4.1 INTRODUCTION

All living beings are beautiful chemical creations and all their activities are controlled by chemicals. The chemical substances used for treatment of disease and for reducing suffering from pain are called medicines or drugs. The word drug is derived from the French word 'drouge' meaning herb. Drugs are chemicals of low molecular mass about 100-500 u. These interact with macromolecular targets in the body and produce a biological response. When the drug has useful actions in the diagnosis, prevention, treatment and cure of a disease then it is called medicine having therapeutic effect. The use of chemicals for therapeutic effect is called chemotherapy. Modern medicine has a great deal to learn the drugs which are collected
from herbs. Many of the plants familiar to the wise woman really do have the healing powers that tradition attaches to them. It is scientifically proved that herbs, flowers and other parts of many plants can be used for health care. 100

Drugs obtained from plant sources are found correlation between the traditional uses of the plant and the therapeutic use of plant derived drug. Drugs which are obtained from plants are discovered as a result of scientific follow up of plants.

Specific research on which herbs are most effective in the detoxification from different drugs should prove most useful. It also appears that detoxification from drugs is not the most difficult variable in dealing with the drug problem.

Relapse prevention is an area that needs further research. The establishment of an alternative life-style in which herbs and whole foods play a significant part may help to prevent disease and increase longevity. This is an another area of research. The acceptance of herbs as effective natural healers by drug programme professionals and paraprofessionals, the medical establishment and the general public is leading to a greater understanding of the nature of health and is having impact on the drug problems the world faces today. On the other hand, if the drug causes harmful effect on the body such as side effect or toxicity, the drug behave as poisons. These side effects or toxicity may also occur if the doses taken are higher than recommended. Drugs or medicine may be administered by oral, intravenous, injections, intramuscular route, subcutaneous route, inhalation or by local applications.

In the above context drugs based on seven membered heterocyclic rings, these drugs are of great interest due to their various biological activities. It has been estimated that over one half of all therapeutic agents consist of heterocyclic compounds. The heterocyclic ring system in many cases comprises the very core of the active moiety or pharmacophore. These pharmacophores and basic nucleus can be suitably exploited for the discovery of new highly effective pharmacologically and therapeutically safer drug.101

**Classification of Drugs:**

Drugs can be classified according to the various criteria. These are classified into the following simple ways:

(i) On the basis of pharmacological effect:

This type of classification is based on the pharmacological effect of the drugs.
For example, analgesics have pain relieving effect, antipyretics help in lowering the body temperature in fever, antiseptics kill or arrest the growth of microorganisms, antidepressants help in changing mood etc. Doctors may use this classification as it is equipped with all possible drugs that can be used for the treatment of a particular type of disease.

(ii) On the basis of action on a particular biochemical process: Action of a drug on a particular biochemical process form the basis of this type of classification. For example, all antihistamine drugs inhibit the action of histamine compound which causes inflammation in body and all allergic reactions. The drugs which are used to block the action of histamines are grouped together as antihistamines. Similarly, drugs related to gastrointestinal motility and secretion process are grouped together.

(iii) On the basis of chemical structure: Chemical structures of drugs are also used for their classification. Drugs with similar chemical structures may have similar pharmacological activity. For example, alcohols are known to possess hypnotic, analgesic and antibiotic action. Sulphanamides show antibacterial properties.

(iv) On the basis of molecular targets

This classification is based on the molecular targets and is considered as the most useful mode of classification for medicinal chemists. Many enzymes and receptors in the cells have some common drug targets.

Chemicals in medicines and health care:

There are some specific classes of drugs used in allopathic system. These are explained as:

I. Antibiotics:

The term antibiotic was given by Vuillemin in 1889 and defined by Waksman in 1944. Antibiotic may be defined as a chemical substance produced by or derived from living cells which is capable in small amount for inhibiting the life process or even destroying the micro-organisms.

Antibiotic may be classified as chemotherapeutic agents. Action of antibiotic is very specific. It means a given antibiotic has been found to be effective against certain types of micro-organisms only. All chemical substances produced or derived from living cells cannot be antibiotics. They have to satisfy certain conditions which are summarized as:
(a) Originally antibiotic must have been a product of metabolism although it might have been synthesized.

(b) If antibiotic is synthetic product, then it should be a structural analogue of naturally occurring antibiotic.

(c) The antibiotic should be effective at low concentrations.

(d) The antibiotic must be antagonize the growth or survival of one or more species of the micro-organisms.

