

# Anti Oxidant Property of New Cephalosporin-Aminoglycoside Fixed Dose Combination

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**Abstract:** Aminoglycosides are known to cause oxidative stress related toxicities and tissue injuries. Present study was planned to evaluate the effect of aminoglycosides on blood oxidative stress parameters in mice and free radical scavenging potential of cephalosporins, when combined as a single injection with aminoglycosides using chemical vector mediated technology. *Mus musculus* mice were divided into five groups: Control group animals (treated with normal saline), amikacin treated group, tobramycin treated group, cefepime + amikacin treated group and ceftazidime + tobramycin treated group. A significant improvement in superoxide dismutase (SOD), catalase activities, along with malonaldehyde (MDA) levels and creatinine levels were observed in fixed dose combination of cephalosporins and aminoglycosides treated groups as compared to aminoglycosides alone (amikacin and tobramycin) treated groups. These findings indicate that on combining cephalosporins with aminoglycosides using chemical vector mediated technology prevents oxidative stress related tissues injury induced by aminoglycosides.

**Key Words:** Cephalosporin, aminoglycoside, free radicals, catalase, superoxide dismutase, malonaldehyde.

## INTRODUCTION

Aminoglycosides are important drugs in clinical use, but these antibiotics are potentially ototoxic and nephrotoxic at levels only slightly above therapeutic range [1,2]. Approximately 5-10% of the people, who are treated with aminoglycosides experience such toxic effects. The toxicity of aminoglycosides has been widely studied [3]. These aminoglycosides are polycationic in nature that cause oxidative stress in living cells [4]. Free radicals play an important role in drug-induced damage to the liver, kidneys and other organs [5]. There are various reports suggesting that aminoglycosides causes ototoxicity [6] and nephrotoxicity [7] due to oxidative stress. The binding of aminoglycosides *in vivo* as well as *in vitro* with negatively charged membranes is associated with impairment of phospholipid catabolism, change in membrane permeability, membrane aggregation [8] and reduces the activities of phospholipases [9,10]. The adverse effect of aminoglycosides has been attributed to the development of an array of alterations in proximal tubule epithelium followed by its destruction, thereby causing kidney dysfunction [11].

Aminoglycosides in combination with cephalosporins synergistically kill various pathogens and broaden the bactericidal spectrum against Gram positive and gram negative bacteria. Cefepime and ceftazidime are cephalosporin class of antibiotics and having free radical scavenging potential [12]. Chemical vector mediated technology is used to provide compatibility of cephalosporins and aminoglycosides without interfering in the pharmacokinetic property of drug component and prevents the oxidation of methionine group and thiazolidine and dihydrothiazine present in antibiotics [13]. The component(s) of chemical vector possess free radi-

cal scavenging potential that leads to reduction in oxidative stress.

The objective of the present study was to evaluate the effect of aminoglycosides on blood oxidative stress parameters in mice and free radical scavenging potential of cephalosporins, when combined as a single injection with aminoglycosides using chemical vector mediated technology.

## MATERIALS AND METHODS

### Chemicals

All the biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. Other chemicals purchased locally were of analytical grade. All the antibiotics such as amikacin, tobramycin, cefepime plus amikacin (Poptox) and ceftazidime plus tobramycin (Tobracef) were obtained from Venus Remedies Ltd. India. The ratio of fixed dose combination of cefepime + amikacin and ceftazidime + tobramycin were 4:1 and 11:1 respectively.

### Animals and Treatments

Twenty *Mus musculus* mice (age 3.5 to 4.0 months; weighing  $30 \pm 5$  g) were used in the experiment. The mice were fed standard pelleted diet and sterile water *ad libitum*. The mice were divided into five groups of four mice each as given below.

