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ORIGINAL ARTICLE

Effect of Metformin on the Kidney Histology of Rats in Gentamicin Induced Toxicity

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ABSTRACT

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Study's background and aim: Metformin, an oral antidiabetic agent has been studied in the past for its protective effects in aminoglycoside induced renal injuries. We hypothesized that the use of metformin may be protective in the aminoglycoside mediated acute renal failure. We thus tried two doses of metformin (M_1; 75mg/kg/day) (M_2; 150mg/kg/day) to evaluate this preventive potential on gentamicin induced acute renal failure in rats.

Study Design: Randomized controlled trial

Place of Study: Animal House of National Institute of Health Islamabad/ Department of Pharmacology, AL Nafees Medical College and Hospital, Islamabad, duration was 1stAugust 2018 to 31stJanuary 2019.

Materials and Methods: The rats were divided into three main groups (n=10) kept under similar conditions for food and temperature. Renal failure was induced by injecting gentamicin (80mg/kg/day) intraperitonealy (ip) for eight days with simultaneous administration of oral metformin for 28 days.Slides of rats' kidneys were prepared for histological comparison at the last day of study.

Results: In gentamicin induced renal failure and simultaneous administration of metformin, the histological findings of rat kidneys showed remarkable tissue necrosis in control group and prevention in metformin treated groups.

Conclusion: Based on the histological results of our study it was concluded that metformin at a dose of 150mg/kg showed a nephroprotective effect in gentamicin induced renal injuries in Sprague-Dawley rats.

Keywords: Metformin, Gentamicin, Nephrotoxicty, Renal injuries, Nephroprotective effect

INTRODUCTION

Medications-induced renal injuries are not rare, and they can range from modest harm to severe and/or complete renal failure [1]. Acute tubular necrosis, acute interstitial nephritis, nephrotic syndrome blockage, electrolyte problems, and chronic renal failure [2] are all possible outcomes in the clinical setting as a result of this condition.

On the basis of these observations, several animal models of acute renal failure have been developed by administering supra-pharmacological doses of drugs such as glycerol (single dose 10mg/kg IM) [3], gentamicin (80 mg/kg per day IP) for 7 days [4], non-steroidal anti-inflammatory drugs (NSAIDs) (single dose 3g/kg po) [5-6], ifosfamide (60mg/kg

Numerous factors, such as the dose and duration of antibiotic therapy [9], the age of the patient [10], concomitant administration of other nephrotoxic drugs, extracellular volume depletion, potassium depletion [11], excessive diuretic doses, and the renal status prior to initiation of treatment are all associated with aminoglycoside nephrotoxicity.

Most cases of renal failure are reversible if the medicine is stopped in the early stages. Creatinine levels, on the other hand, may continue to rise for several days as a result of continuous tubular injury caused by chronically high parenchymal levels of aminoglycosides in the blood. There are several different types of tubular dysfunction conditions, including hypomagnesemia, hypocalcemia, hypokalemia, and full-blown tubular necrosis. Fanconi syndrome has been reported in the context of aminoglycoside poisoning [12].

[13] A large number of studies have been carried out in order to identify herbal extracts and antioxidants that have the ability to decrease or protect kidney damage. There have only been a few experimental studies that have demonstrated that some medications and substances have a preventative and protective role in gentamicin-induced nephropathy in particular. Resveratrol (a natural antioxidant), ROS scavengers, and metformin are the agents involved [14-15]. The use of metformin is frequently restricted due to concerns about lactic acidosis, however the contraindications to metformin have been well studied [16]. It has been discovered that in diabetic individuals with mild to severe renal impairment, there is no influence on their blood lactate levels [17]. Similar to this, serum metformin concentrations rise only when the glomerular filtration rate (GFR) is less than 30ml/min. As a result, it can be administered safely to patients with mild to moderate renal failure [18].

It was our goal with this study to determine the protective effects of metformin on the cellular and histological architecture of gentamicin-induced kidney damage in albino rats, and we were successful.

MATERIALS AND METHODS

For this study 32 Albino rats weighing 100-150 g were purchased from National Institute of Health (NIH), Islamabad. The rats were kept in the animal house of NIH at the standard room temperature of (25^{0}C) under 12h day and night cycles with ad libitum food and water. The rats were given one week to be acclimatized.

