BIOCHEMICAL APPROACH TO STUDY THE EFFECT OF CONTAMINATION BY ANTIMICROBIAL AGENTS ON PHARMACEUTICAL INDUSTRIAL LABOURS

THESIS SUBMITTED BY

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THIS THESIS HAS NOT BEEN

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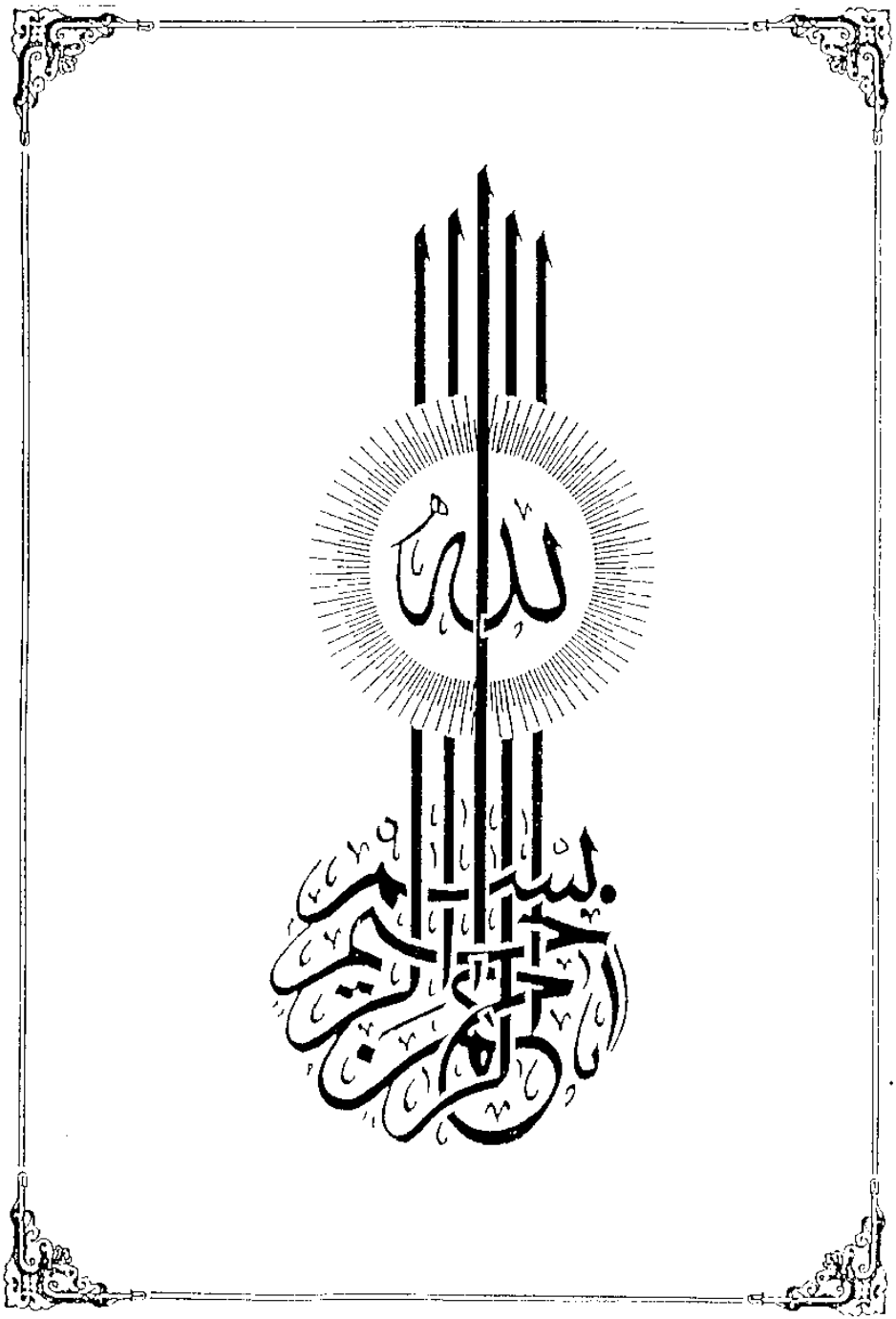

