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EVALUATION OF NEPHRO-PROTECTIVE EFFECT OF DPP4 INHIBITOR AND ANTIOXIDANT AGAINST GENTAMYCIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Background:- Nephrotoxicity is a global health challenge of vast proportion around the world. Recent studies demonstrated the reno-protective effects of two dipeptidyl peptidase-4 (DPP-4) inhibitors, saxagliptin and linagliptin, against gentamycin-induced renal injury. However, none of these studies investigated the combination of DPP 4 inhibitor and antioxidant. This prompted us to test this hypothesis and to assess, for the first time, the potential reno-protective effect of DPP-4 inhibitor and antioxidant. **Objective:-** This study aimed to investigate the potential protective effect of DPP-4 inhibitor and antioxidant on gentamycin-induced nephrotoxicity. **Method:-** Nephrotoxicity was induced in the rats with Gentamycin (100mg/kg). All animals except normal control were intraperitoneally administered with gentamycin at a dose of 100mg/kg once daily for 10 days. Respective treatment were started from day 2nd till day 14th (2 weeks). On the 15th day, blood samples were collected through retro-orbital plexus under anesthesia. Serum was separated to measure creatinine, BUN, uric acid, proteins & MDA (Malondialdehyde). Body weight were also recorded. **Result:-** Administration of combination of DDP-4 inhibitor and antioxidant ameliorated gentamycin induced renal injury and restored renal functional, oxidative, inflammatory, apoptotic & histopathological changes. **Coclusion:-** These findings suggest that combination of DDP-4 inhibitor and antioxidant treatment attenuate renal dysfunction and structural damage through the reduction of oxidative stress, mitochondrial dysfuction and apoptosis in the kidney.

KEYWORDS: Nephrotoxicity, Gentamycin, DDP-4 inhibitor, antioxidant, Renal Biomarkers.

1. INTRODUCTION

Nephrotoxicity can be defined as the adverse effect of substances on renal function.[1,3] These substances can include molds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine.[2,3] Due to relatively large blood flow (20 % of stroke volume) and the ability to extract and concentrate hydrosoluble toxic molecules, the kidney is prone to drug induced damage. The experimental data point to the fact that drug induced nephrotoxicity includes multiple mechanisms that can be classified as vascular, glomerular and tubular. The kidney damage is usually a consequence of tubular obsruction caused by cell swelling or debris deposition.[4,6] Toxic substances can damage various cell types in kidney. The most studied effect is necrosis of tubular epithelial cells.[5,6] One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or urine output.[2,3]

Aminoglycoside antibiotics are commonly used for the treatment of severe gram negative bacterial infections. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects.[7,9] The most widely used drug in this category is gentamycin.[8,9] Nephrotoxicity remains the major side effect hindering the clinical use of the aminoglycoside, gentamycin.[10,14] A small fraction of the administered dose preferentially accumulates in the proximal tubules, inducing oxidative stress, apoptosis, necrosis of renal cells and eventually acute renal injury and damage.[11,14] Therefore, many approaches were adopted to mitigate the progression of the renal injury as once-a-day administration regimen.[10,14] Neprotoxicity induced by GEN is a complex phenomenon characterised by an increase in blood urea nitrogen (BUN) and serum creatinine (Cr) concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure.[15,9]

Incretins; glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are gut hormones secreted from the intestinal cells in response to food intake. Once reaching the circulation, they potentiate the glucose dependent insulin secretion from pancreatic cells and inhibit glucagon secretion.[12,14] The incretin effect is significantly decreased in patients with type 2 diabetes (T2D) and contributes to impaired insulin secretion

and chronic hyperglycemia. GLP-1 and GIP are rapidly inactivated by the dipeptidyl peptidase-4 (DPP-4).[13,14]

Glutathione (GSH) is the most abundant nonprotein thiol and has many functions in vivo. The major role of GSH is the maintenance of cellular redox balance. It plays a role as a substrate of glutathione peroxidase, an antioxidative enzyme that scavenges various peroxides.[16] The physiological role of GSH as an antioxidant has been described and substantiated in studies of numerous disorders reflecting the increased oxidation is a result of abnormal GSH metabolism.[17,18] GSH is thought to be an important factor in cellular function and defense against oxidative stress, such as radiation and drug resistance. Many reports have demonstrated that GSH acts as an endogenous antioxidant.[19,20]

However, there have been no prior studies demonstrating a protective effect of sitagliptin (DPP-4 inhibitor) & GSH against the gentamycin induced nephrotoxicity. In this study, we demonstrated for the first time that combination of sitagilptin and GSH suppresses oxidative stress in vivo, and the impairment of renal function.