Animals were divided randomly into 3 groups (n=10) named C (Control) M_1 (Metformin 75 mg/kg/day) M2(Metformin 150mg/kg/day) while two additional rats were kept as absolute control for comparative histopathological changes.Control group was given distilled water (1ml) by gavage for 28 days as single morning dose starting from day 0 while M_1 and M_2groups received respective doses of Metformin dissolved in 1ml distilled water from day 0 to 28.All animals (except absolute control) were given injection gentamicin (80mg/kg/day) intraperitonealy from day 0 to 7 to develop renal failure[4].

On the last day of study, rats were sacrificed and their kidneys were removed and fixed by immersing them in 10% buffered formalin for 24 hours. Afterwards the kidneys were trimmed sagittaly, dehydrated by passing different grades of ethanol (70%, 80%, 90%, 100% followed by xylene treatment and finally embedded in paraffin wax (melting point =56^{0}C). Blocks were cut into 5 µm thick paraffin sections and fixed on pre-coated glass slides. Specimen slides were stained with Haris Hematoxylin (BDH) and Eosin (BDH).Prepared slides were examined under

light microscope at 40 X magnificationfor morphological changes like massive and diffuse cell necrosis in proximal tubules of kidneys.

RESULTS

Gross Examination of Preserved Kidneys: On gross examination, kidneys of animals in absolute control group were found to be normal in shape and size. The colour of kidneys' external surface (Capsule) at the time of examination was faded from dark to yellowish due to preservation in formalin (Figure 1 A). The kidneys of group (Group C) which were treated with gentamicin only at a dose of 80mg/kg for 28 days, were found to be swollen, with cystic spots on them (Figure1 B).

Gross examination of kidneys of treated group-I (Gentamicin 80mg/kg for 08 days + Metformin 75 mg/kg for 28 days) revealed moderate swelling as compared to absolute control (healthy rat kidneys) (Figure 1C), while in kidneys of treated group-II (Gentamicin 80mg/kg for 08 days + Metformin 150 mg/kg for 28 days), no such gross pathologies were seen (Figure1D).

Microscopic Examination: In control group (Group C) most of the cells of the kidney were massively enlarged along with focal epithelial necrosis. There was breakdown of glomerular capillaries with interruption of tubular basement membranes. There was also infiltration of lymphocytes. Majority of the tubular epithelial cells showed abundant eosinophilic granular cytoplasm while few epithelial cells showed foamy appearance. The degenerative tubules also showed swelling, cytolysis and tubular irregularity (Figure2B) when compared with the slides of normal kidneys (Figure2A).

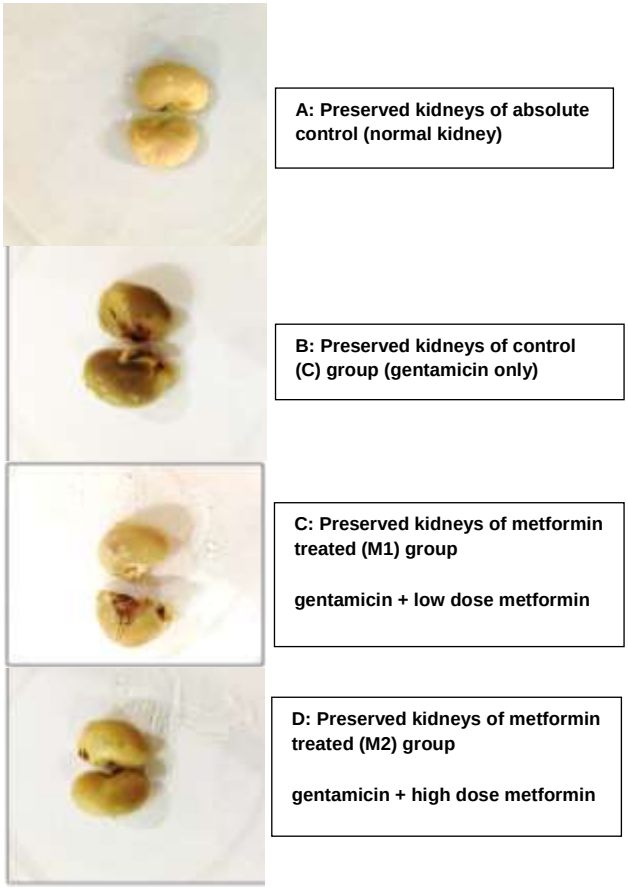

Figure 1: Gross appearance of kidneys of animals of all groups preserved at day 28 of experiment

The slides of kidneys of treated group-I (group M1) showed enlarged cells and interrupted basement membranes to some extent but the signs of necrosis were less (Figure2C) than the control group (Figure2B). Slides of treated group –II (Group M2) showed no major histopathological changes (Figure2D) and they showed normal appearance i-e very similar to healthy kidneys.