For a particular antibiotic to act as a therapeutic agent, it has to satisfy following condition:

(a) It must be effective against a pathogen.

(b) It must not cause significant toxic side-effect.

(c) Its stability must be appreciable high so that it can be isolated and processed in to suitable forms of dosages which are readily absorbed.

(d) It should be stored for a long time period without appreciable loss of its activity.

(e) The rate of detoxification and elimination from body must be such that there exists sufficient time interval between two successive dosages and during that period a proper concentrations level has to be maintained.

(f) The antibiotic should be completely eliminated from the system soon after its administration has been stopped.

2. Antimicrobials:

Chemical substances inhibiting the growth or causing the death of a micro organism are known as antimicrobial agents. Although a wide range of chemicals have these properties if a sufficiently high concentration is used, the term is usually restricted to those substances that are effective at a concentration suitable for practical application. The antimicrobial agents can be subdivided into the following groups according to the action and purposes for which they are employed.

1. If the substance causes a cessation of growth of micro-organism which is reversed when the chemical is removed, it is called a static agent. If the substance kills the micro-
organism, it is called a cidal agent. This distinction is often depends upon the concentration of the drug, a static agent may become cidal if the concentration is increased.

2. A further subdivision can be based upon the group of micro-organisms affected. Thus the agents acting on bacteria are called bacteriostatic or bactericidal and those acting on fungi are called fungistatic or fungicidal.

3. The antimicrobial agents can also be subdivided on the basis of the relationship between potentially pathogenic micro-organisms and their animal hosts.

   (a) **Disinfectants:** This is a term applied to chemicals used to kill potentially infectious organisms. Disinfectants destroy the micro-organisms but these are not safe for living tissues and are not meant to come into direct contact with man because of their toxicity. Disinfectants play a major role in water treatment and in public health situation. They are normally used in the treatment of inanimate objects, surfaces, waters, floors, drainage system, instruments etc.

   (b) **Antiseptics:** This term refers to relatively non-toxic and non-irritant antimicrobial agents that may be applied topically to the body surfaces either to kill or inhibit the growth of the pathogenic micro-organisms. These are safely applied on cuts, wounds, ulcers, diseased skin surfaces. These are also used to reduce odors resulting from bacterial decomposition of the body or in the mouth. They are therefore mixed with deodorants, face powders and breath purifiers.

   (c) **Chemotherapeutic agents:** This term describe the chemicals that are used to kill or inhibit the growth of micro-organisms already established in the tissues of the body. In theory the name may be applied to any substance used for therapeutic purposes but in practice it has been restricted to chemicals used in the treatment of microbial infections.

The disease in human beings and animals may be caused by a variety of micro-organism such as viruses and bacteria etc. The micro-organism are very small which can be seen only with a microscope. These are called microbes. Any organism which causes disease is called pathogen. However, body possesses an efficient natural defense mechanism which operates at all times against potential pathogenic microbes. The skin is impervious to microbes. Many body secretions either kill or inhibit the growth of microbes. The common example of secretions are lysozyme in tears, nasal
secretions, saliva, fatty acids and lactic acid in sweat and sebaceous secretions and HCl in stomach. The pathogens reach the tissue due to a breath in defense mechanism and cause infection. Invasion and multiplication of an organism in infected host destroys the normal cell metabolism and cause physiological disturbances to the body which appears in the form of some disorders or disease. In addition, toxic substances produced by the microbes may adversely affect the tissues or organs of the host.

The control of microbial diseases can be achieved by the following ways:

1. By drugs which kill the organism in the body.
2. By drugs which inhibit or arrest the growth of the organism.
3. By increasing immunity and resistance to infection of the body.

In the early 20th century, the scientist tried to search the chemicals that would adversely affect the invading bacteria but not the host. So scientists are trying to find the relationship between structure and activity of medicinal compounds.

4. Antipyretics:

The chemical substances which are used to lower the temperature of the body in high fever are called antipyretics. Examples: paracetamol, Phenacetin and Aspirin.

5. Analgesics: The chemical substances which reduce pain without causing consciousness, mental confusion or some other disturbances of nervous system are called analgesics.

