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Evaluation of *in vitro* antibacterial activity of Anticonvulsant drugs

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ABSTRACT

The emergence of resistant strains necessitates the search for newer antibitotics from the existing sources.As the non antibiotics such as antihistamines, psychotropics, antihypertensives, anaesthetics, anti- inflammatory drugs were found to possess antibacterial activity. Thus the study was carried out with an objective to analyze the antibacterial activity of anticonvulsant drugs. Antibacterial activity of the anticonvulsant drugs namely Carbamazepine, Primedase, Lobazam, Lonazep,Gabapentine,Valproic acid,Lametec ,Valparin alka were estimated by both the Agar well dilution and Minimum inhibitory concentration method .Also the bactericidal and bacteriostatic property of the effective drugs were determined by estimating the number colony forming units of the microorganisms tested .Gabapentine and carbamazepine were ineffective to the bacterial isolates. Valproic acid was highly effective to Staphylococcus aureus and Proteus vulgaris at the concentration of 100 µg/ml whereas inhibited the other tested organisms greater than 200 µg/ml .Hence of all the tested anticonvulsant drugs Valproic acid was the only drug found to be effective against the tested bacteria by in vitro analysis.

Key words: *Antibacterial, Anticonvulsant, Bactericidal.*

INTRODUCTION

Owing to the emergence of drug resistant microorganisms,the search for new class of drugs either from natural sources (plants, fungi, actinomycetes, algae) or synthetics, semisynthetic drugs, modifications of the already existing drugs is increasing.

Drugs could be either specific in its action or may possess multiple functions .Antibiotics like monorden, erythromycin, chloramphenicol were found to possess tranquilising, antihypertensives and smooth muscle relaxing properties [1]. Tetracycline and its derivatives have effects on inflammation, immunomodulation, cell proliferation, and angiogenesis and also have been used to treat a variety of conditions including acne, cutaneous sarcoid, and rheumatoid arthritis [1, 4]. Similarly drugs belonging to other classes (non antibiotics) such as antihistamines like diphenhydramine, bromodiphenhydramine, methdialazine, promethazine [2, 6, 8-15].

Psychotropics like promazine, chlorpromazine, fluphenarine, trifluoperazine, antihypertensives such as methyl DOPA, local anaesthetics like procaine and anti- inflammatory drugs such as diclofenac;cardiovascular drugs like dobutamine possess antibacterial activity [3].

So far substantial studies had been carried out in antihistamines, psychotropics, antihypertensives, anaesthetics, anti-inflammatory drugs, Cardiovasular drugs dobutamine [3, 5, 7, 16, 17] but the antibacterial activity of anticonvulsant drugs were not studied so far.

Anticonvulsant drugs are drugs used to control seizures in people with epilepsy and can be classified according to their chemical structure into six classes-Barbiturates, hydantoins, Oxazlidinediones, Sucinimides, iminostilbines, benzodiapine. As there is a lack of reports on the antibacterial activities of the anticonvulsant drugs, the study was carried out to explore the *in vitro* antibacterial activity of the commonly used anticonvulsant drugs.

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MATERIALS AND METHODS

Drugs:

Anticonvulsant drugs namely Carbamazepine, Primedase, Lobazam, Lonazep, Gabapentine, Valproic acid, Lametec, Valparin alka were dissolved in either distilled water or dimethyl sulfoxide (DMSO) depending on their solubility and kept at 4°C and the clinical isolates of bacteria belonging to one gram positive (Staphylococcus aureus ATCC 38591) and eight gram negative genera (*Escherichia coli ATCC 25922,Klebsiella pneumoniae, Vibrio cholerae, Shigella dysentriae, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeurginosa, Salmonella paratyphi B*) were obtained from CMC, Vellore. Stock solution was prepared using 1 g of the drug powder in 10 ml normal saline making a stock solution of 100mg/ml.