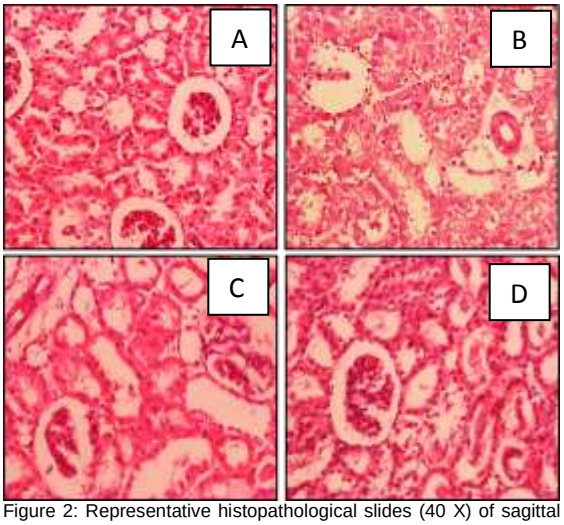

Figure 2: Representative histopathological slides (40 X) of sagittal sections of kidneys of rats

A- Normal Kidney (Absolute Control)
B- Control Group (gentamicin 80mg/kg) C at day 28
C- Treated Group-I (gentamicin 80 mg/kg + metformin 75 mg/kg) M1 at day 28
D- Treated Group-II (gentamicin 80 mg/kg + metformin 150 mg/kg) M2 at day 28

DISCUSSION

Many substances, most of which are employed for therapeutic purposes, might have negative effects on the renal system, which makes it a vulnerable organ. It has been described in the literature that this adverse effect might result in anything from minor renal damage to complete renal failure as a result of this unfavourable effect [1-2].

Gentamicin's nephrotoxicity is typically exhibited as acute tubular necrosis in the majority of instances [19], which is histopathologically confirmed. When exposed to aminoglycosides, patients may have a range of tubular dysfunction conditions, including hypomagnesaemia, hypocalcaemia, hypokalemia, and full-blown Fanconisyndrome [20]. Several studies have been conducted in order to determine the actual cause of the toxicity. Several studies have shown that this toxicity is caused by the buildup of the drug in the proximal convoluted tubular cells, and that the action of the drug is reversed by the secretion of certain enzymes from the brush border of renal tubules [21-22].

Metformin has a powerful antioxidant effect by reducing oxidative stress, and it has been demonstrated to prevent aminoglycoside-induced acute renal failure in rats by inhibiting a mitochondrial-dependent route. As a result of gentamicin toxicity, metformin was shown to prevent histological abnormalities in mice. It was also discovered that metformin repaired gentamicin-induced impairments in mitochondrial biogenesis in the laboratory. Metformin was also shown to reduce the amount of gentamicin-induced mitochondrial oxidative stress, according to the findings of that study [23]. However, in those tests, metformin was added to the drinking water, making it difficult to identify the exact quantity that the animals consumed. Because of the documented anorexia and lethargy in rats during and after induction of gentamicin toxicity, we were concerned about the amount of metformin used in this experiment [4]. This was due to the possibility that reduced

water consumption during the experiment could occur. In order to fill this study void, we planned our experiment with two different dosages of metformin. It was necessary to dilute metformin in one millilitre of distilled water, and the doses were estimated in accordance with the M1 and M2 groups (75 mg/kg per day and 150 mg/kg per day, respectively). These doses were administered to rats once a day for 28 days with the use of a gavage needle directly into their stomachs.

Singh and his colleagues published a review article in 2012 in which they described numerous animal models that were used in conjunction with different medicines to cause renal failure. Using gentamicin and other medications, they were able to filter out sixteen groups of chemicals that caused acute renal injury in rats, dogs, and rabbits. They also determined the dose range of the chemicals that caused acute renal injury in rats, dogs, and rabbits. In their study, the dose range of gentamicin for induction of renal failure was 40-200 milligrammes per kilogramme of body weight per day for 4-10 days, but in our study, we administered gentamicin 80 milligrammes per kilogramme of body weight per day for eight days to induce renal failure [24].