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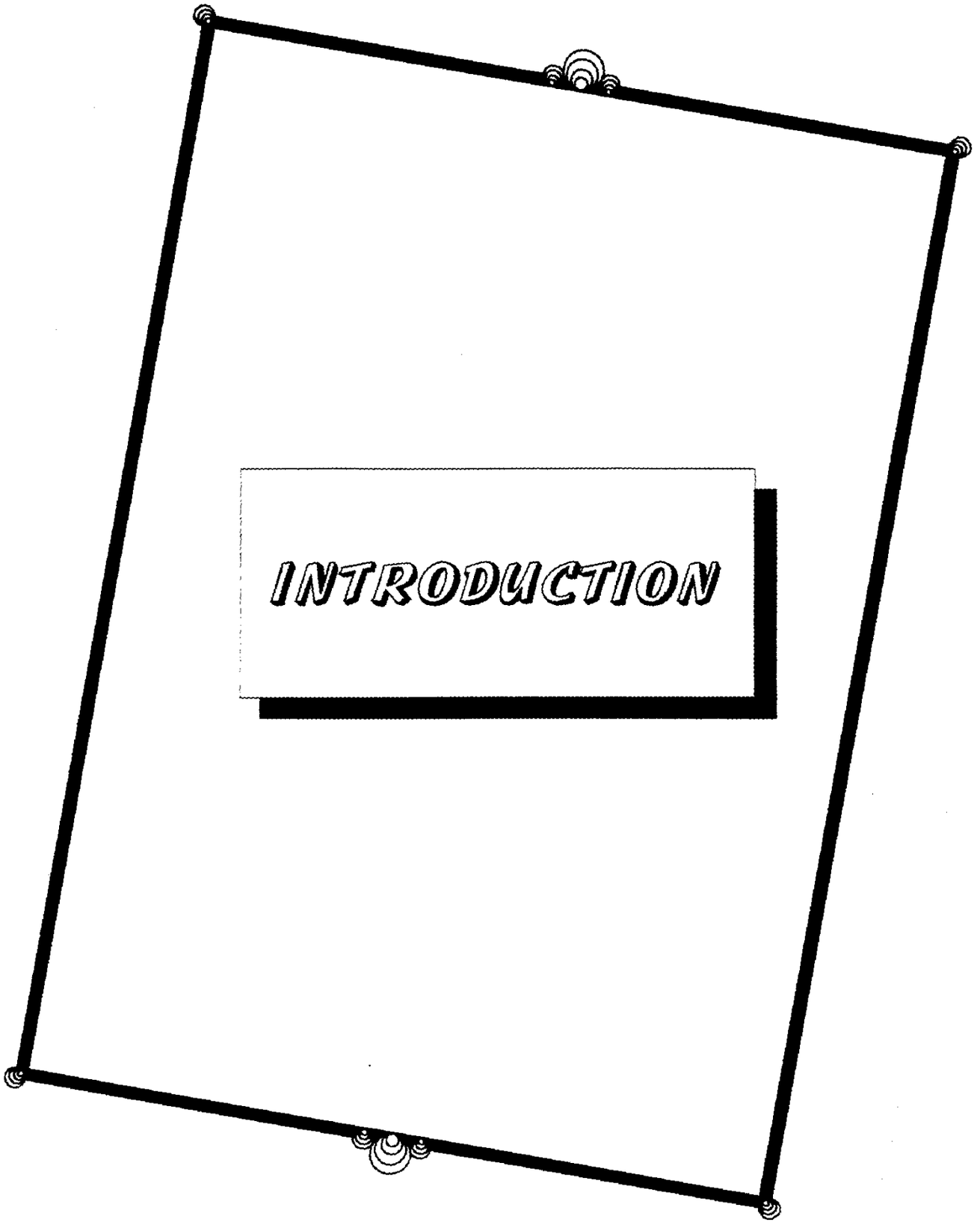
ABBREVIATIONS

