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Original Article

Nephroprotective Activity of Dibenzo-α-Pyrone Derivatives in Gentamicin-Induced Nephrotoxicity and Oxidative Stress in Rats

Shalini K. Sawhney[1,*], Monika Singh[2], Dinesh Puri[3], Sangeeta Hazarika[4], Achal Mishra[5]

[1]Department of Pharmaceutical Chemistry, ITS College of Pharmacy, Muradnagar, Ghaziabad, Uttar Pradesh, INDIA.
[2]Department of Pharmacology, ITS College of Pharmacy, Muradnagar, Ghaziabad, Uttar Pradesh, INDIA.
[3]Department of Pharmaceutics, School of Pharmacy, Graphic Era Hill University, Dehradun, Uttarakhand, INDIA.
[4]Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, INDIA.
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Shri Shankaracharya Technical Campus, Bhilai, Chhattisgarh, INDIA.

ABSTRACT

Objectives: The current work was done to determine the curative and nephroprotective activities of the synthesized butylamine derivative of Dibenzo-α-Pyrone (DAP). **Materials and Methods:** The synthesised drug at a concentration of 10, 20, and 40 mg/kg p.o. was given as a single dose in Albino rats with Gentamicin-induced nephrotoxicity. The changes in body weight of animals, if any, were monitored. Biochemical markers like serum creatinine, blood urea, and BUN concentrations were investigated to detect nephrotoxicity. In the tissues of the kidney, Glutathione (GSH) and lipid peroxidation (MDA) were also assessed. **Results:** 40 mg/kg p.o. of synthesised butylamine derivative of Dibenzo-α-Pyrone (DAP) exhibited significant (p<0.001) nephroprotective activity in the experimental animals. The reduced body weight and augmented biochemical markers (blood urea, serum creatinine, BUN levels), which were the attributes of gentamicin administration, were altered by the test sample. An increase in GSH and a decrease in Malondialdehyde (MDA) production also proved the nephroprotective activity of the synthesized sample. **Conclusion:** The derivatives of Dibenzo-α-Pyrone (DAP) demonstrated nephroprotective activity *in vivo*. This study based on experimental evidence suggests the probable mechanism of nephroprotection of the test sample might be due to its antioxidant property.

Keywords: Dibenzo-α-pyrone, Gentamicin, Nephrotoxicity, Urea, Creatinine, Glutathione, Lipid peroxidation.

Correspondence:
Dr. Shalini Kapoor Sawhney
Department of Pharmaceutical Chemistry, ITS College of Pharmacy, Muradnagar, Ghaziabad-201206, Uttar Pradesh, INDIA.
Email: shalini.sawhney17@gmail.com

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INTRODUCTION

Aminoglycosides, a class of antibiotics popularized at the cost of potent bactericidal activity and cheaper rate are effective in the treatment of bacterial infections.[1] Paromomycin, plazomicin, tobramycin, neomycin, kanamycin, gentamicin, amikacin, etc. are a few examples of aminoglycoside antibiotics.[2] The protein synthesis inside bacteria is believed to be inhibited by these antibiotics. When the concentration of aminoglycosides is higher, more will be the bactriocidal rate. The narrow margin between safe and toxic doses is to be surveiled. Nephrotoxicity and loss of hearing are the most common side effects of aminoglycosides. At the lowest therapeutic doses, gentamicin is reported to cause nephrotoxicity. Endocytic vacuoles and lysosomes fuse as a consequence of proximal tubular cells' poor reabsorption of gentamicin due to adsorptive endocytosis.[3,4] Augmentation in levels of urea, metabolic products and plasma creatinine (enzymuria, proteinuria, glycosuria, aminoaciduria), tubular cytotoxicity, apoptosis, necrosis,[5-7] and an imbalance in electrolytes level (hypermagnesuria, hypercalciuria, hypomagnesemia and hypocalcemia)[8,9] are the characteristics of gentamicin-induced nephrotoxicity. Alterations in urine volume, body weight, rate of lipid peroxidation and renal clearance are an extension of the characteristics. Antioxidant enzymes like lipid peroxides Glutathione (GSH) and SOD (Superoxide Dismutase) act as a protective shield for kidneys from free radicals and super-oxides. Some of the consequences of gentamicin-induced nephrotoxicity include reduced renal dysfunction and glomerular filtration rate as a result of renal free radical production, a decrease in anti-oxidant defence processes, and congestion of glomerular filtration[10,11] and when the kidney fails to filter waste substances