Agar well diffusion method:

Serially diluted drugs were placed onto the wells and the microorganisms were swabbed uniformly onto the agar plates and incubated at 37°C.

Minimum inhibitory concentration:

The drugs which showed significant zones of inhibition were chosen to assay for MIC.A stock solution containing 25 µg/ml of the drug was prepared. Then 1 ml of the drug solution was dispensed into the first tube and it is serially diluted till the last tube to the concentration of 3.125 mg/ml. Overnight cultures of each of the test isolates were prepared in sterile nutrient broth and 1 ml of the inoculums was transferred to each tube.

Agar Dilution method:

The drug was added at concentrations in molten nutrient agar and poured in petridishes and the organisms were grown in peptone water .The overnight culture was spot inoculated on the nutrient agar plates such that each inoculum contained 2x 10^6 CFU as adjusted with the Mcfarland standards. The plates were incubated at 37 °C and examined after 24 hrs for the macroscopic growth. The lowest concentration of drug that failed to show any visible macroscopic growth was considered as its MIC.

Determination of $_P$ H:

After adding the drugs to the media, pH was determined to rule out the possibility that the change in pH could inhibit the

growth of microorganism.

Determination of the mode of action:

Bacterial isolates sensitive to the anticonvulsant drug was chosen and the drug was added at their effective concentration to the logarithmic growth phase of culture. The colony forming units/ml (CFU/ml) was determined at every 6 hrs interval upto 12 hrs.

RESULTS AND DISCUSSION

Owing to the increased resistance of microorganisms to the new generation of antibiotics, this study was attempted to discover the new antimicrobials from the already existing drugs. Although a wide range of non antibiotics had been investigated by other workers, the antimicrobial properties of anticonvulsant drugs that are in use have not been studied so far.

Out of the eight drugs tested in our study, seven drugs did not show promising results and found to be either subinhibitory or either resistant to the microorganisms tested. Valproic acid was the only drug that were found to inhibit *Staphylococcus aureus* and *Proteus vulgaris* at the concentration of 100 ug /ml but were able to inhibit the the growth of *Salmonella Paratyphi B, Shigella dysentriae, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli, Vibrio cholera* only at higher concentration and hence cannot be considered significant.

Gabapentin and Carbamazepine were resistant to the bacterial isolates. Valproic acid alka, Primedase, Lobazam, Lonazep and Lametec were sub inhibitory to *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginoa* and *Proteus Vulagaris*. Valproic acid showed powerful antimicrobial action against all the test bacteria.

Valproic acid showed bactericidal action against *Staphylococcus aureus* and *Proteus vulgaris* at 100 µg/ml level of the drug .*Salmonella Paratyphi B, Shigella dysentriae, Pseudomonas aeruginosa* were inhibited at 400 ug /ml level of the drug. *Klebsiella pneumoniae* and *Escherichia coli* were inhibited at 200 ug /ml level of the drug. *Vibrio cholerae* were sensitive at 800 ug /ml level of the drug. (**Table 1**)

Table No. 1: Antibacterial activity of anticonvulsant drugs as determined by agar well diffusion method

Microorganisms	CBZ	PD	LB	LNZ	GP	VPA	LMT	VA
Staphylococcus aureus	-	+/-	+/-	+/-	-	+ 100µg/ml	+/-	+/-
Proteus vulgaris	-	+/-	+/-	+/-	-	+ 100µg/ml	+/-	+/-
Salmonella paratyphi B	-	+/-	+/-	+/-	-	+ 400µg/ml	+/-	+/-
Shigella Dysenteriae	-	-	-	-	-	+ 400µg/ml	-	-
Pseudomonas Aeruginosa	-	-	+/-	+/-	+/-	- 400µg/ml	-	+
Klebsiella pneumoniae	-	+/-	+/-	+/-	-	+ 200µg/ml	+/-	+/-
Escherichia coli	-	+/-	+/-	+/-	-	+ 200µg/ml	+/-	+/-
Vibrio cholerae	-	-	-	-	-	+ 800µg/ml	-	-