Control group (isotonic saline treated group)

Amikacin sulphate treated group (28.5 mg/Kg body weight/day)

Tobramycin sulphate treated group (4.0 mg/Kg body weight/day)

Ceftazidime plus Tobramycin treated group (34.1mg/Kg body weight/day)

Cefepime plus Amikacin fixed dose combination treated group (35.7mg/Kg body weight/day)

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The respective drugs were administered -intramuscularly for 7 days. At the end of treatment, 1ml blood samples were drawn in heparinized vials from the heart by cardiac puncture under the light ether anesthesia. Blood samples were diluted 10 times with chilled distilled water, left for at least 1 hr at 0- 4°C before the estimation of enzyme assay.

### Enzyme Assays

#### Superoxide Dismutase (SOD) Assay

SOD activity was determined by the Method of Misra and Fradovich [14]. The reaction mixture consisted of 1.0 ml carbonate buffer (0.2M, pH 10.2), 0.8 ml KCl (0.015M), 0.1 ml of blood and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025M). The change in absorbance was recorded at 480 nm at 15 second interval for one minute at 25°C. Suitable control lacking enzyme preparation was run simultaneously.

One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

#### Catalase Assay

Catalase activity was measured by the method of Luck [15]. The reaction mixture consisted of 0.3ml phosphate buffer, (0.2M pH 6.8), 0.1ml H<sub>2</sub>O<sub>2</sub> (1M) and water to make the final volume to 3.0ml. The reaction was started by adding the suitable aliquot of enzyme preparation. The change in the absorbance was recorded at 15 sec interval for one minute at 240nm at 25°C. Suitable control was run simultaneously. One Unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from H<sub>2</sub>O<sub>2</sub> in 100 sec at 25°C.

#### Measurement of Lipid Peroxidation

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of

malonaldehyde (MDA) formed, essentially according to Ohkawa *et al.* [16]. It was determined by thio barbituric reaction. The reaction mixture consisted of 100 µl of diluted blood, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and water to make up the volume to 4.0 ml. The tubes were boiled in water bath at 95°C for one hour and cooled immediately under running tap water. Added 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were centrifuged at 3500 rpm for 30 minutes. The upper layer was aspirated out and optical density was measured at 532nm. The reference standard used was 1,1, 3,3 tetra ethoxy propane.

#### Estimation of Creatinine

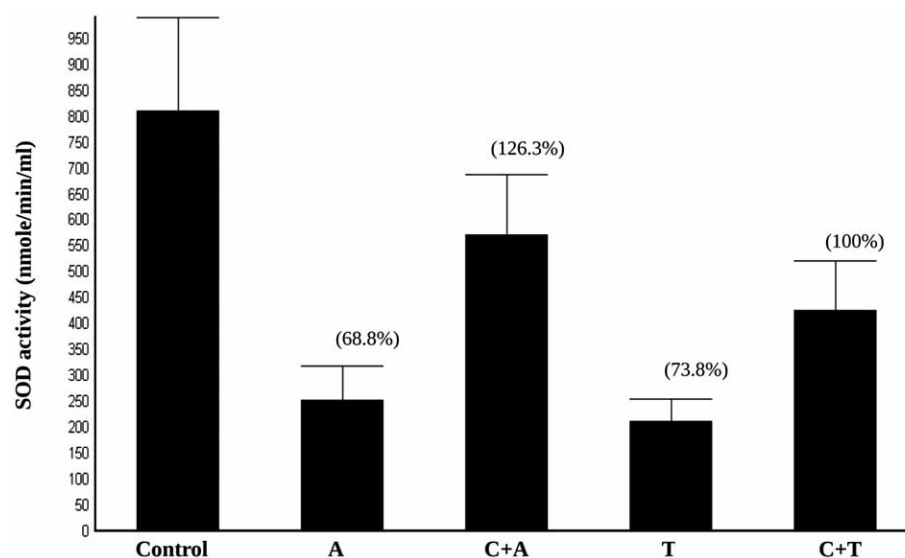
Creatinine levels were determined by the alkaline picrate method using diagnostic kits (Bayer Diagnostics India Ltd., Baroda, Gujrat, India).

