

EVALUATION OF THE POSSIBLE PROTECTIVE EFFECT OF METFORMIN IN CISPLATIN - INDUCED NEPHROTOXICITY IN ADULT MALE ALBINO RATS

By

Mohammed Assasa*, Sayed Abd El-Raheem, Abd El-Lateef Said***, and Ashraf I. Hassan***

Departments of Forensic Medicine*, Pathology** and Pharmacology***, Faculty of Medicine, Al Azhar University, Cairo

ABSTRACT

Backgrounds: Clinical use of cisplatin is limited by its nephrotoxicity as cisplatin -induced oxidative stress which leads ultimately to kidney dysfunction.

Objective: Evaluation of the nephron-protective effect of metformin against nephrotoxicity induced by cisplatin in adult albino rats.

Materials and Methods: Sixty adult male albino rats were divided randomly into 6 equal groups: **Group I** (control group) was injected with 1 ml isotonic saline solution intraperitoneally (I.P). **Group 2** was injected with single dose of cisplatin I.P 3.5mg/kg. **Group 3** was injected with single dose of cisplatin I.P 7.5 mg/kg. **Group 4** was given an oral dose of metformin (350 mg/kg /day for 10 days). **Group 5** was given 100 mg/kg/day metformin for 3 days before cisplatin injection I.P at a dose of 7.5 mg/kg on day four, and metformin continued for 7days after. **Group 6** was given 350 mg/kg/day metformin for 3 days before cisplatin injection I.P at a dose of 7.5 mg/kg on day four, and metformin continued for 7days after. Body weight of each rat was recorded on day 1, 4, 7 and 10 and average weight for each group was calculated. At the end of specified duration (10 days), rats were sacrificed and both kidneys were excised, processed and stained with Hematoxylin and Eosin for histopathological study and with Periodic Acid-Schiff (PAS) and Mallory's stain stains for histochemical study.

Results: Rats injected with high dose of cisplatin (7.5 mg/kg) showed more loss of average body weight and higher blood urea nitrogen (BUN) and serum creatinine (Cr) levels than those rats injected with 3.5 mg/kg cisplatin. Rat group injected with high dose of cisplatin (7.5 mg/kg) and given low dose metformin (100 mg/kg/day) showed less loss of average body weight and improved renal functions than rat group given high dose of cisplatin (7.5 mg/kg) and high dose of metformin (350 mg/kg/ day). Histopathological examination with H&E and histochemical examination with PAS and Mallory stains revealed mild glomerular, tubular and interstitial changes in cisplatin low dose group compared to cisplatin high dose group which showed more renal damages. Group given metformin low dose + cisplatin high dose showed less renal damage than those given high dose of cisplatin+ high dose metformin .

Conclusion: Metformin has protective effects against cisplatin -induced nephrotoxicity in adult male albino rats.

Key words: Cisplatin, nephrotoxicity, metformin.

INTRODUCTION

Cisplatin is an important chemotherapeutic agent useful in the treatment of several cancers. Several side effects of cisplatin have been reported, mainly nephrotoxicity and myelosuppression which limit its clinical use (Florea and Büsselberg, 2011). Inflammation, oxidative stress injury, and apoptosis probably explain part of Cisplatin nephrotoxicity, primarily in the proximal tubular epithelium (Chirino and Pedraza-Chaverri, 2009). Cisplatin or its metabolites may be absorbed by kidney tubular cells through organic cation transporters located on the baso-lateral side of the tubular cells, which will lead to subsequent tubular cell death including apoptosis and necrosis (Gabbiani *et al.*, 2010). Reactive oxygen species production, depletion of antioxidant systems and stimulation of renal accumulation of lipid peroxidation products are the main mechanisms associated with cisplatin-induced nephrotoxicity (Peres and da Cunha, 2013). Cisplatin-induced nephrotoxicity is associated with structural and functional damage to the mitochondria (Zsuzsanna *et al.*, 2012). Cisplatin also binds to DNA and disrupts DNA function (Dasari and Tchounwou, 2014).

Many researches were targeted to find a substance to ameliorate toxic effects of cisplatin on kidney through antagonizing mechanism of cisplatin action. Rutin which has an anti-oxidant and anti-inflammatory effect via decreasing the oxidative stress, and repairing the histopathological changes was tested. It showed minimal histopathological findings in the form of minimal interstitial

congestion and minimal tubular injury, while glomeruli and the blood vessels appeared normal (Alhoshani *et al.*, 2017). Garlic extract and metformin (MF), alone or in a combination, tried to ameliorate gentamycin induced tubular toxicity (Rafieian-Kopaei *et al.*, 2013). Ceftriaxone which had anti-fibrotic potential acts as a nephron-protective agent and could be used as adjuvant therapy to improve cisplatin-induced nephrotoxicity, as Ceftriaxone given before cisplatin treatment improved renal function biomarkers and renal histoarchitecture (Abdel-Daim *et al.*, 2017).

