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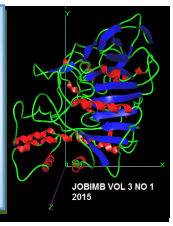


# Antioxidantal and Histopathological effects of Mirazid on Gentamicin-Induced Renal Damage in Rats

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**ABSTRACT**

The present study evaluates the biochemical effect of mirazid(MZ) in rats of GMtamicin(GM)-induced renal damage. Albino male rats *(Rattus norvegicus*), weighing 40–50 g were divided into 6 groups; normal saline, orally trated mirazid 10 mg/kg, GM 100mg/kg, intraperitoneally (i.p) for 10 days, MZ at 2.5, 5, and 10 mg/kg, per oral for 10 days with the same concentration of GM, MZ administered concurrently with GM for 10 days. We investigated the effects of MZ on GM-induced nephrotoxicity. GM treatment caused nephrotoxicity as evidenced by marked elevation in serum Cr and blood urea (152.3 ± 8.6 mg/dL, 1.6 ± 0.12 mg/dL resp) when compared to the saline treated group. GM administration increased renal MDA and NO GMeration but decreased SOD and CAT activities, and GSPx (glutathione peroxidase) compared to normal control. MZ administration with GM injections caused significantly decreased MDA, NO GMeration and increased SOD, CAT activities and GSPx activity when compared with GM alone. Histopathological analysis revealed epithelial loss with intense +granular deGMeration in GM treated rats, whereas MZ decrease the severity of GM-induced renal damage. To conclude, our data suggest that MZ exhibits some antioxidantal effect against GM nephrotoxicity, as it has a potent scavenger of free radicals to prevent the toxic effects of GM at both the biochemical and histological level and further studies on its mechanism of action and side effects are wanted.

## INTRODUCTION

Aminoglycosides continue to represent highly effective antimicrobial agents since their introduction about more than 50 years ago [1,2]**.** Despite the introduction of highly potent, wide-spectrum antibiotics, aminoglycosides are still considered to be very important against many life-threatening infections especially against gram-negative bacterial infections [3]. They are very commonly used antibiotics worldwide because of certain properties as rapid bactericidal effects, clinical effectiveness, a low rate of true resistance, synergism with other β lactam antibiotics, and low cost of therapy [4,5]**.** The most widely used drug in this category is Gentamicin (GM) [6]**.** Amajor complication of GM treatment is nephrotoxicity, accounting for 10–20% of all cases of acute renal failure (ARF) according to experimental results [7,8]. Nephrotoxicity induced by GM is a complex phenomenon characterized by increase in plasma creatinine and urea levels and severe proximal renal tubular necrosis, followed by deterioration and renal failure [2,9]. Although the pathogenesis is still not well understood, the toxicity of aminoglycosides including GM is believed to be related to the Generation of reactive oxygen species (ROS) in the kidney [2,6]**.** The cellular antioxidant status determines the susceptibility to oxidative damage and usually alters in response to oxidative stress [10]**.**

Mirazid(MZ), a drug introduced to the local Egyptian market over the last decade, is prepared from (Arabian or Somali) myrrh, an oleo–gum resin obtained from the stem of

Commiphora molmol Engier and some other thorny trees in the family Bursearceae [11]. Myrrh itself has been used for the treatment of sore throat, bleeding gums, chronic pharyngitis, amenorrhoea, diarrhoea and stomach complaints [12,13]. MZ has been marketed in Egypt as an antischistosomal drug, following reports of its activity against schistosome infections in [14]and humans [15,16]. Treatment of the infected mice with *S.mansoni* with Citrus reticulate extract and MZ ameliorates the levels of the hepatic antioxidants to a great extent. Lipid peroxides were greatly reduced. GSH, levels of Vit C, E and CAT activity were increased [17]. So we may use the antioxidantal effect of MZ in treatment of many diseases caused free radicals.