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and toxins adequately from the blood, it results in a medical condition called kidney failure. Kidney failure has two forms acute and chronic. As there are no symptoms observed physically and noticeably during kidney failure, it might take several years for detection. This is why it is frequently called the "silent killer".[12] Intramuscular, intravenous, or topical administration of gentamicin is recommended for the bacterial infection treatment. However, the insignificant absorption of gentamicin from the small intestine is the reason for its non-proposal for oral use. It is eliminated unchanged through urine by glomerular filtration.[1]

Shilajit, an Ayurvedic rejuvenator, contains Dibenzo-α-Pyrone (DBαP) as an active ingredient. It is found in the Himalayas and Hindukush ranges. Shilajit is formed when some plants gradually deteriorate under the influence of microorganisms.[2] A literature review reveals its anti-inflammatory, anti-ageing, analgesic, anti-oxidant, anti-ulcerogenic, adaptogenic activities, anti-alzheimer, immunomodulator, anti-ulcerogenic, and its use as a nutritive tonic.[13-18]

This study's goal was to determine if synthesized butylamine derivatives of Dibenzo-α- pyrone (DAP) had any curative or protective properties against gentamicin-induced nephrotoxicity in albino rats.[19,20]

MATERIALS AND METHODS

Drugs and Chemicals

Dibenzo- α-pyrone was synthesized according to the procedure given by Sawhney *et al.*[21]

Adult disease-free animals were used in experiments Wistar albino rats of either sex, weighing between 100-150 g, were caged in polypropylene cages and fed under conventional environmental settings (25±3°C; 12 hr day/night cycle; 35-60% humidity). When conducting animal studies and providing them with the adequate care, CPCSEA India ("Committee for the Purpose of Control and Supervision of Experiments on Animals") recommendations were carefully approved. The *in vivo* studies were duly authorized by the IAEC (No. ITS/05/IAEC/2021).

Gentamicin-induced nephrotoxicity

We randomly grouped the adult Wistar albino rats into 5 groups, each with six individuals (n=6).

Normal control (Group I): The saline solution was administered intraperitoneally to the animals.

Disease control (Group II): For seven days in a row, gentamicin (100 mg/kg, i. p.) was given through injection into the animals.

Drug control (Group III): Animals were given DAP (10 mg/kg, p. o.) and gentamicin (100 mg/kg, i. p.) every day for 7-days in a row.

Drug control (Group IV): Gentamicin (100 mg/kg, i. p.) was given to the animals, and DAP (20 mg/kg, po) was given daily for seven days in a row.

Drug control (Group V): Animals received DAP (40 mg/kg, p.o.) and gentamicin (100 mg/kg, i.p.) injections every day for 7-days in a row.

Post 24 hr after the last dose on the 7th day, the retro-orbital plexus of animals was punctured to collect a sample of blood, whereof it was centrifuged to obtain serum to assess the level of creatinine and urea. Kidneys were isolated from euthanized rats. A fraction of the tissue was cleaned using saline and preserved in 10% formalin to study its histopathology while the other was used to quantitatively analyse oxidative stress indicators like decreased levels of lipid peroxidation (TBARS) and GSH.[22,23]

Determination of food intake and body weight

For each group body weight of each rat was checked. It was assessed before the animal was sacrificed and daily from the start of the trial until the end.