Metformin treatment was found to restore significantly diabetic nephropathy-induced oxidative stress mRNA levels in Streptozotocin diabetic nephropathy (Alhaidar *et al.*, 2011). Various studies had emphasized the anti-inflammatory and antioxidant role of metformin through multiple mechanisms. So, metformin can be an appropriate treatment option for many diseases, when inflammatory processes and oxidative stress play a role in their pathogenesis (Dehkordi *et al.*, 2019). Metformin was found to protect against tubular injury by restoring the biochemical alterations. Moreover, metformin protected podocytes in diabetic nephropathy in rats (Kim *et al.*, 2012).

(The present study aimed to investigate the proved protectivity of metformin in different doses of cisplatin- induced nephrotoxicity).

MATERIALS AND METHODS

Animals: This study was done on sixty adult male albino rats of local strain; weighing 200 ± 20 g and aging 70 days

which were obtained from the breeding colony maintained at the animal house of the Nile Company for pharmaceuticals, Cairo, Egypt. All animals procedures were reviewed and approved by the Animals ethics committee, Al-Azhar University, based on the code of practice for the care and use of Animals for Scientific purposes, National Committee for Research, All animal experiments were carried out according to the guidelines and approval of Institutional Animal Ethics Committee. Rats were housed in the animal facility of Faculty of Medicine, Al-Azhar University under the normal conditions, each two rats were housed in a clear stainless steel cages. Each cage sized 20X32X20 cm. Rats were kept under constant and controlled temperature ($23 \pm 3^{\circ}\text{C}$), humidity (about 60%), with normal light/ dark cycle and fed on a standard rat diet and water ad libitum. Rats were acclimated and monitored under lab conditions for a week.

Chemicals and stains:

Cisplatin: was purchased from EMIC pharmaceutical industry (Cairo Egypt) in the form of parenteral vial with a concentration of 50mg/ml. Dose was calculated according to the body weight of each rat.

Metformin hydrochloride (Glucophage): was purchased from Minipharma Merck Serono Company (500 mg metformin hydrochloride tablet). Dose was calculated according to the body weight of each rat.

Periodic Acid-Schiff (PAS): (Product Code: AR165 Staining Interpretation: PAS-positive structures: Magenta, Nuclei: Blue Background: Pink. Control Tissue: Kidney for basement membrane, Dako

Corporation, Denmark). PAS was used to demonstrate the glomerular basement membrane (BM), tubular BM, brush borders, cell borders.

Mallory's stain kit (Staining Interpretation: Collagen fibrils: Deep Blue, Nuclei: Red, Erythrocytes: Gold yellow, Control Tissue: prostate, Dako corporation, Denmark). Mallory's stain was used to demonstrate the collagen fibres in glomerular and tubular BM, and in the interstitium.

Experiment design: Rats were randomly divided into six equal groups:

Group 1: (Control group) was injected with 1 ml isotonic saline solution intraperitoneally (I.P).

Group 2: was injected with cisplatin I.P single dose 3.5 mg/kg on 4th day.

Group 3: was injected with cisplatin I.P single dose 7.5 mg/kg on 4th day.

Group 4: was given an oral dose of metformin (350 mg/kg /day) for 10 days.

Group 5: was given an oral dose of metformin 100 mg kg/day for 10 days starting 3 days before giving cisplatin injection I.P at a dose of 7.5 mg/kg on 4th day and metformin was given for 7 days after.

Group 6: was given an oral dose of 350 mg kg/day metformin for 3 days before cisplatin injection I.P at a dose of 7.5 mg/kg on day four, and metformin was given for 7days after.

Body weight of each rat was recorded on day 1, 4, 7 and 10. Average weight for each group was calculated. Blood urea nitrogen (BUN) and creatinine (Cr) levels were measured by a colorimetric method

using commercial kits on an auto analyzer. On day of sacrificing, serum samples were obtained to measure blood urea nitrogen (BUN) and serum creatinine (Cr) for all the rats (*Amini et al., 2012*). At the end of specified duration (10 days) rats were sacrificed and both kidneys were excised, washed with saline and fixed in 10% neutral buffered formalin for 24 hours. They were processed in a sequence of ethanol solutions and finally embedded in paraffin wax blocks. Tissues blocks were sectioned at thickness of 4 μ m, followed by deparaffinization by xylene

and stained with Hematoxylin & Eosin (H&E), Periodic Acid-Schiff (PAS) and Mallory stains.

Statistical Analysis:

All the statistical analysis were processed using Statistical Program of Social Sciences (SPSS) for windows (version 17, SPSS Inc., Chicago, IL, USA), Values of the measured parameters were expressed as mean value \pm standard deviation (SD).