## MATERIALS AND METHODS

### Animals

Albino rats (Rattus norvegicus), 60 adult males, weighting from 50-60gm, from Theodor Bilharzias Research Institute, ministry of scientific research, and housed in groups of 10 rats in a cage, maintained at the animal house of Genetic Engineering and Biotechnology Research Institute (GERI), Sadat City, Sadat City University . They were kept in laboratory under constant condition of 25°C, and 12h light / dark cycle for one week before the experimental work. All experiments were carried out in accordance with protocol approved by the local experimental animal ethics committee.

### Drugs

Mirazid (Commiphora extract) capsules were purchased. MZ was obtained from the producing company Pharco Pharmaceuticals Company, Alexandria, Egypt. Each capsule containing 300 mg purified Commiphora extract as soft gelatin forms. Mirazid was used in a freshly prepared 2% suspension with saline. Gentamicin (Gentamicin Sulphate) vials were purchased from Sigma company, U.S. A. Each vial contains 80 mg per ml of gentamicin sulphate.

### Experiment Protocol

The experimental animals were divided randomly into six groups, ten rats in each group, normal saline, orally trated MZ 10 mg/kg, GEN 100mg/kg, intraperitoneally (i.p) for 10 days, MZ at10, 5, and 2.5 mg/kg, per oral for 10 days with the same concentration of GM, MZ administered concurrently with GM for 10 days.

### Sample Collection and Biochemical Assays

After 24 hr from the last injection, rats were sacrificed, two blood samples collected by cardiac puncture. Serum samples used for determination of Serum urea, Creatinine according to urease and Jaff´e methods, respectively. Serum NO estimation according to the method of the Biodiagnostic nitrite assay kit, Biodiagnostic company,Dokki, Egypt. Kidney was isolated from each rat, washed in 0.9 % saline; one kidney was frozen at - 80°C till further enzymatic analysis. EDTA sample was centrifuged then plasma was used for determination of CAT activity was determined according to Aebi's method [18]. The erythrocytes (red for determination of glutathione peroxidase (GPX) and super oxide dismutase (SOD) according to standard methods [19,20] using reagents from Biodiagnostic Company, Dokki, Egypt. Kidney tissue was homogenized in ice-cold 150 mM KCl for determination of MDA, according to [21]. Reagents were from Biodiagnostic Company, Dokki, Egypt.

### Histological assessment

Kidneys from rats of all the groups were fixed in 10% formaldehyde, dehydrated in graded alcohol, and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin–eosin (H&E) for light microscopic analyses. The slides were coded and were examined by a histopathologist who was ignorant about the treatment groups.

## RESULTS

Data represented in Table (1) show the results of serum urea and creatinine of control and experimental groups of rats treated with GM and MZ. Marked significant elevation ($p < 0.05$) in both urea and creatinine were observed in the group 3, GM intoxicated rats when compared with group 1, control rats. Activities of urea and creatinine in serum were significantly ($p < 0.05$) maintained at near normal levels in the group 4 rats, pre treated with GM plus MZ (10mg/kg B.W). Non significant ($p > 0.05$) restore to normal levels in the group 5 and 6, rats pre-treated with GM and MZ (5 and 2.5mg/kg B.W) respectively compared to group 3. Group 2, rats administered with MZ alone did not show any changes when compared to group 1, control group.

Data represented in Table (2) show the results of Malondialdhyde (MDA) conc in kidney tissues and Nitric Oxide (NO) in serum of control and experimental groups of rats treated with GM and MZ. Marked significant elevation ($p < 0.05$) in conc of tissue MDA and serum NO were observed in the group 3, GM intoxicated rats when compared with group 1, control rats., also conc of tisuue MDA and serum NO show significant ($p < 0.05$) in group 4 and 5 rats pre treated with GM and MZ (10 and 5mg/kg B.W) respectively and non significant ($p > 0.05$) in group 6 rats pre treated with GM and MZ (2.5mg/kg B.W) compared to group 3. Group 2, rats administered with MZ alone did not show any changes when compared to group 1, control rats.