Food consumption was recorded daily. Extra care was taken to distinguish the spilling of food from the husk while the experimental animals consumed food to assess food intake accurately.[24]

Determination of serum urea level

The amount of urea in a blood sample was determined using a standard diagnostic kit (Span Diagnostic Ltd., India). Upon condensation with NED (Naphthyl Ethylene Diamine) and o-phthaldehyde, urea formed a coloured complex. The amount of urea in the blood closely correlated with the rate at which this complex formed. After 15 min of heating in a water bath, the optical density of samples was noted at 505 nm in an original rate mode (fixed interval).[25]

Determination of serum creatinine level

Determination of serum creatinine level-A Span Diagnostic Limited, India-designed creatinine kit was used to estimate the levels of creatinine in serum by the alkaline picrate method. Creatinine and alkaline picrate combine to form a red complex in a protein-free solution. A colorimetric assessment of this complex was done.[26]

Determination of reduced Glutathione (GSH)

The approach described by Sedlak[26] was followed to determine the non-proteinous total sulfhydryl and reduced GSH contents in the kidneys of rats. Tissue homogenates were produced and precipitated with trichloroacetic acid in cold KCl of 5.0 mL (1.15%) (TCA). Tris-EDTA buffer of 2.0 mL (pH 8.9) and 0.1 mL of 5 were combined with 0.5 mL of the supernatant, 5-dithio-bis-2-nitrobenzoic acid (DTNB) to get the reaction solution. On a UV-Visible spectrophotometer (Merck Thermospectronic), the

absorbance solution was noted at 412 nm. The values were given in µmoles of GSH for every gram of protein.

Determination of lipid peroxidation (MDA)

The TBARS formation (Thiobarbituric Acid Reactive Substances) was examined to determine the levels of lipid peroxidation.[27] TBARS assay gives a measurement of the Malondialdehyde (MDA) present in a sample. 0.5 mL of tissue homogenate, 3.0 mL of H_3PO_4 (1%), and 1.0 mL of thiobarbuturic acid (0.6%) were combined well in a reaction tube and centrifuged. At 520 and 535 nm, the organic layer absorbance was recorded and the values were reported in nanomoles of MDA/g tissue.[28]

Total tissue protein

It can be estimated using Lowry's approach.[24]

Histopathological assessment

Animal kidneys were removed after being sacrificed and examined histopathological. The kidneys were removed after being dissected and preserved in 10% neutral buffered formalin. The features studied include glomerular and tubular histology.

Statistical analysis

The mean as well as SEM (Standard Error of the Mean) were used to represent all findings. To statistically analyse the gathered data, Tukey's post-hoc and ANOVA (one-way analysis of variance) tests were both carried out. Statistics were deemed considerable at $p<0.05$.

RESULTS

DAP effect on food intake and body weight

Animals' body weight and food consumption changed during the trial, as indicated in Table 1. Animal food intake and body weight both increased significantly ($p<0.05$) after DAP treatment.

DAP impact on serum biochemical (serum urea level, nitrogen and creatinine)

In comparison to control groups, gentamicin-treated groups exhibited a substantial ($p<0.001$) rise in BUN as well as blood urea levels, a drop in total serum protein, and a rise in serum creatinine.

Administering 10 mg/kg, 20 mg/Kg and 40 mg/kg of butyl amine derivative of dibenzo-α-pyrone considerably($p<0.001$) declined the amount of serum creatinine, BUN, and urea in gentamicin groups in comparison to the gentamicin controlled group, the total serum protein content rose considerably ($p<0.001$) (Table 2).

DAP effect on oxidative stress indicators (MDA, GSH, and Protein)

Significantly higher GSH levels and lower MDA levels ($p<0.05$) were found in comparison to the negative control (Table 3).