RESULTS

Rat body weight: as seen in table (1) both groups II and III shows that average rat body weight was progressively decreased after giving cisplatin injection. On giving

cisplatin/ metformin in group V and group VI obvious less average body weight decrease was noted.

Table (1): Average Recorded Rat Body Weight of different groups (9)

Days \ Groups	Group I	Group II	Group III	Group IV	Group V	Group VI
Day 1	244	243.6	244.2	247	240.2	246
Day 4	251.4	239.5	235	251.4	238.2	241.2
Day 7	256.4	230	230.5	253.2	238	238.6
Day 10	260	227.2	225	255	236.6	237.5

Biological markers: Blood Urea nitrogen (BUN) and serum creatinine (Cr) levels were markedly increased in both groups II and III as seen in table (2) compared to control group I levels. More increase in BUN and serum Cr on increasing dose of

injected cisplatin as seen in group III than that seen in group II. An obvious decrease in levels of BUN and serum Cr in groups V and group VI compared to group II and group III on giving metformin with cisplatin.

Table (2): Blood Urea nitrogen (BUN) and Creatinine

Groups	Serological Parameters	BUN (mg/dl)	Serum Creatinine (mg/dl)
Group I		22.6 ± 6.3	0.60± 0.3
Group II		56.7± 5.5	1.34± 0.4
Group III		66.8± 6.7	2.23± 1.4
Group IV		46.4± 2.2	1.15± 0.02
Group V		51.2± 3.2	1.26± 0.23
Group VI		59.6± 2.6	2.1± 0.3

Histopathological results:

Light microscopical examination of H&E stained slides of kidneys showed the following:

- **Group 1 (Control):** normal histological structure of cortex and medulla (Figure 1 & Table 3).
- **Group II:** (cisplatin low dose): Mild glomerular deformity and hypercellularity with mild narrowing of Bowman's spaces. Tubular epithelium was markedly oedematous with scattered necrotic\ apoptotic epithelial cells. The interstitium showed markedly dilated congested blood vessels (Figure 2 & Table 3)
- **Group III:** (cisplatin high dose): Marked cortical necrosis, marked deformity and hypercellularity with obliterated Bowman's spaces were noted in viable glomeruli. Tubular epithelium is markedly edematous with numerous necrotic\ apoptotic tubular cells with numerous intra-tubular hyaline casts. Interstitium showed markedly dilated

congested blood vessels (Figures 3, 4 & Table 3)

- **Group IV:** (metformin high dose): Mild glomerular cellularity with patent Bowman's space and normal histological architecture of tubules were noted (Figure 6 & Table 3)
- **Group V:** (cisplatin+ metformin low dose): as seen in (Figure 5 & Table 3) mild glomerular deformity and cellularity with patent Bowman's spaces, less oedematous proximal tubular epithelium with scattered necrotic\ apoptotic epithelial cells in contrary to distal tubular epithelium which were markedly edematous with few intra-tubular hyaline casts
- **Group VI:** (cisplatin+ metformin high dose): Marked glomerular deformity and moderate hypercellularity with narrow to patent Bowman's spaces are seen. Tubular epithelium is mildly oedematous with scattered necrotic\ apoptotic epithelial cells and few intra-tubular hyaline casts (Figure 6 & Table 3).

Table (3): Histopathological results

Parameters Groups	Glomeruli			Tubules			Interstitium
	Deformity	Cellularity	Bowman's space	Edema	Necrosis	Casts	BV
Group I	0	0	0	0	0	0	0
Group II	+	++	+	+++	+	0	+++
Group III	+++	+++	+++	+++	+++	+++	+++
Group IV	0	+	0	0	0	0	0
Group V	+	+	0	+	+	+	0
Group VI	+++	++	+	+	+	+	0