Data represented in Table (2) show the results of the activities of antioxidant enzymes SOD, GPx in erythrocyte and CAT activity in plasma of control and experimental groups of rats treated with GM and MZ. Marked significant decrease ($p < 0.05$) in the activities of these enzymes were observed in the group 3, GM intoxicated rats when compared with group 1, control rats., also activities of these enzymes show high significant elevation ($p < 0.05$) in group 4 rats treated with GM and MZ (10 mg/kg B.W) compared to group 3. Moderate significant elevation ($p < 0.05$) in the activity of GPx in group 5, rats pre treated with GM plus MZ (5 mg/kg B.W) and low significant ($p<0.05$) in group 6, rats pre treated with GM and MZ (2.5 mg/kg B.W) compared to group 3. Moderate significant elevation ($p < 0.05$) in the activity of SOD in group 5, rats pre- treated with GM and MZ (5 mg/kg B.W) and non significant ($p > 0.05$) in group 6, rats pre- treated with GM and MZ (2.5 mg/kg B.W) compared to group 3. Activity of plasma CAT gives non significant ($p > 0.05$) in the in group 5, 6 rats pre- treated with GM and MZ (5 and 2.5 mg/kg B.W) respectively compared to group 3. Group 2, rats administered with MZ alone did not show any changes when compared to group 1, control rats in the activities of SOD and CAT, but give significant elevation of ($p < 0.05$) in GPx. The histological changes in the kidney of all the groups were graded and the results are expressed in (Figure 1). It is further evident from the microscopical study of kidney sections that the kidneys from the saline treated appeared histologically normal (Figure 1.A) whereas the GM-treated group showed tubular epithelial loss with intense granular degeneration involving >50% renal cortex as shown in (Figure 1.C). In addition to the tubular epithelial loss, some of the tubular epithelium contains tubular casts. The histomorphology of rats treated with MZ (10 mg/kg) alone at

high dose appeared histologically normal as shown in (Figure1.B). The histomorphology of rats treated with MZ (10 mg/kg) showed moderate tubular epithelial degeneration with desquamation in patchy areas of the renal cortex. Treatment with the two lower doses of MZ (5mg/kg) appeared to mitigate the severity of the GM treatment-induced renal necrosis whereas the high dose (2.5 mg/kg) treatment almost completely protected the histological features of kidney from GM-induced alterations as shown in (Figure 1.D,E,F).

Table.1: Effect of GM different concentrations of MZ on serum urea and creatinine among different studied groups.

| Analytical parameter | Group I (n=10) | | Group II (n=10) | | Group III (n=10) | | Group IV (n=10) | | Group V (n=10) | | Group VI (n=10) | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| Urea mg/dl | 57.3# | 2.5 | 57.3 | 3.1 | 152.3*# | 8.6 | 59* | 1 | 65* | 4.6 | 100.3* | 2.5 | <0.05 |
| Creatinine mg/dl | 0.3# | 0.03 | 0.4 | 0.01 | 1.6*# | 0.2 | 0.5* | 0.02 | 0.5* | 0.05 | 0.9* | 0.05 | |

Table. 2: Effect of GM and different concentrations of MZ on erythrocytes SOD, GPx , plasma CAT, kidney MDA and serum NO among different studied groups.

| Analytical parameters | Group I (n=10) | | Group II (n=10) | | Group III (n=10) | | Group IV (n=10) | | Group V (n=10) | | Group VI (n=10) | | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| (SOD) (U/g Hb | 26.4# | 1.5 | 29.7 | 1 | 11.8*# | 0.7 | 26.2* | 2.4 | 23.5* | 2.7 | 14.4* | 2.1 | <0.05 |
| (GPx) (mU/g Hb) | 19.3# | 0.2 | 21.7 | 1.1 | 9.6*# | 0.4 | 18.8* | 1.3 | 16.8* | 0.7 | 12* | 0.1 | |
| Plasma (CAT) (U/L) | 61.6# | 4.5 | 61.7 | 3.4 | 34.1*# | 2.8 | 55.5* | 3.3 | 41.4* | 1.4 | 38.4* | 1.6 | |
| MDA (nmol/ml) | 60.6# | 0.6 | 60.3 | 1.2 | 80.5*# | 0.5 | 63.7* | 1.8 | 66* | 2.6 | 79.7* | 1 | |
| NO (µmol/L) | 33.6# | 3.3 | 32.7 | 1.7 | 58.3*# | 2.3 | 41* | 2.8 | 43.9* | 3.5 | 54.8* | 4.2 | |