### STATISTICAL ANALYSIS

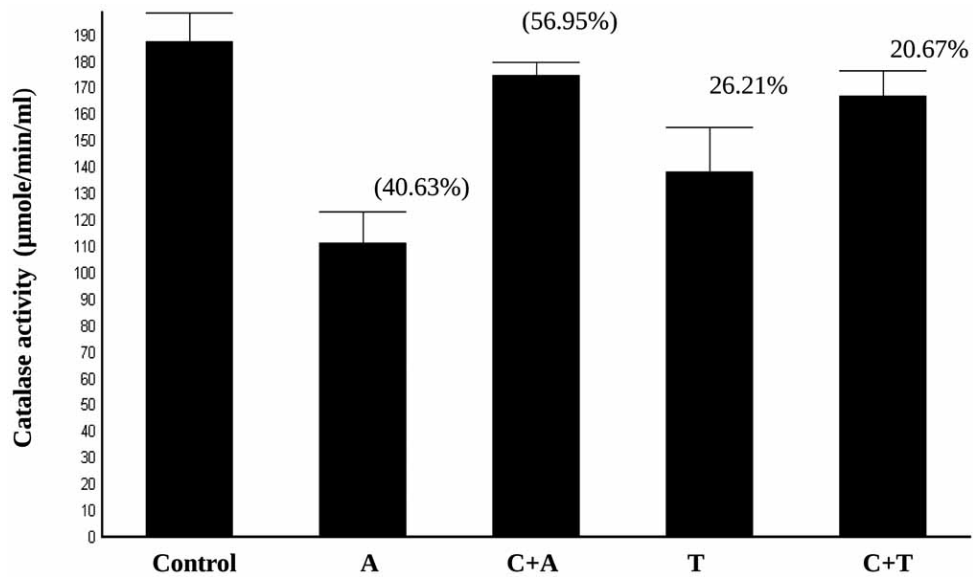
All values are expressed in mean ± SD. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between control and treated groups. P values <0.05 were considered statistically significant.

### RESULTS

A significant (p<0.001) decrease in blood superoxide dismutase and catalase activities were observed in amikacin (68.8%, 40.63%) and tobramycin (73.8%, 26.21%) treated groups as compared to control group. Activities of these enzymes were significantly increased in cefepime + amikacin treated group (p<0.01, 126.3% ; p<0.001, 56.95%) as well as ceftazidime + tobramycin treated group (p<0.05, 100%; p<0.01, 20.67% ) as compared to amikacin and tobramycin alone treated group respectively (Figs. (1) and (2)).



**Fig. (1).** Values are expressed in Mean ± SD. Value in parenthesis represent % changes in control vs amikacin and tobramycin alone treated group and amikacin and tobramycin vs fixed dose combination treated group. A= amikacin treated group, C+A (Potentox)= Cefepime plus amikacin treated group, T= Tobramycin treated group and C+T (Tobracesf)= Ceftazidime plus tobramycin treated group.



**Fig. (2).** Values are expressed in Mean ± SD. Value in parenthesis represent % changes in control vs amikacin and tobramycin alone treated group and amikacin and tobramycin vs fixed dose combination treated group. A= amikacin treated group, C+A (Potentox)= Cefepime plus amikacin treated group, T= Tobramycin treated group and C+T (Tobracesf)= Ceftazidime plus tobramycin treated group.