A/G ratio	Albumin per globulin ratio
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine tri phosphate
BSP	Bromsulphalein
C	Concentration
CSF	Cerebro spinal fluid
dl	deciliter
DNA	Deoxy ribonucleic acid
DON	6-diazo-5-oxo-L- norleucin
Fig.	Figure
g	Gram
GGT	Gamma glutamate transferase
h	hour
HPLC	High performance liquid chromatography
IgG	Immunoglobulin G
IgM	Immunoglobulin M
i.v.	intravenous
L	Liter
LD	Lactate dehydrogenase
µg	Microgram
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute
ml	Milliliter
mmol	Millimole

mol	Mole
N	Normality
n	Number of individuals
NADH	Reduced nicotinamide adenine dinucleotide
nm	Nanometer
N.S	Nonsignificant
O.D	Optical density
P	Probability
PHA	Phytohemaglutinin
PPD	Purified protein derivative
R	Reagent
RNA	Ribonucleic acid
r.p.m.	Revolution per minute
S.D	Standard deviation
S.E	Standard error
U	Unit
v/v	Volume per volume



INTRODUCTION

The antimicrobial agents play a vital role in the control of various infectious diseases. The use of these agents is an important landmark indicative of the rapid development of synthetic techniques.

The main target of using chemically synthesized agents is to modify different functions and interfere with the biological functions of microorganisms. Therefore, synthetic compound represents the largest group of therapeutic agents, they are compounds neither occurring naturally, nor derived from natural products. Many drug resembling agents are manufactured synthetically rather than by extraction from tissues (Briggs and Briggs, 1974).

A chemotherapeutic agent may be defined as "A synthetic chemical substance designed to treat infections by destroying the infecting organism when administered to the host without injuring its tissues. The range therefore, of these agents effect extends from that on multicellular forms (as molds and malignant tumors) to unicellular bacteria and viruses (Busch and Lane, 1967).

Toxicity of antimicrobial agents:-

A low toxicity is essential, whether the antimicrobial agent is used for short term or prolonged therapy. The ideal agent should not act as a sensitizing or a photo-

sensitizing agent and if it is to be applied topically, it should not act as a primary irritant. Antimicrobial agents should not affect the metabolism, or functions of the vital organs, and an ideal agent in general should not be limited to the absence of immediate toxic effects (Goldsten, 1968).

Choice of an antimicrobial agent:

When the organism has been identified bacteriologically, or there is a strong suspicion that it is involved, then, the most effective antimicrobial against the organism be used to treat the infection (Sykes and Matthew, 1976).

There may be reasons, however, which prevent the selection of a particular antimicrobial agent for therapeutic use against microorganisms, for example, it may not be immediately available, or the organism of a particular strain is resistant to it, under these conditions, an antimicrobial agent of a second or even third choice may need to be considered. The *in vivo* activity of the antimicrobial agent judged by its minimum inhibitory concentration for the organism in question or the particular strain of the organism is a guide to effectiveness. This, rather than *in vitro* data, must be the final criterion in selecting the antimicrobial agent for treating an infection.

For some organisms selectivity test are essential as some strains may be susceptible to the action of a particular antimicrobial agent, while other strains of the same organisms may be resistant e.g. some *Staphylococci, Escherichia coli and Proteus species.*

There are many diseases which are caused by an infection with a specific type of organisms such as gonorrhoea, syphilis, typhoid fever and tetanus. In such cases it is relatively easy to make an accurate diagnosis clinically although bacteriological confirmation is necessary. The organism concerned in such infections is susceptible to the action of certain antimicrobial agents. Other diseases as pneumonia, meningitis, endocarditis or urinary tract infections may be caused by one of variety of many organisms. It is essential therefore, in the treatment of such infections that a bacteriological diagnosis be made if the most suitable antimicrobial agent is to be selected (Blacow and Wade, 1972).

Bacterial resistance against antimicrobial agents:-

Sensitive microorganisms are inhibited by low concentrations of the antimicrobial agent, while resistant organisms are not inhibited by those concentrations. In the early days of the development of penicillin for therapeutic use, it was clear that certain bacteria were not killed by this antimicrobial agent (Falkow, 1975).