These are two types:

a. Non-narcotic drugs       b. Narcotic drugs

a. Non-narcotic drugs: The common non-narcotics are aspirin and paracetamol. Aspirin hinder the synthesis of compounds known as prostaglandins which stimulate inflammation in the tissues and cause pain. These drugs are effective in reducing skeletal pain. Aspirin has antipyretic properties. Now it is used in the prevention of heart attack because it has anti-blood clotting action. However aspirin is supposed to be toxic to the liver. It gets hydrolyzed in stomach giving salicylic acid which sometimes causes bleeding in stomach. So over dosage and its use in empty stomach should be avoided. Naproxen, ibuprofen may use as alternative of aspirin.
b. Narcotic drugs: These drugs produce sleep and unconsciousness. Certain narcotics are also used as analgesic. For example: morphine, codeine, heroin are used in severe pain as analgesics. These are known to be habit forming. When used in medicinal doses these reduce pain and produce sleep, but in excessive doses these may leading to death.

6. Antimalarials:

The chemical substances used for the treatment of malaria are called Antimalarials. The alkaloid quinine has been used as an Antimalarials for a long time. These days a number of synthetic drugs like chloroquine, paraquine, primaquine etc. have been prepared for the treatment of malaria.

7. Anesthetics: These are chemical substances which produce general or local insensibility to pain and other sensations. Cocaine, novocaine are local Anesthetics. Chloroform, diethyl and vinyl ethers etc. are general anesthetics.

8. Antihistamines: These are chemical substances which decrease or nullify the main actions of histamine released in the body and hence prevent the allergic reactions.

A number of different sensitizing substances called antigens derived from food or environment may cause allergic reactions in human beings. This is due to the release of a chemical substance called histamine in the body. Synthetic drugs such as seldane, dimetapp are used as antihistamines. Antihistamines are also called anti-allergic drugs. These are used to treat allergy like skin rashes, conjunctivitis, rhinitis.

9. Antacids: These give relief from acid indigestion, acidity, heart burns and gastric ulcers. Acidic stomach is necessary for good health, but excessive acidity may cause discomfort. Thus antacids remove excess acid and raise the pH to appropriate level in stomach. Baking soda in water is very common antacid. Al(OH)$_3$, Mg(OH)$_3$, NaHCO$_3$, CaCO$_3$, KHCO$_3$ etc. are widely used as antacids.

10. Tranquillizers: The chemical substances used for the treatment of stress, mild and severe mental diseases are called tranquillizers. These are used to release mental tension and anxiety. These are the constituents of sleeping pills. They act on higher centers of nervous system. These are also called psychotherapeutic drugs. These drugs make the patient passive & help to control their emotional distress or depression. They also help to restore confidence and the patient's work with full capacities which they already have.
Examples. There are various types of tranquillizers which functions by different mechanisms. The most common one is noradrenaline which induces a feeling of well being and helps in changing mood. It is an important neurotransmitters and helps in mood changes. The signal sending activity of the hormone becomes low due to low level of the noradrenaline under certain reasons and thus cause depression. To overcome this depression, patient needs some antidepressant drugs which inhibit the enzymes which catalyze the degradation of nor adrenalin and slow down the metabolism of important neurotransmitter. This results in the activation of receptor for longer periods of time and remove the effect of depression. The commonly used antidepressant drugs are iproniazid and phenelzine. Some other most commonly used tranquillizers are barbituric acid and its derivatives such as veronal, amytal, membutal, seconal and luminal. These derivatives are called barbiturates. These are hypnotic. In addition to barbiturates, a large number of other non-hypnotic tranquillizers are known.

Various approaches used in drugs designing: (1) Random screening of synthetic compounds or chemicals and natural products by bioassay procedures.

(2) Novel compounds preparation based on the known structure of biologically active natural substances of plant and animal origin i.e. lead skeleton.

(3) Preparation of structural analogue of lead with increasing biological activity and application of bio-isosteric principle.

The main compound has the desired biological or pharmacological activity but may have many undesirable characteristics, e.g. high toxicity, other pharmacological activities and pharmacokinetic problems. A rational approach is used for identifying a main compound which is based on a molecular understanding of the drug and its receptors. Once a main compound has been obtained it is subjected to structural variation with the aim of optimizing its biological properties in the desired direction. Identification of a nucleus of main compound depends upon the consideration of the following points:

(i) Molecular structure of the drug.

(ii) Behavior of drug in bio-phase.

(iii) Geometry of the receptor.

(iv) Drug receptor interaction.
(v) Change in the structure on binding.

(vi) The observed biological action.

