an inhibition of oxidative effects of free radicals[33] and increased the activity of the antioxidant enzymes as dismutase.[34]

The results demonstrated that treatment with mirabegron caused a statistically significant reduction of the elevated serum urea and serum uric acid. Mirabegron caused also a non-significant reduction in serum creatinine. This is an indicator that mirabegron may have a nephroprotective effect against gentamicin-induced nephrotoxicity. Mirabegron caused a non-significant reduction in renal MDA levels. It also caused an increase in renal GSH. The results also indicated that mirabegron caused a reduction of the serum nitrite which is an indicator for the reduction in the formation of the toxic peroxy nitrite which is a potent free radical. Most of the results of the renal function tests and antioxidant activity indicated that mirabegron caused a mild improvement in the renal function tests and the drug had a modest antioxidant activity in comparison to the gentamicin treated group. There are no sufficient data that can confirm or exclude the possible antioxidant and renoprotective effect for mirabegron. However, there was a study that indicated that β3-adrenoceptor agonist that contained aryloxypropanolamine moiety may have antioxidant activity in vitro.[16]

The combination of mirabegron with NAC caused a statistically significant reduction of renal parameters in comparison to the gentamicin treated group. The reduction in renal parameters was slight higher than the reduction caused by the treatment with NAC alone. The reduction in renal MDA levels was statistically significant and was higher than the reduction caused by NAC alone. The increase in renal GSH was much higher than the increase that caused by NAC alone. The results also indicated that the combination of NAC and mirabegron caused statistically significantly reduction of the serum nitrite which was higher than that of NAC alone.

The above results indicated that the combination of NAC and mirabegron caused a slight higher antioxidant activity and renoprotection against gentamicin-induced nephrotoxicity. This is an indicator that the addition of mirabegron to NAC in cases of gentamicin induced nephrotoxicity may an additional benefit.

CONCLUSION

This study had shown that mirabegron which is a novel β3-agonist has a modest antioxidant activity and renoprotection against gentamicin nephrotoxicity in rats. The combination of NAC and mirabegron caused additional benefits in the management of kidney toxicity caused by the use of gentamicin. Further studies in human are needed to investigate any possible nephroprotective effect for mirabegron in human.

Funding

This research did not receive any grant.

Conflict of Interests

The author declares no conflict of interests in preparing this research.

ACKNOWLEDGEMENT

The author is gratefully acknowledge Dr. Hala Mostafa, Biochemistry Department, College of Medicine, Assiut University for her helping during biochemical tests and Dr. Mona Sedeek Department of Physiology, Faculty of Medicine, Aswan University, Aswan, Egypt for her kind help during the research.

REFERENCES

1. Igawa Y, Michel MC. Pharmacological profile of β3-adrenoceptor agonists in clinical development for the treatment of overactive bladder syndrome. Naunyn Schmiedebergs Arch Pharmacol, 2013; 386: 177–183.
2. Robinson D, Thiagamoorthy G, Cardozo L. A drug safety evaluation of mirabegron in the management of overactive bladder. Expert Opin Drug Saf, 2016; 15(5): 689–696.
3. Mund RA, Frishman WH. Brown adipose tissue thermogenesis: beta3-adrenoreceptors as a potential target for the treatment of obesity in humans. Cardiol Rev, 2013; 21: 265–269.
4. Chapple CR, Cardozo L, Nitti VW, Siddiqui E, Michel MC. Mirabegron in overactive bladder: a review of efficacy, safety, and tolerability. Neurourol Urodyn, 2015; 33: 17–30.
5. Dessy C, Balligand JL. Beta3-adrenergic receptors