Monitoring of MDA, as well as GSH, has indicated that the derivative has noteworthy *in vivo* antioxidant activity. Nephrotoxicity induced by gentamicin shows reduced tubular injury and glomerular filtration rate because of the ROS formation (Reactive Oxygen Species), which might be affected by changes in the ultrafiltration coefficient, membrane lipid peroxidation, filtration surface area alteration, mesangial cells contraction, and reduces the glomerular filtration rate.[29] Such renal hemodynamic anomaly may be managed, and lowering it is crucial to preventing the decline of normal kidney functioning. The degradation of the proteins and nucleic acids cause nitrogenous compound formation that are not proteins, including creatinine and urea.[30] The variations in kidney function shown in the rat system and the nephrotoxic properties of gentamicin in humans are closely correlated.[31] Nephroprotective activity can be attributed to the anti-oxidant property thereby preventing the damage caused by reactive oxygen species.[32-36]

Uric acid, creatinine, and serum urea level variations strongly indicate the impairment of renal function. Dibenzo-α-pyrone derivatives were administered, and this considerably reduced the concentrations of uric acid, creatinine, protein, and serum urea in the treated groups. Numerous studies point to the presence of free radicals and lipid peroxidation in gentamicin-induced nephrotoxicity, even though the exact mechanism is not fully defined. Although free radical formation is not the primary reason of gentamicin-induced renal damage, it is essential for gentamicin-nephrotoxicity, since *in vivo* antioxidant therapy in rats prevents renal damage. Administration of gentamicin results in renal lipid peroxidation, which can be observed with reduced GSH and an increase in the MDA amount in comparison to the control group. Due to its ability to stop the destruction caused by

Table 1: Change in food Intake and Body weight.

Groups	Treatment design	Body weight (gm)	Food Consumption (gm)
I	Normal Control	195.08±6.7[a]	12.33±0.31[a]
II	Gentamicin(100 mg/Kg)	141±8.1[b]	5.41±0.23[b]
III	Butylamine Der. (10 mg/Kg)	189±2.1[b]	8.95±0.51[b]
IV	Butylamine Der. (20 mg/Kg)	196.19±6.8[b]	10.61±0.73[b]
V	Butylamine Der. (40 mg/Kg)	197±3.2[b]	12.49±0.31[b]

The value is given as mean±SEM, *n*=rat number.[a] considerably varied from control group ($p<0.05$).[b] considerably varied from gentamicin group ($p<0.05$).

Table 2: Effect of Butylamine derivative of Dibenzo-α-pyrone on Serum creatinine, blood urea, and BUN in trial groups.

Groups	Treatment design	Blood urea (mg/100 mL)	BUN (mg/100 mL)	Serum Creatinine (mg/100 mL)	Total Protein (nm/dL)
I	Normal Control	19.0±1.2	9.4±0.8	0.6±0.1	6.3±0.5
II	Gentamicin(100 mg/Kg)	68.1±1.3[a]	31.6±1.5[a]	2.3±0.3[a]	2.5±0.2[a]
III	Butylamine Der.(10 mg/Kg)	45.4±1.5[b]	20.9±0.9[b]	0.7±0.0[b]	5.2±0.3[b]
IV	Butylamine Der.(20 mg/Kg)	60.2±2.0[c]	27.5±0.8[c]	1.4±0.2[b]	3.7±0.2
V	Butylamine Der.(40 mg/Kg)	39±1.1	18±0.8[b]	0.9±0.1[b]	4.1±0.2

Values are presented as mean±SEM, n=6; [a]p<0.001 deemed statistically considerable in comparison to the normal group; [b]p<0.001 and [c]p<0.05 deemed statistically considerable in comparison to the gentamicin control group.

Table 3: Derivative effect on MDA and GSH.

Groups	Treatment design	GSH(µmol/mg protein)	MDA (µmol/mg protein)
I	Normal Control	214.11±38.11	89.45±18.77
II	Gentamicin(100 mg/Kg)	53.61214.11±15.21[a]	614.83±43.21[a]
III	Butylamine Der.(10 mg/Kg)	137.4±9.75[b]	127.21±13.78[b]
IV	Butylamine Der.(20 mg/Kg)	155.72±26.28[b]	91.01±11.87[b]
V	Butylamine Der.(40 mg/Kg)	180.33±05.06[b]	84.24±14.65[b]

The value is given as mean±SEM, n=rat number.[a] considerably varied from control group (p<0.05).[b] considerably varied from gentamicin group (p<0.05).