Structural modification has been the mainstay of drug synthesis since the earliest days. Many efforts have been done to identify the relationship between chemical structure and biological activity. A chemical structure known to have a particular biological activity is chosen and attempts are made to improve it by modification based on chemical intuition and isosteric consideration to get highly potent compounds with minimal side effects. Now a day with ever increasing advancement in the use of computer software as a tool in design & screening of new main compound on the basis of receptor structure on molecular level, QSAR studies, molecule modeling plays a major role in drug discovery process. After years of hard work and dedication a new drug is approved. Many other skills such as packaging, distribution and marketing are important in bringing new drugs to the patients.

Screening approaches are also important for the drug discovery process. One form of this approach uses a biological end point to identify lead without concern for the structure or mechanism by which the agent is acting to achieve the end result. Hence drug discovery encompasses all studies necessary to find and characterize a drug, including technology development and all activities from disease and target identification through phase-2 clinical trial studies. More recently automated high output screening systems utilizing cell culture system with linked enzyme assay and receptor molecules derived from gene cloning have greatly increased the efficacy of random screening. This only becomes possible with the advent of computer controlled robotic system for the assays and combinatorial chemistry techniques.

**Drug Design:** is a process that starts with the identification of a disease and therapeutic target of interest and includes methodology, assay development, leads identification and characterization, animal pharmacological studies, pharmacokinetic and safety studies in animal models. It is an attempt to discover substances effective in a given pathological condition with as favorable a therapeutic index as possible.

**Designing of a Drug:**

There are two main consideration for designing a drug. These are

(i) Drug target     (ii) Drug metabolism
(1) Drug target: The drugs combine with biological macromolecules such as carbohydrates, proteins, lipids, fats and nucleic acids present in the body. These biological macromolecules are called targets. For getting a desired therapeutic effect of a drug, the right choice of the molecular target is very important. The site on which the drug acts is called receptor. A receptor is usually a protein or proteinaceous material.

(ii) Drug metabolism: When a drug is administered, it travels through the body in order to reach the target. During its route up to the site of action, it passes through a number of complex membranes. These membranes play an important role in determining the manner by which the drugs are distributed or in some cases may serve as a site of action. Therefore, the drug has to be designed, in such a way that it reaches the target without being metabolized in between. Moreover, the drug should be such that after its action, it must be excreted without harming the body. Ordinarily, the drugs remain bound to protein and therefore, do not easily excreted. However most of the drugs undergo metabolic transformations to form more water soluble products and are easily excreted. Therefore, metabolism of drugs not only makes them inactive but also forms more water soluble products for excretion. The metabolism mainly takes place in liver through kidney, lungs, skin, placenta, adrenal cortex and lymphocytes also take part in the metabolism process but to a small extent.

For designing a drug, the knowledge of physiological function of the drug target in the body is also essential. This information is helpful in selecting a compound which interacts with the target and has therapeutically effects. Such compounds are called lead or main compounds and serve as starting points for designing a drug. These main compounds may be obtained from natural sources such as plants, tress, bushes, venoms and metabolites of micro-organisms. These may also be obtained by chemical modifications of the naturally occurring compounds or may be synthesized.

A large number of main compounds have been isolated from fish, coral, sponges and marine micro-organisms. Synthetic drugs became available during the 20th century. The potency of synthetic drugs is generally greater than crude plant drugs. These are prepared to get compounds with improved activity and lesser side effects. To improve drug activity and to reduce side effects, mechanism of drug action, active sites and metabolic pathways in the biological systems are also considered before designing a drug.

**Distribution of drugs:**

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In therapeutic practice, many drugs are taken by patients by different modes of administration and after that it is absorbed in the blood. After absorption of drugs in body there is distribution of drugs throughout the body; so that drugs may reach to various tissues by means of circulation process. So drug distribution is to and from movements of drug to show their therapeutic effects from blood to various tissues of the body and its relative concentration in the tissues.

All drugs are not equally distributed in all parts of body. They may distribute to different tissues with different speed that is depends on various factors like solubility of drugs, permeability of membranes, level of concentration of drugs in blood and tissues, position of equilibrium etc.

1. Water soluble drugs mainly concentrate in the blood and fat soluble drugs accumulate in fatty tissues.

2. Ability of drugs to cross the membranes also affect the distribution of drugs. It is found that highly fat soluble drugs enter the brain where as water soluble drugs does not enters in the brain. It means water soluble drugs are not so easily and quickly cross the cell membranes as done by fat soluble drugs.

3. Some drugs are tightly bound to blood proteins and enter the tissues very slowly. Drugs which are loosely bound to blood proteins leaves the blood very easily and enter the tissues and thus concentration of drugs falls in blood quickly.

