

## Research Article



## Estimation of Anti Microbial Activity of Ascorbic Acid in Superoxide Dismutase Level in Microbial Culture with Amino Penicillin - Ampicillin

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### ABSTRACT

The *in vitro* activity of ascorbic acid on the antibacterial effect of ampicillin on gram positive and gram negative organism was investigated using *in vitro* standardized method of the national committee for clinical laboratory standard (NCCLS). The study was conducted to improve the efficacy of ampicillin with ascorbic acid against *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2345 by enhancing their membrane permeability. The Minimum inhibitory concentration and disc diffusion method were determined to identify the efficacy of ascorbic acid. In the present study was to determine the super oxide dismutase level in microbes with the addition of ampicillin and ascorbic acid. The cells were suspended in fresh Luria Bertani broth was treated with antibiotics 100µg/ml and different concentrations of ascorbic acid 50, 100, 200µg/ml. This SOD level was tested with the amount of protein presents identified by protein estimation method. Decreased SOD level in the ampicillin treated or ampicillin, ascorbic acid combination treated microorganisms indicates a low level of growth. An ampicillin and ascorbic acid 100 µg/ml should have 42% reduction of SOD as an additive effect. But in combination 44 % activity was observed which indicates a low degree of synergy.

**Keywords:** Ampicillin, Ascorbic acid, Super oxide dismutase, anti oxidants.

### INTRODUCTION

Overuse of antibiotics is that bacteria reproduce and mutate rapidly that they develop immunity to antibiotics. This results in antibiotics being useless against some bacteria. These resistant bacteria may then infect a non abuser of antibiotics and though this person does not routinely use antibiotics, has the same difficulty in fighting the infection with antibiotics because the bacteria are already resistant to antibiotics. When antibiotics are given, there may be a major shift in balance with a killing out, of large numbers of the beneficial bacteria. This, in turn, potentially allows overgrowth of the pathogens. Current development in the treatment of microbial infections includes antioxidants along with antibiotics. Antioxidants are usually prescribed to restore the health in the patients. Moreover there is a possibility of potentiating the antibacterial activities of the antibiotic by antioxidants. In the earlier literature the effect of dietary antioxidants such as ascorbic acid, Vitamin E, and β-carotene on the antibacterial activity of the amino penicillin, ampicillin was identified<sup>1-7</sup>. Therefore in the present study, an attempt was made to reveal the level of superoxide dismutase in the microbial cells when treated with ascorbic acid and ampicillin.

### MATERIALS AND METHODS

#### Micro organism

*Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2345 were obtained from National Chemical Laboratory, Pune, India.

#### Estimation of Superoxide dismutase activity in microbial culture<sup>8</sup>

*Staphylococcus aureus* cells were grown in suspension of Luria bertani medium. The inoculated culture at mid-log phase a density of  $2 \times 10^6$  cells/ml with 95% viable cells was taken for the experiments. Exponential phase culture 2ml, were centrifuged at 2420rpm for 5 mts at 4°C. The pellets were washed thrice with potassium phosphate (kk2) buffer<sup>9</sup>. The cells were suspended in fresh LB broth and treated with antibiotics 100µg/ml and different concentrations of antioxidants 50, 100, 200µg/ml incubated at 22°C for one hour. The cells were incubated at 22°C with shaking at 120 rpm for 24 hours and centrifuged at 12000g for 10 mts and collect the cell lysate into a tube.

Levels of SOD (Superoxide dismutase) in the cell free supernatant were measured by 1.3 ml of solution A (0.1mM EDTA, 0.05M Na<sub>2</sub> Co<sub>3</sub> pH 10.5) 0.05ml of solution B (9.0M NBT – Nitro Blue tetrazolium ) 0.1ml of solution C (0.6% Triton X-100 in solution A) 0.1ml of solution D (20mM hydroxylamine hydrochloride) all the above solutions were mixed and the rate of nitro blue tetrazolium reduction were recorded for one minute at 560 nm. 10µl of the cell free supernatant were added to the test cuvette, reference cuvette does not contain hydroxylamine hydrochloride. The percentage inhibition in the rate of reduction of NBT was recorded as amount of protein required inhibiting reduction rate by 50% one minute.

#### Protein estimation

Estimation of protein in the cell free supernatant Reagent A: 0.1N Sodium hydroxide in 2% Sodium carbonate



Reagent B: 1% Sodium potassium tartrate. (1gm in 100ml distilled water) Reagent C: 0.5% Copper sulphate in 1% sodium potassium tartrate. Reagent D: 48ml of reagent A +1ml of reagent B+1ml of reagent C. Reagent E Folin - ciocaltue reagent (1:1) 10 $\mu$ l of the sample and standard solution were taken for analysis. To this addition

of 2ml of reagent D containing solution of A, B and C and waiting for 10 minutes as the reaction time. Following the reaction time addition of 0.1ml Folin ciocaltue reagent and waiting for 30 minutes. Then made up to 10ml with distilled water and reading were measured by spectrophotometer at 600 nm<sup>10</sup>.

**Table 1:** Effect of Ascorbic acid on antibacterial activity of ampicillin against *Staphylococcus aureus* by tube assay method.