According to our findings, the microscopic findings of our investigation are compatible with the findings of Padmini and Kumar's study. In their research, they demonstrated the effects of two dosages of gentamicin on the kidneys of albino rats, as well as histological abnormalities in the kidneys of albino rats. Gentamicin was administered intraperitoneally at doses of 60 mg/kg and 80 mg/kg, respectively. During the course of their research, they discovered that normal saline-treated (healthy) kidneys did not exhibit any histological abnormalities. The Bowman capsules and tubular basement membranes were also found to be intact in the gentamicin-treated group, whereas their findings were identical to our findings in the untreated group. Gentamacine triggered the disintegration of the kidney's vasculature and the disruption of the basement membranes of glomeruli, according to their findings. Moreover, they detected glomerular congestion in conjunction with lymphocyte infiltration, and these findings were consistent with ours [25].

It was discovered in our study that the treatment with metformin reduced histological damage in a dose-dependent way. The histopathological findings of our investigation are consistent with the findings of Amini and colleagues, who investigated the ameliorative effects of metformin on renal histologic and biochemical abnormalities caused by gentamicin-induced renal toxicities in a Wistar rat model of renal toxicity. They discovered that rats treated with metformin had no structural changes in their renal tissues, whereas large and diffuse cell necrosis was found in the proximal tubules of the kidneys of rats injected with gentamicin, according to their findings. They also discovered that the tubular lumen was commonly filled with hyaline casts or heterogeneous cellular debris in the control group, whereas in the metformin-treated group, the majority of the tubules were preserved and the majority of the cells were devoid of necrotic alterations. Their study employed higher doses of gentamicin than our trial, which was a significant difference. Their protocol included not only gentamicin at a dose of 100mg/kg, but also metformin at a dose of 100mg/kg for 10 days, which was in contrast to our regimen, which included gentamicin at an initial dose of 80mg/kg for 8 days, followed by metformin at two doses (75mg/kg and 150 mg/kg) for a total of 28 days of administration. We administered metformin by a gavage syringe, whereas Amini and his colleagues blended the medication into drinking water [26].

In 2014, Janjua did a similar study in which they utilised rabbits as experimental animals, which was published in the journal Science. In this study, they employed gentamicin at doses of 40 mg/Kg and 150 mg/Kg, respectively, and metformin at a level of 100 mg/Kg. They discovered that metformin provided total nephroprotection at low toxic dose levels of gentamicin (40 mg/Kg), but it only moderately attenuated the nephrotoxic insult at a dose of 150 mg/Kg [27].

CONCLUSION

Our results showed histologic damage in gentamicin treated group which was markedly less in metformin treated groups,providing the evidence and conclusion that that metformin at a dose of 75 mg/kg produced partial and at dose of 150 mg/kg produced almost complete nephroprotective effect in gentamicin induced renal injuries in Sprague-Dawley rats.