- =Resistant /ineffective; +/- = subinhibitory; + = Inhibitory /effective; CBZ- Carbamazepine; PD- Primedase; LB-Lobazam; LNZ- Lonazep; GP-Gabapentine; VPA-Valproic acid; LMT- Lametec; VA- Valparin alka

At the logarithmic growth phase of the culture, the CFU count of the *Staphylococcus aureus* was $4.0x\ 10^8$ and it subsequently declined to $3.0x10^4$ after 6 hrs, $2.0x10^2$ after 12 hrs of incubation with 100 ug /ml of the drug, Valproic acid.

Proteus vulgaris showed a marked decline in the colony forming units from $3.0x10^5$ to $2.0x10^3$ after 6 hrs, followed by $2.0x10^2$ after 12 hours of incubation with 100 ug /ml of the drug, Valproic acid.

E.coli at the logarithmic growth phase of the culture showed $3.0x10^9$ colony forming units and it declined to $3.0\ x10^6$ after 6 hrs, $2.0x10^3$ after 12 hrs of incubation with 200 ug /ml of the drug, Valproic acid.(**Fig. 1**)

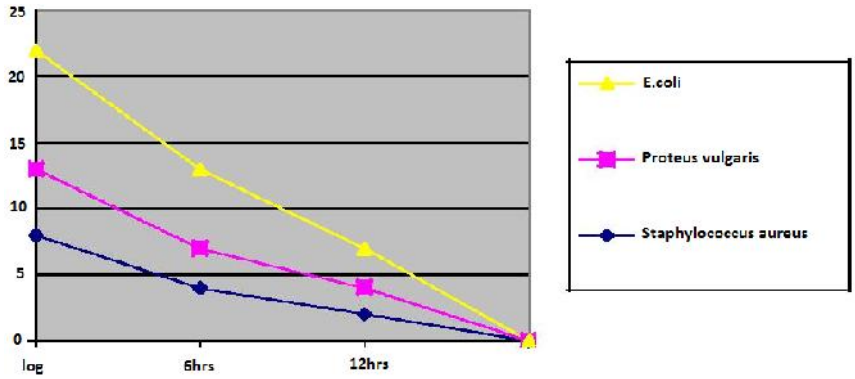

Fig. 1: Bacterial growth reduction (in log) at various time intervals after the addition of the drug Valproic acid

Further investigation on the inhibitory kinetics of Valproic acid towards *Staphylococcus aureus* and *Proteus vulgaris* had shown that Valproic acid were able to inhibit and decrease the growth of *Staphylococcus aureus* by 3 fold after 12 hrs whereas *proteus vulgaris* declined by two fold growth.

As the inhibitory concentration was not significant to the other microorganisms tested and hence due to the possible development of resistant strains, the inhibitory kinetics were not studied further for the other microorganisms.

Valproic acid, chemically called as 2-propyl pentanoic cid or 2 propyl valeric acid has been used as an anticonvulsant drug in the treatment of epilepsy, bipolar disorder, depression, migraine headaches. VPA being a histone diacetylase inhibitor has been recently investigated for the inhibitory potential to HIV [18] .Besides these,VPA is also found to be investigated in clinical trials for the treatment of colorectal polyps, treatment of inflammatory skin disorders and also used in direct reprogramming in generation of induced pluoripotent stem cells [19]. Owing to these potential pharmacological properties in the treatment of various disorders, thus further *in vivo* investigation of valproic acid followed by clinical studies could lead valproic acid as a potential antibacterial candidate.

CONCLUSION

Valproic acid was the only drugs that were found to be effective against *Staphylococcus aureus* and *Proteus vulgaris* of the tested 8 anticonvulsant drugs .Hence further in *vitro* and clinical investigation of Valproic acid on antibacterial activity lead it to a potential antibacterial candidate.

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