0 = No, + = Mild, ++ = Moderate, +++ = Marked

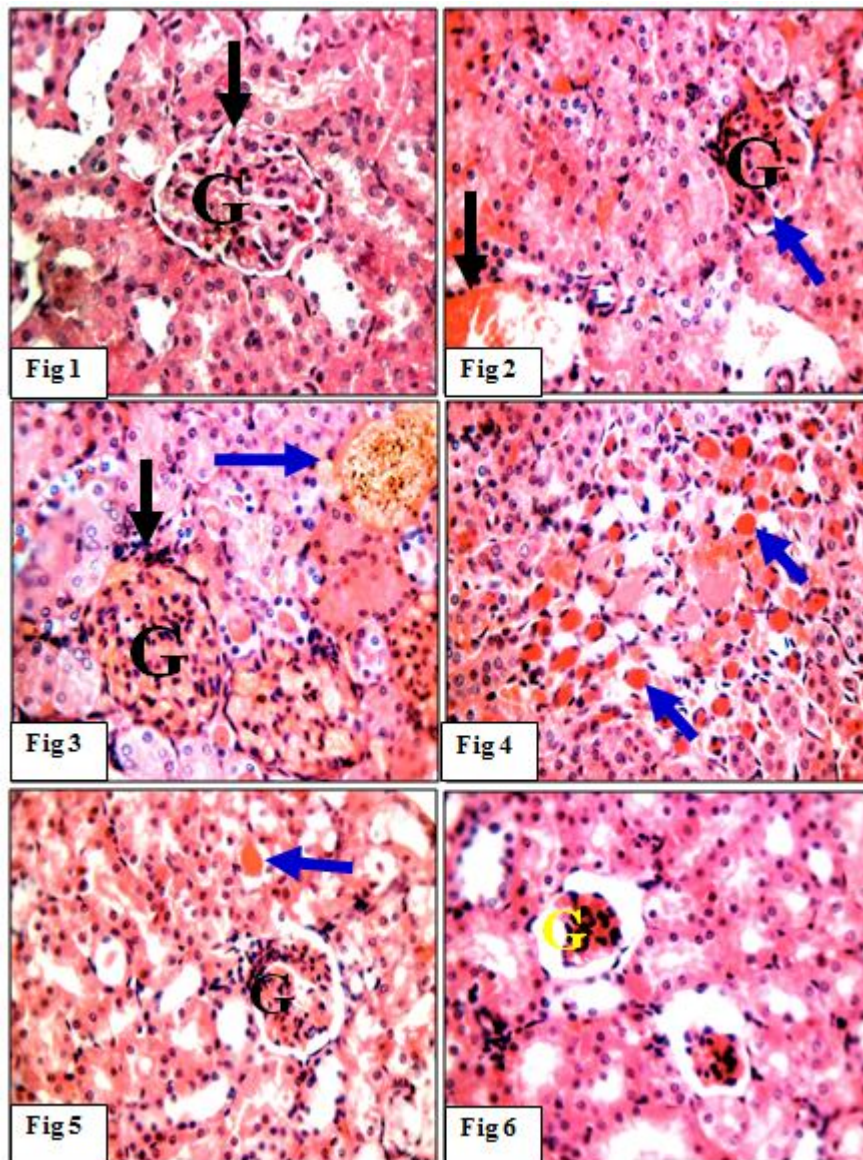


Fig (1): (Control). Fig (2) (Cisplatin low dose): Mild glomerular deformity and hypercellularity with markedly dilated congested blood vessels. **Fig (3) (Cisplatin high dose):** Marked glomerular deformity and hypercellularity with obliterated Bowman's spaces and markedly dilated congested blood vessels **Fig (4):** Numerous intra-tubular hyaline casts. **Fig 5 (Cisplatin + Metformin low dose):** Mild glomerular deformity with intra-tubular hyaline casts. **Fig 6 (Cisplatin + Metformin high dose):** Marked glomerular deformity and hypercellularity with mild tubular edema (H&E X400)

II- Histochemical results:

Light microscopical examination of PAS and Mallory stained slides of kidneys showed the following:

- **Group 1 (Control):** PAS showed average glomerular and tubular BM with

preserved brush borders, cell borders, and nuclear details (Fig 7 & Table 4). Mallory's stain showed no fibrosis.

- **Group II:** (Cisplatin low dose): PAS showed mild thickening of glomerular BM. Renal tubules showed partially

preserved brush borders with partially preserved nuclear details and indistinct (poor) cell borders (Fig 8 & Table 4). Mallory's stain showed thin irregular glomerular and tubular BM (Fig 13 & Table 5)

- **Group III:** (Cisplatin high dose): PAS showed marked thickening of glomerular BM., Renal tubules showed partially preserved brush borders with loss of nuclear details (Fig 9 & Tables 4). Mallory's stain showed thin irregular Bowman, s capsule and tubular BM (Fig 14 & Table 5)
- **Group IV:** (Metformin high dose): PAS showed mild thickening of glomerular BM, with preserved tubular BM and brush

borders (Fig 10 & Table 4). Mallory's stain: showed thin irregular Bowman, s capsule and tubular BM (Fig 15 & Tables 5)

- **Group V:** (Cisplatin + Metformin low dose) PAS showed mild thickening of glomerular and tubular BM with partially preserved brush borders (Fig 11 & Tables 4). Mallory's stain showed thick irregular Bowman, s capsule and tubular BM (Fig 16 & Table 5).
- **Group VI:** (Cisplatin + Metformin high dose) PAS showed marked thickening of glomerular BM with preserved brush borders with loss of nuclear details of renal tubules (Fig 12 & Table 4).