Group I: Control, Group II: Rat treated with MZ (10mg/kg B.W), Group III: Rat treated with GM (100mg/kgB.W), Group IV: Rat treated with GM + MZ (100mg/kg B.W and 10mg/kg B.W respectively) Group V: Rat treated with GM + MZ (100mg/kg B.W and 5mg/kg B.W respectively) Group VI: Rat treated with GM + MZ (100mg/kg B.W and 2.5mg/kg B.W respect) #: Comparisons are made between group I & group III, (*): Comparisons are made between group III & group IV, V, VI and P: is considered significant when < 0.05

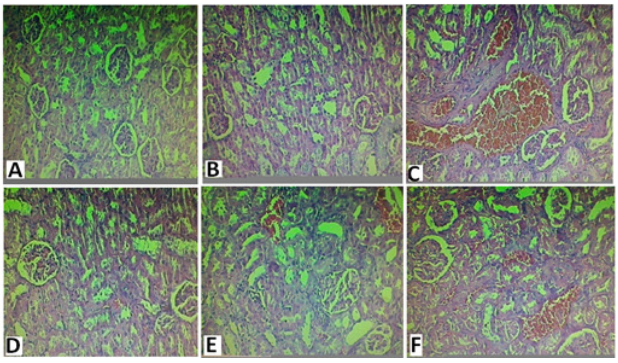


Figure (1): Histological changes in the kidney of rats; (A) control group, (B) MZ group, (C) GM group, (D) GM +MZ 100mg/10mg group, (E) GM +MZ 100mg/5mg group, (F) GM +MZ 100mg/2.5mg group.

## DISCUSSION

Results of the present study showed that interperotoneial (i.p) administration of 100 mg/kg body weight of GM daily for 10 days led to a significant increase in serum urea and creatinine levels as compared to those of the control group. The elevation in serum urea and creatinine levels in GM treated rats is considered as significant markers of renal dysfunction (glomerular damage marker). These findings are in agreement with other reports [22–25]. However, our results are in contrast with that of Guidet and Shah [26] who did not observe any change in these parameters following treatment with gentamicin (100 mg/kg/day) for 5 consecutive days. In our study, a dose of 100 mg/kg/day gentamicin for 10 days was administered to rats as opposed to 5-day treatment in the study of Guidet and Shah [26]. Our results are in line with the observation that a decline in glomerular filtration rate and Increase in serum creatinine and urea are not usually apparent until 10 days of treatment with GM [27]. In addition, these results showed that there is a significant decrease in urea and serum creatinine level in rats that were orally treated with MZ (10 mg/kg B.W) daily for 10 days as compared to those of GM-treated group, and shows no significant changes in urea and creatinine when compared to control group. In addition, we showed that there are different changes in the level of urea and creatinin in groups treated with MZ plus GM, as there are significant decrease ($p< 0.05$) in urea and creatinine level at high concentration of MZ (10 mg/kg B.W) better than at concentration 5 mg/kg body wt, and non significant decrease ($p> 0.05$) at concentration 2.5 mg/kg bwt when compared to GM intoxicated group. These results are in accordance with that obtained by Hanan [17] that treatment of rats infected with bilharzias (*S. mansoni*) by MZ and citrus reticulate extract showed enhancement levels of kidney functions.