2. MATERIALS AND METHODS

2.1. Animals:- The study was approved by the Institutional ethics committee. 30 Wistar Albino rats (200-250gm) were selected for present study. The animals were housed at room temperature (22-28 ^{o}C) 12 hr dark and light cycle and given standard laboratory feed and water *ad - libitum*. Experiments were conducted in strict accordance with CPCSEA guidelines.

2.2. Drugs and Chemicals:- Gentamycin sulfate ampoules was obtained from Abbott (Mumbai), Sitagliptin was purchased from Sun Pharmaceutical Industries Ltd., Glutathione were purchased from HK Vitals, Chloroform were purchased from commercial vendors.

2.3. Experimental protocol:- Animals were divided into five groups, six animals each. Control Group received 2 ml/kg/day vehicle orally. Toxicant Control Group received gentamycin (100 mg/kg/day) intraperitoneally. Test I Group received sitagliptin (30 mg/kg/day) by oral gavage simultaneously with gentamycin. Test II Group received glutathione (300 mg/kg/day) intraperitoneally with gentamycin. Test III Group received combination of sitagliptin (30 mg/kg/day) and glutathione (300 mg/kg/day) with

gentamycin. Treatment continued for 14 days. Respective treatment were started from day 2nd till day 14th (2 weeks).

2.4. Sample collection:- On the 14th day, after the last gentamicin injection was applied, rats were placed in individual metabolic cages. On 15th day blood samples were collected through retro-orbital plexus under anaesthesia. Serum was separated to measure creatinine, BUN, urea, uric acid, MDA.

2.5. Biochemical determination:- The determination of serum creatinine (CliniQuant-FSR, Jaffe,s Method, Initial Rate, Creatinine assay kit), serum uric acid (CliniQuant-FSR, Uricase – Tinder, End Point, Uric acid assay kit), blood urea and blood urea nitrogen (CliniQuant-FSR, Urease – GLDH, Fixed Time, Urea (BUN) kit), MDA (abbexa MDA ELISA Kit) was done as instructed by manufacturer.

2.6. Data Analysis and Statistics:- Data were expressed as means ± SD. Statistical significance was tested with the one-way analysis of variance (ANOVA) followed by Bonferroni's Test as a post hoc test using GraphPad Prism version 5.00. Probability < 0.05 was considered significant.

3. RESULTS

Effect of sitagliptin, glutathione & combination of both on body weight

After two weeks of treatment, rats showed significant change in body weight because of inflammation of kidney. Final weight of rats in positive control group was significantly higher than initial weight of rats as compared to control group, whereas test group I, II & III showed reduction in gain of weight of rats than that of toxicant control group.

Effect of sitagliptin, Glutathione & Combination of both on renal biomarkers (Blood Urea, Blood Urea Nitrogen, Serum Creatinine, Serum Uric acid, MDA)

As shown in Table No. 1, Group II (Toxicant control) were injected with gentamycin (100 mg/kg). It showed significantly elevated levels of urea, BUN, creatinine, uric acid, MDA as compared to the control group.

Group III (Test I) were treated with sitagiptin (30 mg/kg). It showed slightly reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

Group IV (Test II) were treated with glutathione (300 mg/kg). It showed slightly readuction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

Group V (Test III) were treated with combination of sitagliptin (30 mg/kg) & glutathione (300 mg/kg). It showed significant reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group. There were no statistically difference between Group I and Group IV.

Effect of sitagliptin, glutathione & combination of both on serum proteins

There is no significant difference in the levels of serum proteins (albumin, globulin) in all gropus.

Table 1: Effect of the Test I, II & III group on body weight, Renal Biomarkers & Serum protein.

Parameter	Group I (Control)	Group II (Toxicant control)	Group III (Test I)	Group IV (Test II)	Group V (Test III)
Body Weight					
Initial Body Weight (gm)	223 ± 0.86	227* ± 0.71	234** ± 0.49	238** ± 0.32	231** ± 0.56
Final Body Weight (gm)	234 ± 0.61	256* ± 0.94	250** ± 0.72	249** ± 0.65	246** ± 0.69
Renal Biomarkers					
Blood Urea (mg/dl)	33.2 ± 0.94	179.9* ± 1.81	32.6** ± 0.87	34.7** ± 0.63	31.5** ± 0.79
Blood Urea Nitrogen (mg/dl)	15.5 ± 0.48	84.01* ±1.08	17.8** ± 0.37	16.5** ± 0.55	14.10** ± 0.61
Serum Creatinine (mg/dl)	0.50 ± 0.02	2.56* ± 0.05	0.62** ± 0.03	0.58** ± 0.04	0.55** ± 0.02
Serum Uric Acid (mg/dl)	4.7 ± 0.13	8.7* ± 0.37	5.1** ± 0.12	4.9** ± 0.15	4.6** ± 0.20
MDA Levels (µmol/L)	7.1 ± 0.37	11.4 ±0. 40	7.9 ±0. 39	7.8 ±0. 23	7.5 ±0. 18
Serum Proteins					
Serum Albumin (g/dl)	3.6 ± 0.14	3.4 ± 0.45	3.7 ± 0.96	3.8 ± 0.56	3.5 ± 0.35
Serum Globulin (g/dl)	-3.6 ± 0.25	-3.4 ± 0.52	-3.7 ± 0.38	-3.8 ± 0.69	-3.5 ± 0.58
A/G Ratio	3.6 : 1 ± 0.46	3.4 : 1 ± 0.71	3.7 : 1 ± 0.27	3.8 : 1 ± 0.21	3.5 : 1 ± 0.45