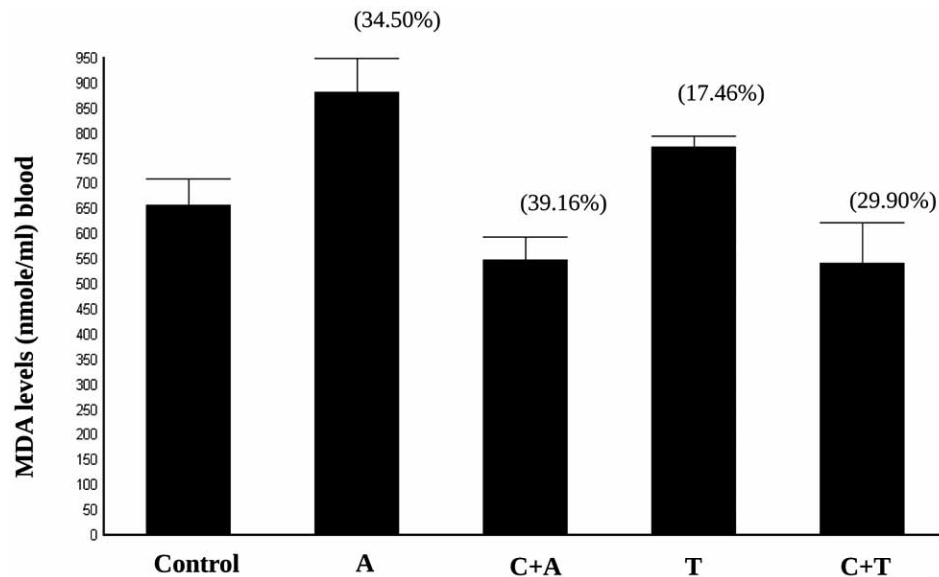
A significant increase in MDA levels were observed in amikacin ( $p < 0.001$ , 34.50%) and tobramycin ( $p < 0.05$ , 17.46%) treated groups as compared to control group. MDA levels were significantly lowered in cefepime + amikacin ( $p < 0.001$ , 39.16%) as well as ceftazidime + tobramycin ( $p < 0.001$ , 29.90%) treated groups as compared to amikacin and tobramycin alone treated group respectively (Fig. (3)).

Both the aminoglycosides (amikacin and tobramycin) caused a significant increase ( $p < 0.001$ , 66.3%; 52.6%) in blood creatinine levels as compared to control group. Blood creatinine levels were significantly ( $p < 0.001$ ) decreased in

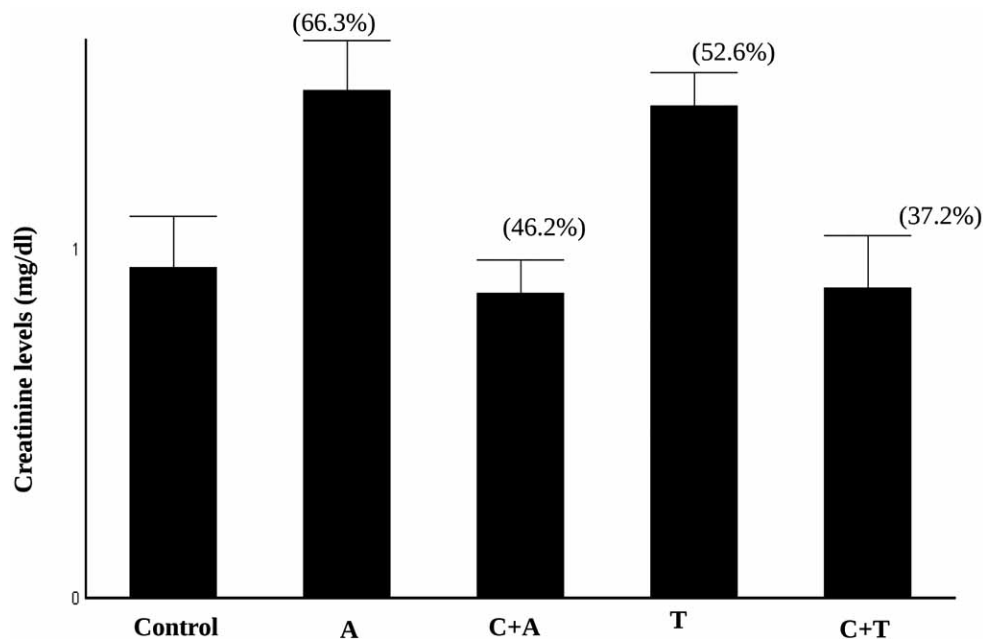
cefepime + amikacin (46.2%) as well as ceftazidime + tobramycin (37.2%) treated group as compared to amikacin alone and tobramycin alone treated group respectively. No significant change in blood creatinine levels were observed in cefepime + amikacin treated group as well as ceftazidime + tobramycin treated group as compared to control group (Fig. (4)).