ROS created by gentamicin, dibenzo-α-pyrone derivatives exhibit *in vivo* antioxidant activity. The therapy with gentamicin caused oxidative stress, which led to a drop in kidney GSH levels.

Dibenzo-α-pyrone compounds may have nephroprotective properties as a result of their anti-oxidant action through a potential mechanism of cellular protection.

Effect of DAP on histopathological evaluation

Such abnormalities in kidney histology were reduced by the butyl amine derivative of DAP at dosages of 10, 20, and 40 mg/kg, p.o. Normal control rats in the gentamicin-induced nephrotoxicity model had normal tubular histology and glomerular function, while the gentamycin-treated group had abnormal blood vessel and glomerular congestion, tubular shape distortion, and the detection of inflammatory cells in the kidney. These alterations in kidney histology were reduced by concurrently administering the derivative (40 mg/kg, p.o.) (Figure 1).

DISCUSSION

The formation of ROS in gentamicin-induced renal toxicity in animals is suggestive of the involvement of oxidative stress. Nephrotoxic conditions are well known to cause a rise in GSH depletion, lipid peroxidation, decreased food consumption, altered weight, and a rise in blood levels of creatinine as well as urea. Bowman's capsule space loss and glomerular destruction that results in glomerular congestion are also seen. The current work was done to determine the curative and nephroprotective activities of the synthesized). The synthesised drug (butylamine derivative of DAP) at a concentration of 10, 20, and 40 mg/kg p.o. was given as a single dose in Albino rats with Gentamicin-induced nephrotoxicity. The changes in body weight of animals, if any, were monitored. Biochemical markers like serum creatinine, blood urea, and BUN concentrations were investigated to detect nephrotoxicity. In the tissues of the kidney, Glutathione (GSH) and lipid peroxidation (MDA) were also assessed.

It was observed that the 40 mg/kg p.o. of synthesised butylamine derivative of DAP exhibited significant (p<0.001) nephroprotective activity in the experimental animals. The reduced body weight and augmented biochemical markers (blood urea, serum creatinine, BUN levels), which were the attributes of gentamicin administration, were altered by the test sample. An increase in GSH and a decrease in MDA production also proved the nephroprotective activity of the synthesized sample.

By functioning as a preventive pharmacological substance and reducing the adverse effects of gentamicin-induced nephrotoxicity, dibenzo-α-pyrone derivatives may be an option in the fight against gentamicin-induced nephrotoxicity. This nephroprotective can be due to its antioxidant as well as anti-inflammatory properties. All treatment given with gentamicin renders nephroprotective activity unlike the treatment offered post-gentamicin-induced nephrotoxicity. The amount of serum creatinine, blood urea, and MDA levels was considerably decreased, it was also noted. These alterations are indicative of the effect on the functioning of glomeruli. Although additional research is needed to understand the precise process of gentamicin-induced nephrotoxicity, the role of free radicals and lipid peroxidation are reported in several