Data represent means ± SD of 6 rats in each group. *p < 0.05 compared with control group. **p < 0.05 compared with gentamycin group.

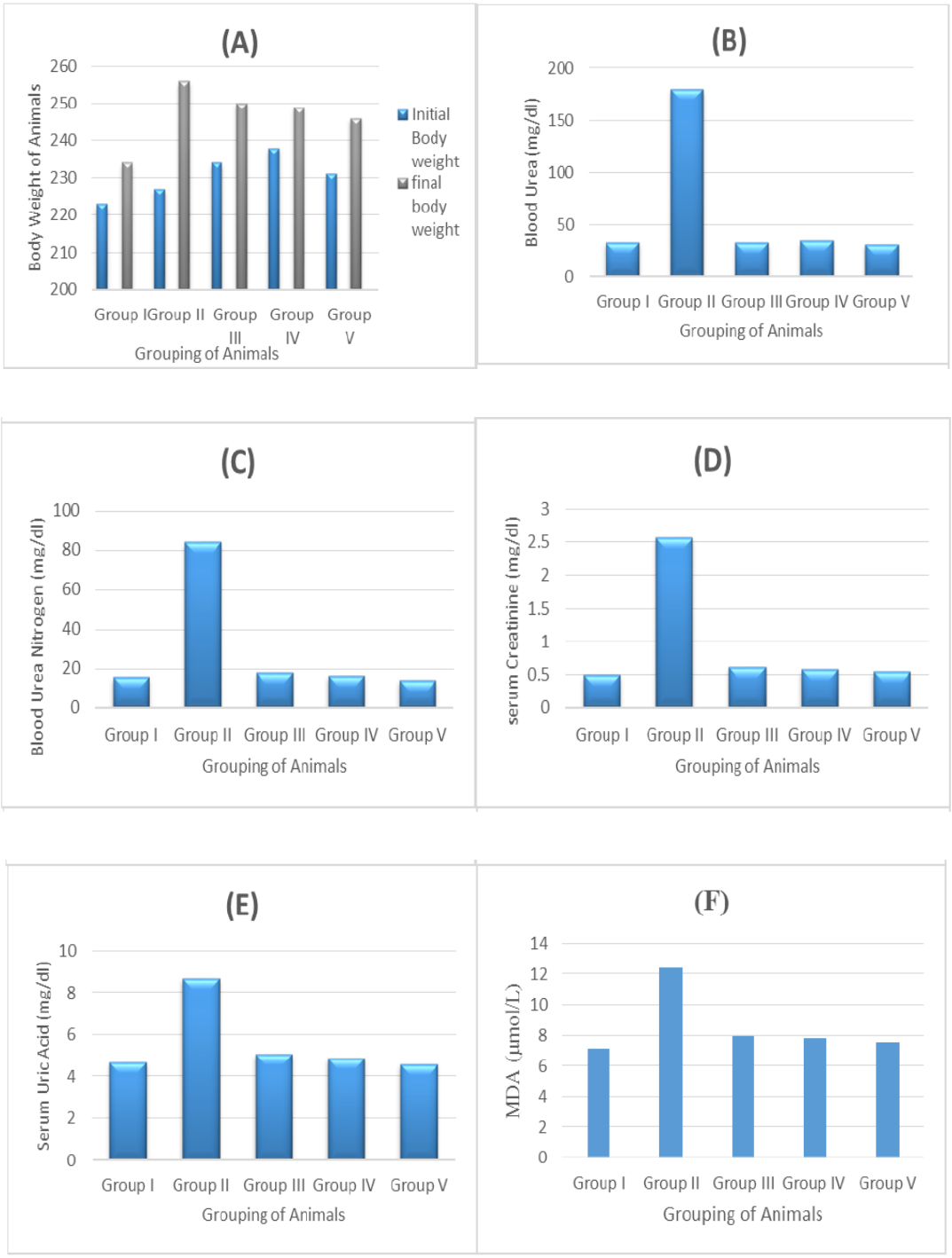

Fig. 1: Effect of sitagliptin, glutathione, combination of sitagliptin and glutathione on gentamycin – induced changes in renal biomarkers. A) Body weight B) Blood urea C) Blood urea nitrogen D) Serum creatinine E) Serum uric acid F) Malondialdehyde. Data represent means ± SD of 6 rats in each group.