REFERENCES

1. Barnett L, Cummings BS. Nephrotoxicity and Renal Pathophysiology: A Contemporary Perspective.Toxicological Sciences2018 ;164(2):379-390.
2. Morales-Alvarez MC. Nephrotoxicity of Antimicrobials and Antibiotics. Advances in chronic kidney disease - Elsevier 2020; 27(1) :31-37.
3. Sun G,WangJ,WangP,RenH,YueY,Song Z. Donepezil protects glycerol-induced acute renal failure through the cholinergic anti-inflammatory and nitric oxide pathway in rats. ImmunopharmacolImmunotoxicol2020 ;42(6):625-631.
4. Huang H,JinW,HuangM,JiH,CapenD,Xia Y. Gentamicin-Induced Acute Kidney Injury in an Animal Model Involves Programmed Necrosis of the Collecting Duct.JASN 2020;31 (9) :2097-2115.
5. Ansari S, Azarmehr N, Barmoudeh Z, Moslemi Z, Ghahremani H, Mirzaei A. Evaluation of the protective potential of hydroalcoholic extract of Thymus daenensis on acetaminophen-induced nephrotoxicity in rats.Heliyon May 2020;6(5): e03898.
6. N.E. Bektur, E. Sahin, C. Baycu, G. Unver .Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. Toxicol. Ind. Health 2016; 32 (4) : 589-600.
7. Dobrek L, Skowron B, Baranowska A, PloszajK,Badziul D, Thor P. The influence of oxazaphosphorine agents on kidney function in rats. Medicina 2017;53(3) : 179-189.
8. Nikolic T, Petrovic D, Matic S, Turnic T, Jeremic J, Radonjic K. et al. The influence of folic acid-induced acute kidney injury on cardiac function and redox status in rats. Naunyn-Schmiedeberg's Arch Pharmacol 2020;393: 99–109.
9. Mahi-Birjand M, Yaghoubi S , Abdollahpour-Alitappeh M , Keshtkaran Z, Bagheri N , Pirouzi A .et al. Protective effects of pharmacological agents against aminoglycoside-induced nephrotoxicity: A systematic review. Expert Opin Drug Saf2020 ;19(2):167-186.
10. Kwiatkowska E,DomanSski L,DziedziejkoV,Kajdy A,Stefanska K,S KwiatkowskiS.The Mechanism of Drug Nephrotoxicity and the Methods for Preventing Kidney Damage. Int. J. Mol. Sci 2021;22(11):6109.
11. Al-Ani I, Algantri K, Nafie E, Al-Mahmood S. The nephrotoxicity of concurrent use of enalapril and gentamicin in rats. Asian J Pharm Clin Res 2018;11(9):348-352.
12. Kelly N, Byrne C. Nephrotoxins and drugs in renal insufficiency. Medicine 2019;47(8) :517-522
13. Salama A, Abd El-Wahed A, Mostafa A. Protective effect of some plants against the toxicity of kidneys caused by gentamicin. J med Sci Res2020;3(1):5-11
14. Dorestan, Nozar; Manzouri, Leila; Bahadoram, Mohammad et.al. Metformin protects renal tubular cells; mechanisms and new concepts.J Nephropathology 2018; 7 (3):132-136.
15. Beshay O, Ewees M, Abdel Hafiz S, AbdelrehimaA, BayoumiaA.Resveratrol reduces gentamicin-induced EMT in the kidney via inhibition of reactive oxygen species and involving TGF-β/Smad pathway. Life Scis2020; 258(1) :118178.
16. Salvatore T, Pafundi P, Marfella R, Sardu C, RinaldiL,Monaco L.et.al. Metformin lactic acidosis: Should we still be afraid? J.diabres.2019;157:107879
17. Bipi P K, George J, Gomathy S, Gracious N, Kumar S, Mohandas M K. Lactate levels and risk of lactic acidosis with metformin in diabetic kidney disease patients. Saudi J Kidney Dis Transpl 2017;28:1356-61
18. Sepahi, Akhavan M, Lakkakula, Bhaskar V. K. S.; Kellner, James S. Administration of metformin in type 2 diabetes mellitus patients with chronic kidney disease; facts and myths. J Nephpath 2020; 9 (1): 1-6
19. Hussain T, Gupta RK, Sweety K, Eswaran B, Vijayakumar M, Rao CV Nephroprotective activity of Solanumxanthocarpum fruit extract against gentamicin-induced nephrotoxicity and renal dysfunction in experimental rodents. Asian Pac J Trop Med. 2012 ;5(9):686-9
20. McWilliam, S.J., Antoine, D.J., Smyth, R.L. et al. Aminoglycoside-induced nephrotoxicity in children. PediatrNephrol 2017;32: 2015–2025
21. Mishra, P., Mandlik, D., Arulmozhi, Mahadik K. Nephroprotective role of diosgenin in gentamicin-induced renal toxicity: biochemical, antioxidant, immunological and histopathological approach. Futur J Pharm Sci2021; 7:169
22. Abbaszadeh, Abolfazl ,Koushki, Sahar , Koushki, Shirin.et.al Metformin; a mini-review to its antioxidative and anti-inflammatory properties Metformin; a mini-review to its antioxidative and anti-inflammatory properties. J RenInj Prev.2018; 7 (1): 7-10
23. De BroeM ,Kajbaf F, Lalau J. Renoprotective Effects of Metformin. Nephron 2018;138:261–274
24. Singh AP,MuthuramanA,JaggiIAS,SinghN,GroverK,Dhawan R. Animal models of acute renal failure.Pharmacol Rep 2012;64(1):31-44
25. PadminiMP , Kumar JV. A histopathological study on gentamicin induced nephrotoxicity in experimental albino rats. IOSR J Dent Med Sci. 2012; 1: 14-17.
26. Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci. 2012; 7: 621-5.
27. Janjua A, Waheed A, Bakhtiar S. Protective effect of metformin against gentamicin induced nephrotoxicity in rabbits. Pak J Pharm Sci. 2014; 27(6): 1863-72.