in cardiac and vascular tissues emerging concepts and therapeutic perspectives. Adv Pharmacol, 2010; 59: 135–163.
6. Kaufmann J, Martinka P, Moede O, Sendeski M, Steege A, Fahling M, et al. Noradrenaline enhances angiotensin II responses via p38 MAPK activation after hypoxia/re-oxygenation in renal interlobar arteries. Acta. Physiol. (Oxf), 2015; 213: 920–932.
7. Rojek A, Nielsen J, Brooks HL, Gong H, Kim Y, Kwon T, et al. Altered expression of selected genes in kidney of rats with lithium-induced NDI. Am J Physiol Renal Physiol, 2005; 288: F1276–F1289.
8. Michel-Reher MB, Michel MC. Agonist-induced desensitization of human beta$_3$-adrenoceptors expressed in human embryonic kidney cells. Naunyn Schmiedebergs Arch Pharmacol, 2013; 386: 843851.
9. Fujimura T, Tamura K, Tsutsumi T, Yamamoto T, Nakamura K, Koibuchi Y, et al. Expression and possible functional role of the β3-adrenoceptor in human and rat detrusor muscle. J Urol, 1999; 161:680–685.
10. Mishima K, Baba A, Matsuo M, Itoh Y, Oishi R. Protective effect of cyclic AMP against cisplatin-induced nephrotoxicity. Free Rad Biol Med, 2006; 40: 1564–1577.
11. Heyman SN, Goldfarb M, Shina A, Karmeli F, Rosen S. N-acetylcysteine ameliorates renal microcirculation: studies in rats. Kidney Int, 2003; 63: 634–641.
12. Ali BH, Al Zaabi M, Blunden G, Nemmar A. Experimental Gentamicin Nephrotoxicity and Agents that Modify it: A Mini-Review of Recent Research. Basic Clin Pharmacol Toxicol, 2011; 109: 225–232.
13. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of Acute Kidney Injury. Annu Rev Pharmacol Toxicol, 2008; 48:463–493.
14. Geleilete TJ, Melo GC, Costa RS, Volpini RA, Soares TJ, Coimbra TM. Role of myofibroblasts, macrophages, transforming growth factor-beta endothelin, angiotensin-II, and fibronectin in the progression of tubulointerstitial nephritis induced by gentamicin. J Nephrol, 2001; 15: 633–642.
15. Yoshida T, Sakane N, Wakabayashi Y, Umekawa T, Kondo M. Anti-obesity effect of CL 316,243, a highly specific beta 3-adrenoceptor agonist, in mice with monosodium-L-glutamate-induced obesity. Eur J Endocrinol, 1994; 131 (1): 97–102.
16. Sichrovska L, Malik I, Sedlarova E, J Csollei J, Muselik J. In vitro Antioxidant Properties of Novel β3-Adrenoceptor Agonists Bearing Benzenesulfonamide Fragment. Dhaka Univ J Pharm Sci., 2013; 12(1): 23–28.
17. Hasanvand A, Kharazmkia A, Mir S, Khorramabadi RM, Darabi S. Ameliorative effect of ferulic acid on gentamicin induced nephrotoxicity in a rat model; role of antioxidant effects. J Renal Inj Prev, 2018; 7(2): 73–77.
18. Sawadaa N, Nomiya M, Hood B, Kosloy D, Zarifpour M, Andresson K. Protective Effect of a beta 3-Adrenoceptor Agonist on Bladder Function in a Rat Model of Chronic Bladder Ischemia. Europ Urol, 2013; 64: 664–671.
19. Abdelrahman AM, Al Salam S, AlMahruqi AS Al Husseni IS, Mansour MA, Ali BH. N-acetylcysteine improves renal hemodynamics in rats with cisplatin-induced nephrotoxicity. J Appl Toxicol, 2010; 30: 15–21.
20. Boyne AF, Ellman GL. A methodology for analysis of tissue sulfhydryl components. Anal Biochem, 1972; 46(2): 639–653.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351–358.
22. Tynan RJ, Weidenhofer J, Hinwood M, Cairns MJ, Day TA, Walker FR. A comparative examination of the antiinflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. Brain Behav Immun, 2012; 26: 469–479.
23. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 2001; 5: 62–71.
24. Randjelovic P, Veljkovic S, Stojiljkovic N, Jankovic-Velickovic L, Sokolovic D, Stoiljkovic M, et al. Salicylic Acid Attenuates Gentamicin-Induced Nephrotoxicity in Rats. The Scientific World J, 2012; Article ID 390613: 1–6.
25. Patel Manali B, Deshpande S, Shah G. Evaluation of efficacy of vitamin E and N-acetyl cysteine in gentamicin-induced nephrotoxicity in rats. Ren Fail, 2011; 33(3): 341–347.
26. Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MA, Hernandez-Pando R, et al. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidant enzymes. Free Radic Biol Med., 2000; 29(7): 602–611.
27. Passauer J, Pistrosch F, Büssemaker E. Nitric oxide in chronic renal failure. Kidney Int, 2005; 67(5): 1665–1667.
28. Fujihara CK, Sena CR, Malheiros DM, Mattar AL, Zatz R. Short-term nitric oxide inhibition induces progressive nephropathy after regression of initial renal injury. Am J Physiol Renal Physiol, 2006; 290(3):632–640.
29. Hishida A, Nakajima T, Yamada M, Kato A, Honda N. Roles of hemodynamic and tubular factors in gentamicin-mediated nephropathy. Ren Fail, 1994; 16: 109–116.
30. Nagai J, Takano M. Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet, 2004; 19: 159–170.
31. Skopicki HA, Zikos D, Sukowski EJ, Fisher KA, Peterson DR. Gentamicin inhibits carrier-mediated dipeptide transport in kidney. Am J Physiol, 1996; 270: F531–V538.
32. Auroma OI, Halliwell B, Hoey BM. The antioxidant action of N-acetylcysteine: its reaction with

hydrogen peroxide, hydroxyl radical, superoxyde, and hypochlorous acid. J Free Radicals Biol Med, 1989; 6: 593-597.
33. Galley HF, Howdle PD, Walker BE. The effect of intravenous antioxidants in patients with septic shock. Free Radical Biol Med., 1997; 23: 768-774.
34. Schillier HJ, Reilly PM, Bulkley GB. Antioxidant therapy. Crit Care Med., 1993; 21: 92-102.