Table (4): PAS scoring

PAS Groups	Glomerular BM	Tubules			
		Brush border	BM	Cell borders	Nuclear details
Group I	0	0	0	0	0
Group II	+	+	+	++	+
Group III	++	++	+	+	++
Group IV	+	0	0	0	0
Group V	+	+	+	+	++
Group VI	++	+	+	+	++

Glomerular BM: 0 = Average + = Mild/moderate thickening ++ = Marked thickening
Brush border: 0 = Preserved + = Partially preserves ++ = Lost
Tubular BM: 0 = Average + = Mild/moderate thickening ++ = Marked thickening
Cell borders: 0 = Preserved + = Partially preserves ++ = Lost
Nuclear details: 0 = Preserved + = Partially preserves ++ = Lost

• Mallory's stain showed thick irregular glomerular and tubular BM, thick irregular Bowman, s capsule with scar

formation (Fig 17) and medullary fibrosis (Fig 18 & Tables 5).

Table (5): Mallory's stain results

Collagen fibers Stained deep blue	Group I	Group II	Group III	Group IV	Group V	Group VI
	-	+	++	+	++	++

- = Negative: thin continuous collagen fibers.
 + = Thin irregular collagen fibers.
 ++ = Markedly thick irregular collagen fibers.

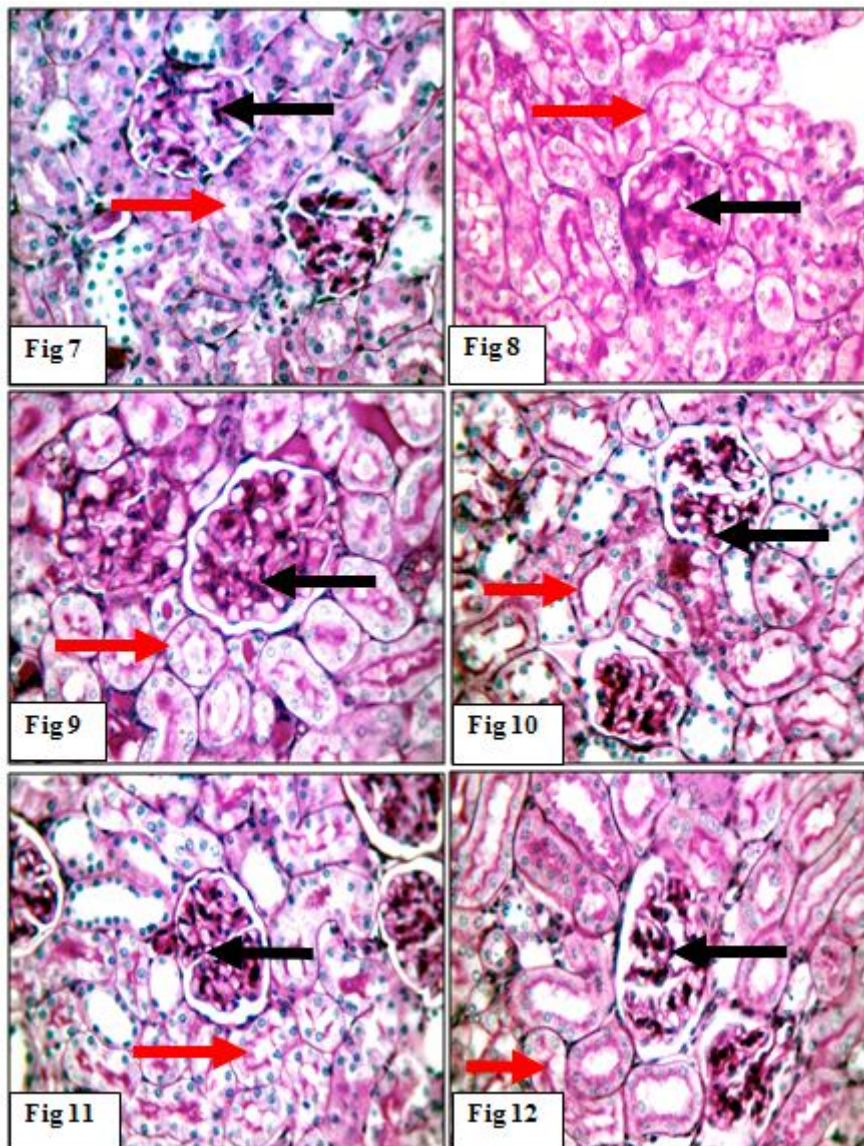


Fig 7 (Control): Average glomerular BM with preserved brush borders. **Fig 8 (Cisplatin low dose):** Mild thickening of glomerular BM with partially preserved brush borders. **Fig 9 (Cisplatin high dose):** Marked thickening of glomerular BM with partially preserved brush borders. **Fig 10 (Metformin high dose):** Mild thickening of glomerular BM with preserved brush borders. **Fig 11 (Cisplatin + Metformin low dose):** Mild thickening of glomerular BM with partially preserved brush borders. **Fig 12 (Cisplatin + Metformin high dose):** Marked thickening of glomerular BM with preserved brush borders (PAS stain X400)