**DISCUSSION**

Aminoglycosides such as amikacin and tobramycin are one of the common drug which cause nephrotoxicity and ototoxicity. Various workers have reported that amikacin and



**Fig. (3).** Values are expressed in Mean ± SD. Value in parenthesis represent % changes in control vs amikacin and tobramycin alone treated group and amikacin and tobramycin vs fixed dose combination treated group. A= amikacin treated group, C+A (Potentox)= Cefepime plus amikacin treated group, T= Tobramycin treated group and C+T (Tobracesf)= Ceftazidime plus tobramycin treated group.



**Fig. (4).** Values are expressed in Mean  $\pm$  SD. Value in parenthesis represent % changes in control vs amikacin and tobramycin alone treated group and amikacin and tobramycin vs fixed dose combination treated group. A= amikacin treated group, C+A (Potentox)= Cefepime plus amikacin treated group, T= Tobramycin treated group and C+T (Tobracesf)= Cefazidime plus tobramycin treated group.

tobramycin alter antioxidant defence mechanism [17]. Aminoglycosides generate free oxygen radicals, leading to tissue injury such as nephrotoxicity and ototoxicity [18]. Sha and Schacht established *in vitro* free radical generation by aminoglycosides [19]. The molecular hypothesis proposed was that the aminoglycosides disrupt signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids [20]. Amikacin and tobramycin penetrate cell membrane of polyunsaturated fatty acids (PUFAs) and causes tissue injury [21].

In the present study, aminoglycosides administration significantly decreased SOD, Catalase activities and increased the MDA and creatinine levels in the blood of treated mice suggesting that the aminoglycosides induced oxidative stress could be the cause of nephrotoxicity. These observations correlated well with previously reported results. It has been reported that aminoglycosides such as streptomycin and gentamycin increase renal MDA levels and depress the antioxidant enzymes activities in kidney and heart [22,23]. It was stated that oxygen free radicals were involved in aminoglycosides induced nephrotoxicity and singlet oxygen might directly inactivate the antioxidant enzymes.

Cephalosporin class of antibiotics such as cefepime and ceftazidime have free radical scavenging potential. Cefepime has low *in vitro* affinity for the major chromosomally mediated lactamases and good stability against enzymatic hydrolysis [24]. It has been also reported that cephalosporins protects against HOCl-driven oxidative injury, this defence is a consequence of a direct drug scavenging capacity towards HOCl [25]. Aminoglycosides are rich in primary amines and possess cytoprotective properties but would not be expected to protect extracellular sulfhydryl group against free radical-mediated oxidation. Cephalosporins are thioether

group containing antibiotics which are very effective in preventing the free radical-mediated oxidation of sulfhydryl group [12].

The data thus support the concept that fixed dose combination of cephalosporin with aminoglycosides prepared using chemical vector mediated technology had antioxidant and free radical scavenging potential. It is postulated that combination therapy of cefepime plus amikacin (Potentox) and cefetazidime plus tobramycin (Tobracesf) scavenged aminoglycoside induced free radical generation, oxidative stress and tissue injury. In addition to proven synergy of ceftazidime + tobramycin (Tobracesf) as well as cefepime + amikacin (Potentox) antioxidant characteristics of these combinations will also scavenge oxidative stress induced by pathogens thus will improve efficacy of the treatment. In conclusion, fixed dose combinations of cephalosporins and aminoglycosides prepared using chemical vector mediated technology possess anti-oxidative and free radical scavenging potential and contribute in improving the efficacy and safety profiles of these combinations.

#### ACKNOWLEDGEMENT

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