4. Some tissues in the body like heart and skeletal muscles etc. store the drugs and act as a reservoir for them. When level of drugs in blood decreases rapidly then these reservoir slowly release the drugs and prolong the effect of action.

5. Some drugs are deposit in fatty tissues and leaves the drugs very slowly and circulate in the blood even after takeoff drugs by patients.

Magnitude of effects of drugs depends on the following factors:

(a) The special attraction between drugs and tissues site.

(b) The internal capacity of drugs to cause an effect.

(c) Time taken by target site to respond the changes occurs.
(d) The effectiveness of cellular and nervous system in maintaining or changing the effects or reactions induced by drugs.

Sometime there is delay in onset of action even after the administration of effective dose of drugs. This can be explained as:

1. Drugs have been taken in form which cannot be absorbed in the body or has been taken by unsuitable route.

2. Drugs may be present in inactive form and has to pass through many metabolic transformation to change in their active forms and all this make delay in actions shown by drugs.

3. Drugs may tightly bound to blood proteins or stored in certain tissues in body and slowly released in blood.

Delay in action shown by drugs under certain conditions; is advantage of different types of therapy as this cause the prolonged action of drugs even after taken off drugs. The benefit of prolong in the action of drugs is that the number of dose of drugs may be reduced that is essential to maintain an effective blood level of drugs and hence constant blood level is attained.

Interaction of drugs with target:

Carbohydrates, protein, lipids and nucleic acid etc. macromolecules are interacted by drug. These macromolecules are known to perform different functions in the body. For examples, different proteins perform several roles in the body. Enzymes which are present in body are made up of proteins. On the other hand, the proteins which are very vital for communication system in the body are called receptors. The protein which carry the polar molecules across the cell membranes are called carrier proteins. Nucleic acids are responsible for genetic information in the cell while carbohydrates and fats form cell membranes.

Drugs act within the cell by modifying normal biochemical reactions. The drugs that enter the human body tend to stimulate certain receptors, act on enzymes or transporter proteins. The interactions of drugs cause protein displacement or enzyme inhibition resulting decrease in its activity.
There are two types of chemical messengers which are involved in the message transfer. These are:

1) Hormones         2) Neurotransmitters

1) Hormones: These are chemical substance which are produced in ductless glands known as endocrine glands. They enter the blood stream and are carried to different parts of the body by the blood stream where they activate all the receptors which recognize them for message transfer. They are not deactivated very quickly.

2) Neurotransmitters:

These are small molecules which help in the transfer of nerve messages. Some common example of neurotransmitters are serotonin, dopamine, acetylcholine etc. A neurotransmitters is released by nerve endings and get bound to the active site of the closely placed target receptor for a very short time to transfer message to it. It then departs quickly unchanged after transferring the message. The receptor, then forwards the message inside the cell. After leaving the active site, the neurotransmitters undergo degradation and lose their capabilities of transferring message, they are deactivated. The degraded products go back to the nerve endings to become active messages again. In this way, the cycle of message transfer is repeated again. The mechanism of activating a receptor is same regardless of whether the messenger is a hormone or a neurotransmitter.

Advances in elucidation of receptors for specific pharmacological response\textsuperscript{106} at the molecular level also lead to drug discovery. A drug possesses certain structure complimentary to the receptor in order to bind with high affinity. The receptor macromolecule recognizes the arrangement of certain functional groups in three dimensional space and their electronic density. It is the recognition of these groups rather the structure of the entire drug molecule that results in an interaction.

**Drug-interaction:** Some time two or more than two drugs are given to patients at the same time in therapeutic practice. During this situation, there is a chance of interactions among the drugs and it is called drug-drug interaction in the body\textsuperscript{107}. This interaction may increase or decrease the therapeutic effects of the drugs than the drugs act as independently on other separate site in the body and cause their side effects also and bring unwanted results.
As the number of drugs are increasing day by day and at the same rate drug interactions is also increasing rapidly in the form irregular heartbeat, headache, allergy, stomach upset, allergy, dangerous fall in blood pressure, damage of liver and heart due to formation of toxins etc. Drug interaction is a complex subject.

Drug interaction can be categories as:

1. Drug-drug interactions: This type of interaction occurs when more than one drug is taken at the same time and patients feel unexpected side effects. Mixing of such drugs may make patients sleep, cause allergy in form of histamines and slow body reactions.

2. Drug-food/beverages interactions: Sometime drugs may interact with food or beverages like calcium rich food products (milk, cheese, ice-cream), grapes juice, vitamins containing iron etc. intake by patients and thus cause low absorption rate of antibiotics in body and decrease the desire effect of drugs.