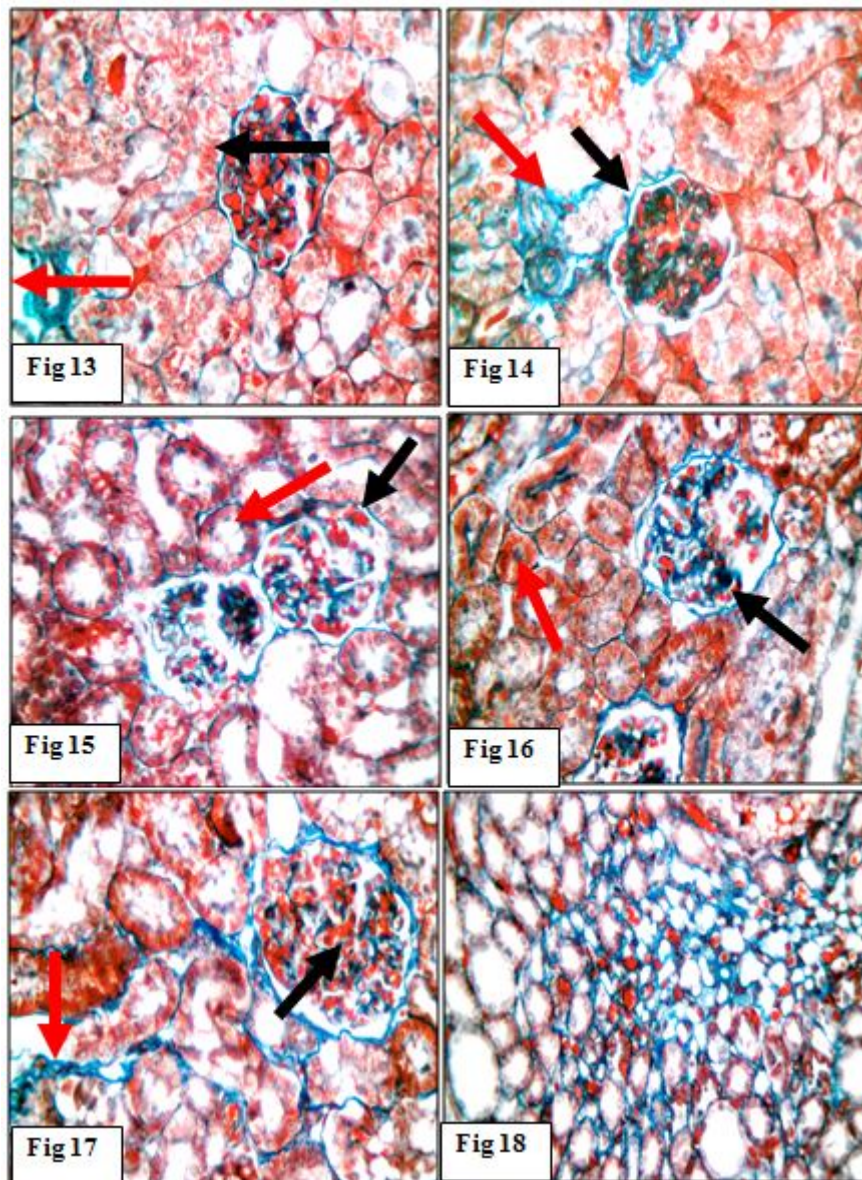


Fig 13 (Cisplatin low dose): Thin irregular glomerular & tubular BM. **Fig 14 (Cisplatin high dose):** thick irregular Bowman's capsule and tubular BM. **Fig 15 (Metformin high dose):** Thin irregular Bowman, s capsule and tubular BM. **Fig 16 (Cisplatin + Metformin low dose):** Thick irregular Bowman, s capsule and tubular BM. **Fig 17 (Cisplatin + Metformin high dose):** Thick irregular glomerular BM, Bowman, s capsule and tubular BM. **Fig 18 (Cisplatin + Metformin high dose):** Medullary fibrosis (Mallory's stain X400)

DISCUSSION

Cisplatin is well known for its nephrotoxic effects producing many histopathological changes like vascular congestion, focal mononuclear cell inflammatory, cellular infiltrate, acute tubular injury with reactive atypia and apoptotic cells (*Alhoshani et al. (2017)*) marked necrosis of proximal tubules and degeneration of the tubular epithelial cells which is related to an increase in lipid peroxidation, oxygen-free radicals, and inflammation in kidney (*Kundan et al. (2013)*). *Pezeszki et al., 2017*, suggested that the cisplatin -induced nephrotoxicity started to develop almost 3 days after administration of the drug in rats.