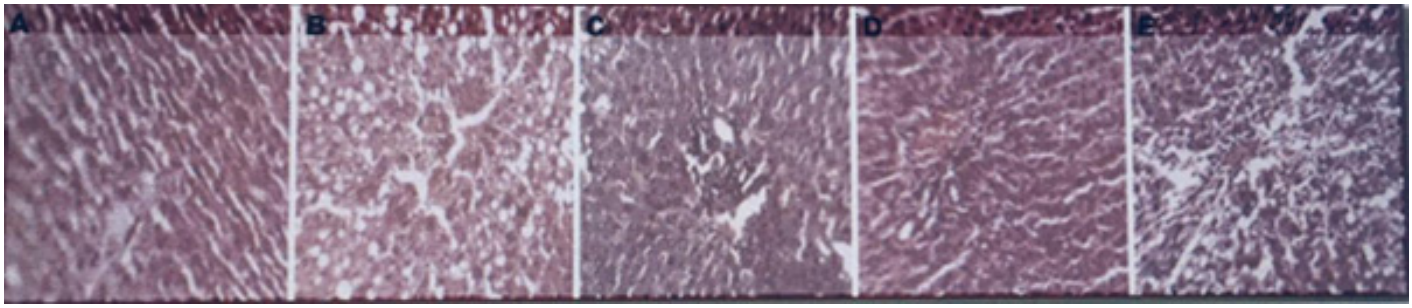

Figure 1: DAP effect on Gentamicin induced hepatotoxicity in rats A: Normal group; B: Gentamicin control group (100 mg/kg, i.p); C: DAP controlled group (10 mg/kg, p.o); D: DAP controlled group (20 mg/kg, p.o); E: DAP controlled group (40 mg/kg, p.o).

studies. A rise in MDA and a reduction in GSH concentration when contrasted with the control group showed that the single dosage of gentamicin caused renal lipid peroxidation. Glutamine is one of the essential precursors for the formation of the endogenous anti-oxidant GSH. Due to its anti-oxidant properties, glutamine has also been documented to demonstrate a preventive role in renal damage. The synthesised derivatives of DAP exhibited antioxidant activity. The nephroprotective activity of the DAP derivatives can be attributed to the anti-oxidant property thereby preventing the damage caused by reactive oxygen species.

CONCLUSION

By functioning as a preventive pharmacological substance and reducing the adverse effects of gentamicin-induced nephrotoxicity, dibenzo-α-pyrone derivatives demonstrated nephroprotective activity *in vivo*. DAP derivatives may be an option in the fight against gentamicin-induced nephrotoxicity. This study based on experimental evidence suggests the probable mechanism of nephroprotection of the test sample might be due to its antioxidant property Thus, it can be concluded that the nephroprotective activity of the DAP derivatives can be attributed to the anti-oxidant property thereby preventing the damage caused by reactive oxygen species.

Also, there is the need to standardize the methods of assessment of nephrotoxicity and nephroprotection and improve *in vivo* animal model commonly employed. This also includes measurement of novel and promising biomarkers with superior sensitivity and specificity over plasma creatinine and BUN for assessing acute or chronic tubulotoxicity alongside detailed histopathological analyses.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Hrs: Hours; **i.p:** Intraperitoneally; **DAP:** Dibenzo-α- pyrone; **p.o.:** per os or by mouth; **mins:** Minutes; **NED:** Naphthyl ethylene diamine; **GSH:** Glutathione; **TCA:** Trichloroacetic acid; **KCl:** Potassium Chloride; **EDTA:** Ethylene diamine tetraacetic acid; **DTNB:** 5-dithio-bis-2-nitrobenzoic acid; **TBARS:** Thiobarbituric Acid Reactive Substances; **MDA:** Malondialdehyde; **H_3PO_4:** Hydrogen Phosphate; **BUN:** Blood Urea Nitrogen; **Der:** Derivatives; **SEM:** Standard Error of Mean; **ROS:** Reactive Oxygen Species.

SUMMARY

The curative and nephroprotective activities of the synthesized butylamine derivative of Dibenzo-α-pyrone (DAP).

The synthesised drug given as a single dose in Albino rats with Gentamicin-induced nephrotoxicity, changes in body weight of animals. Biochemical markers like serum creatinine, blood urea, and BUN concentrations were checked to detect nephrotoxicity. In the tissues of the kidney, Glutathione (GSH) and lipid peroxidation (MDA) were also assessed.

The reduced body weight and augmented biochemical markers (blood urea, serum creatinine, BUN levels) were altered by the test sample. An increase in GSH and a decrease in Malondialdehyde (MDA) production was observed

The nephroprotective activity *in vivo*. suggests the probable mechanism of nephroprotection of the test sample might be due to its antioxidant property.

ETHICAL APPROVAL

The IAEC Committee and the CPCSEA Committee of the Ministry of Social Justice and Empowerment of the Indian Government authorized every experimental method.

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