Ampicillin ( $\mu$ g)	Percentage Inhibition				
	Ampicillin Without Ascorbic acid	Ascorbic acid 15 $\mu$ g	Ascorbic acid 30 $\mu$ g	Ascorbic acid 50 $\mu$ g	Ascorbic acid 100 $\mu$ g
2 $\mu$ g	23.60 $\pm$ 2.64	56.65 $\pm$ 2.05	56.65 $\pm$ 1.89*	57.08 $\pm$ 1.55*	58.31 $\pm$ 1.75*
4 $\mu$ g	73.3 $\pm$ 1.944	75.32 $\pm$ 2.11	77.47 $\pm$ 2.12*	78.68 $\pm$ 1.52*	79.61 $\pm$ 1.55*
8 $\mu$ g	80.83 $\pm$ 1.72	81.69 $\pm$ 1.36	81.74 $\pm$ 1.57	82.89 $\pm$ 1.49	82.97 $\pm$ 1.58
10 $\mu$ g	81.9 $\pm$ 0.70	82.08 $\pm$ 1.8	81.82 $\pm$ 1.26	83.99 $\pm$ 1.63	83.83 $\pm$ 1.58
15 $\mu$ g	86.05 $\pm$ 1.71	87.56 $\pm$ 1.41	87.55 $\pm$ 1.62	90.41 $\pm$ 1.62*	91.7 $\pm$ 2.3*

\* Values are compared with ampicillin significant at P<0.05; Superscript \* indicate statistical significance when the values are compared with that of ampicillin alone at the same concentration at P<0.05.

**Table 2:** Effects of ampicillin alone and combined ampicillin and ascorbic acid activity estimated by superoxide dismutase in the cell free supernatant against *Staphylococcus aureus*

Sl. No.	Treatment group	SOD U/mg of protein	Percentage reduction in SOD levels
1.	Control	183.33 $\pm$ 6.48	
2.	Ampicillin 100 $\mu$ g	135.54 $\pm$ 7.0678*	26 %
3.	Ampicillin 100 $\mu$ g with Ascorbic acid 50 $\mu$ g	111.11 $\pm$ 8.9223*	39%
4.	Ampicillin 100 $\mu$ g with Ascorbic acid 100 $\mu$ g	103.17 $\pm$ 7.8335*	44%
5.	Ampicillin 100 $\mu$ g with Ascorbic acid 200 $\mu$ g	88.88 $\pm$ 9.0684*	51.99%
6.	Ascorbic acid 100 $\mu$ g	153.88 $\pm$ 7.5358	16%
7.	Ascorbic acid 200 $\mu$ g	125.71 $\pm$ 7.5408	31%

\* Values are compared with ampicillin significant at P<0.05; Superscript \* indicate statistical significance when the values are compared with that of ampicillin alone at the same concentration at P<0.05.

## RESULTS AND DISCUSSION

Ampicillin, oral amino penicillin is effective against gram positive and gram negative organisms<sup>11</sup>. Hence in the present study a gram positive microorganism *Staphylococcus aureus* and a gram negative microorganism *Escherichia coli* were chosen to assess the interaction between ampicillin and ascorbic acid. Moreover the selection of these two organisms can be justified on the basis that both the organisms may develop resistance to the antibiotic<sup>12</sup>. So the additive or synergistic effect of the ascorbic acid if any will be useful therapeutically in ampicillin resistant infections. The susceptibility of different microorganisms to antibiotics and antibiotic sensitivity of the organisms can be determined by various antimicrobial assays. Serial dilution method, tube assay method and cylinder plate method were the *in vitro* tests performed in the earlier study.

In tube assay method, the percentage inhibition was determined from the following formula:

Percentage inhibition

$$= \frac{\text{absorbance of positive control (without ampicillin)} - \text{absorbance of test solution}}{\text{absorbance of positive control}} \times 100$$

The percentage inhibition of the growth of *Staphylococcus aureus* at various concentrations of ampicillin with ascorbic acid was determined at very low concentration. The growth inhibition of *Staphylococcus aureus* by 2 $\mu$ g/ml level of ampicillin was rapidly increased even with a small amount of ascorbic acid in combination but further increase in ascorbic acid concentration did not have a proportionate enhancement of antibacterial activity shown in table 1.

To elucidate Ascorbic acid shows synergy with ampicillin against *Staphylococcus aureus*. The reason may be the prevention of penicillinase activity of *Staphylococcus aureus* by ascorbic acid as it is already reported that *Enterobacter cloacae* ATCC 13047 showed increasing susceptibility to ampicillin when incubated anaerobically in the presence of increasing concentrations of ascorbic acid through inhibition of  $\beta$ -lactamase. Another molecule

found in literature, with which  $\beta$ -lactamase have improved antibacterial activity is epigallocatechin gallate<sup>13-18</sup>. The synergy was contributed to the interference of epigallocatechin gallate with the integrative and biosynthesis of the bacterial cell wall through direct binding to peptidoglycan and inhibition of penicillinase activity. The antioxidant enzyme superoxide dismutase levels in the microbes were determined by kono et al method. Decreased SOD levels in the ampicillin treated or ampicillin, ascorbic acid combination treated microorganisms indicate a low level of growth. A combination of ampicillin and ascorbic acid 100 should have 42% reduction of SOD as an additive effect shown in table 2. But 44 % activity was observed which indicates a low degree of synergy.

## CONCLUSION

To elucidate the interaction between ampicillin sodium and ascorbic acid, with different concentrations was used and estimate the results. The growth inhibition of *Staphylococcus aureus* by 2 $\mu$ g/ml level of ampicillin was rapidly increased even with a small amount of ascorbic acid in combination but further increase in ascorbic acid concentration did not have a proportionate enhancement of antibacterial activities. The super oxide dismutase level was tested with the combination which exhibits a additive, synergic effect. From our study we found out that the ascorbic acid was a better antioxidant and it may be used in the treatment of infectious diseases with the antibiotics. Based on these studies smaller doses of ascorbic acid were shown to enhance the activity of ampicillin against many bacteria as well as reduce allergic reaction to antibiotics. Ascorbic acid also decreases the usage of antibiotics needed to kill bacteria.

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