A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models. In GM-treated rats, a significant increase ($p<0.05$) in lipid peroxidation products malondialdehyde (MDA) suggesting that the involvement of oxidative stress has been reported, and there is a role of lipid peroxidation in GM-induced acute renal failure. Biological membranes contain a large amount of polyunsaturated fatty acids, which are particularly susceptible to peroxidative attacks to produce lipid peroxides. Lipid peroxides have been used as a sensitive indicator of oxidant-induced cell injury [28]. Moreover, treatment of rats with hydroxyl radical scavengers has been shown to protect against GM induced acute renal failure. Also GM induced nephrotoxicity is proposed to be due to the overproduction of nitric oxide (NO). Results of the present study showed that injection of albino rats with 100 mg/kg body weight of GM daily for 10 days caused a significant elevation in the level of renal MDA and significant increase ($P< 0. 05$) in NO with significant decrease ($P< 0. 05$) in the level of renal CAT, SOD activities and GPx as compared to those of normal group. The reduction in the activities of antioxidant enzymes could reflect the harmful effect of GM. This is in agreement with Abdel-Zaher et al. [29], Shifow et al., [22], when studied the protective effect of Melatonin, a pineal hormone against GM nephrotoxicity and Vijay Kumar et al. [30], when studied the protective effect of Probucol against GM-induced nephrotoxicity in rats, and also in agreement with Preethi et al. [25] when was studied the protective effect of urosolic acid against GM nephrotoxicity. Our study showed that the activities of antioxidant enzymes SOD, GPx activity in erythrocyte and CAT activity in plasma of control and experimental groups of rats treated with GM and MZ. Marked significant decrease ($p < 0.05$) in the activities of these enzymes were observed in GM- treated group when compared with control rats., also activities of these enzymes show significant elevation ($p < 0.05$) in rats treated with GM and MZ (10 mg/kg B.W) group compared to GM- treated group. Significant elevation ($p < 0.05$) in the activity of GPx in rats of groups treated with GM and MZ (5 and 2.5 mg/kg B.W) respectively compared to GM- treated group. Non significant elevation ($p > 0.05$) in the activity of plasma CAT in rats of groups treated with GM and MZ (5 and 2.5 mg/kg B.W) respectively compared to GM- treated group. Significant elevation ($p < 0.05$)

in the activity of SOD in rats of group treated with GM and MZ (5 mg/kg B.W) and non significant ($p > 0.05$) in rats of group treated with GM and MZ (2.5 mg/kg B.W) compared to GM-treated group. rats of group administered MZ alone did not show any changes when compared to normal control in the activities of SOD and CAT, but give significant elevation of ($p < 0.05$) GPx. This is in agreement with Hanan [17], that treatment of rats infected with Bilharzia (*S. mansoni*) by MZ and citrus reticulate extract shows enhancement levels liver antioxidants to a great extent.

Meanwhile, MZ inhibited MDA production in renal cells induced by GM and decrease the level of NO production. In GM + MZ 100mg/10mg/kg B.W group it was clear that there is a significant decrease with a P value < 0.05compared to GM-treated group. In addition, amounts of tissue MDA and serum NO show significant decrease ($p < 0.05$) in rats of groups treated with GM and MZ (10 and 5mg/kg B.W) respectively and non significant ($p > 0.05$) in rats treated with GM and MZ (2.5mg/kg B.W) compared to group 3. Group 2, rats administered MZ alone did not show any changes when compared to normal control. This is in agreement with Hanan [17] that treatment of rats infected with bilharzia (*S. mansoni*) by MZ and citrus reticulate extract shows enhancement levels liver antioxidants to a great extent, also agree with El-Rigal and Hetta [31]. The nephrotoxic effects of GM seem to be related to the generation of destructive ROS. In these cells, ROS have been implicated in a wide range of biological functions, but they can express both beneficial and highly toxic effect on cellular homeostasis [23]. ROS have been proposed as a cause of cell death in many different pathological states as well as in glomerular disease, in renal ischemia and reperfusion injury [2].The protective effect might be due to ability of MZ to inhibit hydrogen peroxide-induced oxidative injury in renal cell line [23]. One of the main side effects of GM is that it increased apoptosis in mesangial cells in vivo and also in vitro. This apoptosis seems to be mediated by an elevation in ROS production (at least, O2-). Simultaneously, GM induces mesangial cell proliferation and apoptosis (mainly in the mesangium).

## CONCLUSION

This study provides scientific evidence of the nephroprotective effects of orally administered mirazid in an albino rat model employing gentamicin as a toxicant that directly induces renal damage. It further proposes that observed protective effects of mirazid in gentamicin nephrotoxicity could be attributed to its well-known antioxidant potential, and the best renoprotective concentration of mirazid is 10 mg/kg body weight and not less than this dose for best free radical scavenger effect of mirazid.

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