Metformin had been suggested to have therapeutic or reno-protective effects against nephrotoxic agents (*Nasri, 2013* and *Rafieian-Kopaei & Nasri, 2013*). *Li et al. (2016)* demonstrated that metformin may protect against cisplatin-induced tubular cell apoptosis. *Sahu et al., (2013)* showed in their study marked ameliorative effects of metformin on renal functions and attributed this improvement to antioxidant and cytoprotective effects of metformin.

Our study showed obvious decrease in average total body weight of rats given cisplatin injection. These results agree with results of the study of *Akunna et al. (2018)* who showed a significant decrease in final body weight of cisplatin treated rats. Our study showed that this average loss of body weight was noted to be less on giving metformin with cisplatin.

Sahu et al. (2013) reported that a single injection of cisplatin (7.5 mg/kg, i.p.) caused a significant increase in blood urea nitrogen and serum creatinine. These results were in accordance to our findings which showed marked increase in renal functions on giving cisplatin injection which were more elevated on increasing cisplatin injected dose from 3.5 mg/kg to 7.5 mg/kg. When metformin was given, blood urea nitrogen and serum creatinine levels were decreased. *Amini et al. (2012)* showed that post-treatment with metformin or co-treatment could prevent the elevation of serum BUN and that metformin may prevent or ameliorate gentamycin induced acute renal failure.

Our histopathological study showed marked histological changes as seen by H& E on giving cisplatin injection (3.5 mg/kg) and when the injected dose of cisplatin was increased from 3.5 mg to 7.5 mg; more cortical necrosis occurred, viable glomeruli showed marked deformity and hypercellularity with obliterated Bowman's spaces, tubular epithelia showed numerous necrotic/apoptotic tubular cells with numerous intra-tubular hyaline casts and Interstitial tissues showed markedly dilated congested blood vessels which agrees with the study of *Sahu et al. (2013)* who reported that a single injection of cisplatin (7.5 mg/kg, i.p.) caused a significant renal damage.

On giving single injection of 7.5 mg cisplatin + metformin 100 mg/kg/day, mild glomerular deformity and cellularity and patent Bowman's spaces were seen. Proximal tubular epithelia were less

oedematous with scattered necrotic/apoptotic epithelial cells, while distal tubular epithelium was edematous with few intra-tubular hyaline casts. Obvious less necrosis/ apoptosis was seen with narrow to patent Bowman's spaces and mildly oedematous tubular epithelial cells and less interstitial congestion and few intra-tubular hyaline casts were seen in rats which were given 7.5 mg cisplatin+ metformin 350 mg /kg/day for 10 days. It was also notable that the effects of 100mg/kg metformin + 7.5 mg/ kg cisplatin was much better than giving 350 mg/kg metformin with same high dose of cisplatin (7.5 mg /kg) for same 10 days period.

Our histochemical study using PAS and Mallory stains showed that 3.5 mg/kg injected dose of cisplatin led to mild thickening of glomerular BM, partially preserved cell and brush borders of tubules and that on increasing the injected dose of cisplatin to 7.5mg/kg marked thickening of glomerular BM with more loss of renal tubular brush borders were noted. Cisplatin + metformin groups showed more preservation of brush borders especially on increasing the dose of metformin to 350 mg/day where better preservation of brush borders of tubules was noted. Our findings were in accordance with other studies that proved that metformin preserved brush borders, decreased cell death and the damaging effects of cisplatin. Metformin proved to minimize the damaging effects of cisplatin. These findings were similar to findings of *Fatemeh et al. (2012)* who concluded from their study that metformin is a nephron-protective drug that could attenuate the tubular damage caused by gentamycin or other nephrotoxic agents.

Reduction of apoptosis, induced by oxidative stress, in endothelial cells and prevention of vascular dysfunction was found with metformin treatment (*Nasri, 2013*). Also, *Taheri et al., 2012*, reported found that there was an ameliorative property of metformin against unilateral ischemia–reperfusion induced injury in rats.

CONCLUSION

Metformin produced significant protection against cisplatin-induced nephrotoxicity especially in low dose.

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تقييم التأثيرات الواقية المحتملة للميتفورمين على التسمم الكلوي المحدث من السيسبلاتين على الجرذان البيضاء

محمد عساسة*، سيد عبد الرحيم**، عبد اللطيف سيد***، أشرف إبراهيم حسن*

أقسام الطب الشرعي* والباثولوجي** والفارماكولوجي***، كلية طب، جامعة الأزهر

خلفية البحث: استخدام مركب السيسبلاتين محدود نتيجة سُميته على الكلى لما يُحدثه من زيادة إضطراب الأكسدة، مما يؤدي في النهاية لخلل وظائف الكلى.