4. DISCUSSION

Iatrogenic renal failure is commonly seen as a complication of many therapeutic agents. This can be likely explained by the capability of kidney to extract and concentrate toxic substances

and its high share of cardiac output. Thus, it is well-documented to be a target for many toxic xenobiotics.[21]

Nephrotoxicity as a side effects of all aminoglycosides, especially Gentamycin, limits its therapeutic use.[22] Aminoglycosides are not metabolized and are essentially eliminated by glomerular filtration. About 10% of the intravenously administered dose is accumulated in the kidney.[23] Gentamycin is largely accumulated in lysosomes, the Golgi and endoplasmic reticulum.[24,25] The serious effect occurs when the concentration of gentamycin inside the previously mentioned organelles exceeds a threshold followed by subsequent destabilization of their membranes with accumulation then release of gentamicin to cytosol.[26,27] Thus, the gentamycin in the cytosol will act on mitochondria and provoke the mitochondrial pathway of inducing oxidative stress, apoptosis and diminish the ATP.[28,29] Gentamycin induces similar morphological alterations in kidneys of both humans and experimental animal.[30] Gentamycin administration produced a elevation of kidney injury markers exhibited as a significant increase of serum BUN and creatinine levels.

In the present study, we aimed to investigate the effect of Sitagliptin and Glutathione against gentamycin-induced nephrotoxicity, hoping to achieve a new therapeutic approach that can protect or reverse gentamycin-induced nephrotoxicity. Sitagliptin significantly counteracted the nephrotoxic effects of gentamicin and retained all the injury markers near the normal levels.[31] Treatment with GM produces oxidative stress in tubular cells, both in vivo in rats[32] and in cultured tubular cells.[33] This oxidative stress is likely to be mediated by hydroxyl radicals, hydrogen peroxide and by superoxide anions[34,35] from mitochondrial origin.[36] GM directly increases the production of mitochondrial ROS from the respiratory chain.[28] The deleterious effect of overproduction of ROS and the process of lipid peroxidation, respectively, damage the protein molecules and degrade the membrane-bound phospholipids.[37] The decreased antioxidant activity in GM-induced nephrotoxicity can be explained by an increase in the generation of free radicals. This is followed by subsequent depletion of antioxidant enzymes during the process of counteracting oxidative stress.[38] Sitagliptin significantly ameliorated all these changes.[31]

Apoptosis contributes in the pathological process of different renal diseases and drug-induced nephrotoxicity.[39,40] About 20% of patients receiving GM treatment could be complicated by acute renal failure with evidence of acute tubular necrosis[33] Experimental studies with GM revealed signs of apoptosis.[41] Earlier studies have pointed that attenuating of apoptosis

suppresses renal injury which focus on the importance of inhibition of apoptosis as a critical clinical target in renal diseases.[42,43] The intrinsic pathway of apoptosis is found to be initiated by mitochondrial dysfunction.[28] Briefly, GM promotes bax aggregation and translocation to the mitochondria, causing activation of caspase-9, which then activates caspase-3. These events lead to a loss of mitochondrial membrane potential and initiate apoptotic process.[39] Sitagliptin prevented renal tubular apoptosis induced by GM exhibited by a significant decrease in the number of positive brownish caspase-3 and bax immunoreactive cells in kidney sections. These results were supported with the previous reports confirming the anti-apoptotic effects of Sita.[44,45]

Protective roles for antioxidants in genral against free radicals have been demonstrated in a num. ber of in vitro and in vivo experiments. Among the species acting as scanegers, GSH's importance has been widely stressed, depletion of tissue GSH causing hypersusceptibility to some toxic chemicals and radication. Renal function, as indicated by glomerular filtration, etc., is also effected by depletion of GSH.[46] In mammalian cells and tissues, GSH is the most abundant nonprotein thiol; it is usually present in millimolar concentrations.[47,48] As the key intracellular antioxidant, GSH reacts with electrophilic compounds and serves as a reductant for eliminating hydrogen peroxide and lipid hydroperoxides.[49] The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the membrane's structure and permeability.[50]

5. CONCLUSION

The present study suggest that sitagliptin alone has renal beneficial effect & that it may serve as an adjuvant to reduce gentamycin induced renal injury in rats. This also suggest that glutathione alone acts as a pontetiate scavenger of free radicals to prevent the toxic effect of gentamycin. But the present study mainly suggest that the combination of boh sitagliptin and glutathione has a more nephropotective potential against gentamycin induced nephrotoxicity. This may be ascribed to their antioxidant, antimitochondrial dysfunction and anti – apoptotic effects. Nonetheless, further studies are needed to investigate different doses of the combination against gentamycin induced nephrotoxicity before safe application in humans.

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