A So-called resistant strain may be susceptible to the antimicrobial agent if massive doses are given. The resistance of bacteria to antimicrobial agents may result from tolerance to the action of the antimicrobial agent in question, or to the elaboration of enzymes which have a destructive effect on it. Drug-tolerant organisms grow in the presence of increasing concentrations of the antimicrobial agents, a phenomenon seen *in vitro* with all antimicrobial agents and clinically with most of them (Falkow, 1975).

Antimicrobial or antibiotic resistant variants may arise from spontaneous gene-mutation with the antimicrobial agent acting as a selective agent (Eriksson and Grennberg, 1968). Another view is that resistance results from a process of adaptation to environment, the sensitive strains being supplied by the antimicrobial agent were suppresed while the naturally resistant strains multiply unchecked.

Antimicrobial agent or antibiotic resistant strain may also result from basic metabolic changes in the organisms which may use an alternative metabolic pathway unaffected by the antimicrobial agent. In the process of becoming resistant to one antimicrobial agent, organisms may become resistant to others, particularly if they are chemically related. Resistance can be transferred from one organism to another. Thus, resistance among enterobacteria can be transferred by conjugation from *Escherichia coli* (Sykes and Matthew, 1976).

Misuse and over dosage of antimicrobial agents:-

There are still some infections that do not respond to any of the currently available antimicrobial agents. It is therefore useless to treat such infections with an timicrobial agent or an antibiotic. However, some viral infections respond to treatment with suitable antimicrobial agent, especially the broad spectrum type and to certain chemotherapeutic agent.

Antimicrobial agent or antibiotic therapy may fail because of incorrect dosage. The dosage may be too small or the correct dose may be given for too short time or by the wrong route. The dose will depend on the severity of the infection. Resistance to antimicrobial agents may be also a cause of therapy failure.

Generally, failure following antimicrobial therapy may be due to:-

1- The organism is not susceptible to the action of the antimicrobial agent used.
2- Although generally susceptible, the organism may have become resistant.
3- Lack of bacteriological control.
4- Incorrect dosage, incorrect spacing of doses.
5- Multiple infection with insufficient antimicrobial agent cover.
6- Superinfection and re-infection.

7- Toxicity of the selected antimicrobial agent is sufficient to negate its therapeutic usefulness (Davies, 1957).

Biochemical target For antimicrobial agents:-

Antimicrobial agents that affect the growth and multiplication of certain types of cells may interact with various targets ("Chemoreceptors" in Ehrlich's terminology) in the sensitive cell. Theoretically, these targets might be numerous by with decreasing concentrations of the antimicrobial agent, the number of targe ts should also decreased. Finally, when limiting concentrations are used, the antimicrobial agents may affect a single target. However, in many instances a single target/multi-target contraversion may occur. For example, tetracyclines are inhibitors of protein synthesis on prokaryotic ribosomes. At higher concentrations, however, tetracycline bind metal ions which react with the tricarboxymethane function of the A ring of their molecule (Williamson and Everett, 1975). This may have far-reaching biochemical consequences in the cell.

As metabolic processes depend on enzymic catalysis, it can be inferred that enzymes are obvious targets for antimicrobial action. A number of possibilities of such an action can exist, direct action at the substrate binding site, direct action at a co-factor binding site,

modification of substrate (or co-factor) binding by false operation of specific systems that regulate enzyme-substrate interactions; and disorganization of the active center by nonspecification elsewhere in the enzyne (Gale *et al.*, 1981).

A direct action at the substrate binding site can be expected in the instance of a chemical analogue of the substrate that acts as a competitive (isosteric) inhibitor.

Examples of such inhibitors among antibiotics are known. L-azaserine and 6-diazo -5- Oxo-L- norleucin (DON) are analogues of L-glutamine and inhibit purine biosynthesis at the stage where L-glutamine acts as a donor of the - NH_2 group. Hadacidin, a structural analogue of L-aspartic acid, is a specific inhibitor of adenylosuccinate synthetase.

Non protein co-factors are essential to the function of many enzymes and represent another possibility of direct action of inhibitors. More frequently, such as action takes place by inhibiting co-factor biosynthesis rather than by binding at a co-factor binding site. For example, several biotin analogues have been isolated from streptomycetes that act as biotin antagonists in its co-factor function in various carboxylating reactions.