الهدف من البحث: تقييم التأثير الواقى لمادة الميتفورمين من التسمم الكلوي المُحدث من مادة السيسبلاتين لجرذان التجارب البيضاء البالغة.

مواد وطرق البحث: قُسم ستون جرذاً أبيضاً بالغاً بشكل عشوائي إلى ست مجموعات متساوية: المجموعة الأولى حُققت بجرعة 1 مليلتر من محلول ملح متساوي التوتر بداخل تجويف البريتون، والمجموعة الثانية حُققت بجرعة واحدة من السيسبلاتين 3.5 مليجرام لكل كيلوجرام بداخل تجويف البريتون. المجموعة الثالثة حُققت بجرعة واحدة 7.5 مليجرام لكل كيلوجرام من مادة السيسبلاتين بداخل تجويف البريتون. المجموعة الرابعة أُعطيت جرعة 350 مليجرام لكل كيلوجرام يومياً من مادة الميتفورمين عن طريق الفم لمدة 10 أيام. المجموعة الخامسة أُعطيت جرعة 100 مليجرام لكل كيلوجرام يومياً من مادة الميتفورمين عن طريق الفم لمدة 10 أيام، ثلاثة أيام قبل حقنها اليوم الرابع مرة واحدة بمادة السيسبلاتين 7.5 مليجرام لكل كيلوجرام بداخل تجويف البريتون، وسبعة أيام بعدها. أما المجموعة السادسة فأعطيت 350 مليجرام لكل كيلوجرام يومياً من الميتفورمين عن طريق الفم لمدة 10 أيام، ثلاثة أيام قبل حقنها مرة واحدة اليوم الرابع بمادة السيسبلاتين 7.5 مليجرام لكل كيلوجرام، وسبعة أيام بعدها. وتم وزن الجرذان في اليوم الأول والرابع والسابع والعاشر من أيام التجربة، وتم حساب متوسط أوزان أجسام الجرذان لكل مجموعة. وعند نهاية المدة المحددة (10 أيام) ذُبحت الجرذان وتم إستئصال الكليتين من كل جرذ، ثم تم تحضيرها وصبغها بصبغتي الأيوسين والهيما توكسيلين لكي يتم دراسة أنسجتها. وكذلك صبغها بصبغتي المالوري وشيف الحامضية الدورية لفحص أنسجتها كيميائياً.

نتائج البحث: أظهرت مجموعة الجرذان التي حُقنت بجرعة 7.5 مليجرام لكل كيلوجرام من مادة السييسبلاتين فقد أكبر بمتوسط أوزان أجسامها ومستويات أعلى بيوريا الدم والكرياتنين عن مجموعة الجرذان التي حُقنت بجرعة 3.5 مليجرام لكل كيلوجرام من مادة السييسبلاتين. كما أظهرت مجموعة الجرذان التي حُقنت بجرعة 7.5 مليجرام لكل كيلوجرام من السييسبلاتين وأُعطيت جرعة 100 مليجرام لكل كيلوجرام يومياً من مادة الميتفورمين فقد أقل بمتوسط أوزان أجسامها وتحسن بوظائف الكلى عن مجموعة الجرذان التي حُقنت 7.5 مليجرام لكل كيلوجرام من مادة السييسبلاتين وأُعطيت جرعة 350 مليجرام لكل كيلوجرام يومياً من مادة الميتفورمين. كما أظهر الفحص النسيجي بصبغتي الهيماتوكسيلين و الأيوسين والفحص النسيجي الكيميائي بصبغتي مالوري وشبف الدوري الحامضي حدوث تغيرات أقل بأنسجة وكبيبات وأنابيب الكلى لجرذان المجموعة التي حُقنت بجرعة 3.5 مليجرام لكل كيلوجرام من مادة السييسبلاتين عن جرذان المجموعة التي حُقنت بجرعة 7.5 مليجرام لكل كيلوجرام والتي ظهر بأنسجتها تضرر شديد بأنسجة الكلى. أما المجموعة التي حُقنت بجرعة 7.5 مليجرام لكل كيلوجرام من مادة السييسبلاتين وأُعطيت جرعة 100 مليجرام لكل كيلوجرام من مادة الميتفورمين يومياً فأظهرت تضرراً أقل من جرذان المجموعة التي حُقنت بجرعة 7.5 مليجرام لكل كيلوجرام من السييسبلاتين وأُعطيت جرعة 350 مليجرام لكل كيلوجرام يومياً من مادة الميتفورمين.

الخلاصة: مادة الميتفورمين لها تأثيرات تقلل من التأثير السمي لمادة السييسبلاتين على كلى الجرذان الذكور البيضاء البالغة.