3. Drug-condition interactions: Under certain medical conditions, if some drugs are given to patients and patients feel unwanted reactions and make these drugs potentially harmful under such clinical conditions then it is known as drug-condition interactions. For example: Someone may feel unwanted reactions if he/she take nasal decongestant even if he/she has high blood pressure.

4. There is a pharmacodynamic response that might be called drug-patient interaction in which an adverse drug reaction occurs because of individual sensitivity to a specific type of drug.

The risk of drug-drug interactions increases as there is increase in age of patients. Diet, exercise, genetics, current medications underlying diseases also affect the drug interaction. Older patients who take more medicines are more affected by drug interactions than the younger ones. So older person have higher rate of prescription and usage of psychoactive drugs than do people in other age group. The mental disorders diagnosed most frequently among older persons are depression, dementia, paranoia, hypochondriasis and iatrogenic drug problems. The latter diagnosis frequently includes the elderly who are prescribed antidepressants or minor tranquilizers who develop clear drug dependencies. Depression in older individuals is not always easily diagnosed; apathy and disorientation may be indicative of depression but they may also indicate brain syndrome. Malnutrition, memory impairments and confusions also complicate the clinical picture. Depression may also go unrecognized when an
older person presents with apathy, withdrawal and self-devaluation regarded by the diagnostician as an appropriate response to aging.

Insomnia is another issue; it is common concomitant of aging and one class of the most widely used psychoactive drugs are sedatives and hypnotics. Insomnia may be due illness, psychiatric or emotional disorders, lifestyle patterns and lessened need for sleep among older persons. Insomnia person is not life threatening, sleeplessness may increase the person's feelings of isolation and depression. Psychoactive drugs used commonly with elderly patients include antidepressants and sedatives, hypnotics and anti-anxiety agents are also widely prescribed. Sometime patients may feel drug interactions when administrated by new drugs or even when they are withdrawn from medication.

So we should take better care about protecting and promoting good health as by knowing about medicines intake and reactions caused by them in case of experiencing unwanted symptoms. Patients and doctors need to aware about the combinations of drugs taken and given in every turn.

So to prevent drug interactions is serious task for all patients and doctors. For making this a successful task, we should keep some following tips in our mind as:

1. Patients should have a list of all medicines, food supplements and diet items and show to their doctors in each visit. This is the first step that may overcome the drug interaction. It will make the doctor to form more educated treatment plan for their patients.

2. Doctors should have to check the issue of drug interactions when giving new medicines to their patients; whether there is possibilities of drug interactions or not with current medicines. Doctor may slip all this due to their busy health care setting. So it is also the responsibility of patients to take care about his/her health and discuss all this with doctors.

3. Patients can use online tool and check drug-interactions, dietary supplements also.

4. It is important that anyone prescribing drugs for a patients asks about other medications the patient take regularly such as query should include questions about over the counter drugs as well as prescription medication.

5. In case of unwanted or adverse drug-interaction, patients have right to demand for compensating medical bills, damages for mental and physical pain and death also to doctors
and pharmacy companies through personal injury lawyer. All this may prevent adverse effects of drug-interactions to large extent.

The biological activity of the compound may be enhanced, decreased or changed even with a slight change in the structure. For a useful drug discovery methods like group addition and changing the type and position of the substituent are employed. Introduction of halo, amino, nitro groups etc. can cause significant changes in the biological activity. Some time it is found that drugs may show unwanted reactions in the body if directly given in their normal or active form. So there is a provision for use of prodrugs also.

Prodrug is the inactive or partially active form of a drug. It is the precursor of a active drug. They are converted into active form in the body by normal metabolic process as by undergoing enzymatic or chemical transformation and then show desired reactions in form of pharmacologically active compounds. So we can say that prodrug has drug like properties like absorption, distribution, metabolism etc. in inactive form.109

In drug discovery and development, the use of prodrug is rapidly increasing. Today drugs are designed on the basis of their properties and so it is also becomes the necessity to design the prodrug in drug discovery and development process. The use of prodrug was rapidly increased in the beginning of 21st century. So designing of prodrugs is very challenging task in research area. Review of many journals, articles, patents showed that research of prodrugs may improve the required properties in investigations of drugs or in the drugs that are available in the market. The increasing use of prodrug can be marked by knowing that millions of prodrugs are in the rows of clinical trials.

Depending on the specific prodrug enzyme interaction, the transformation of prodrug in active form may occur in the body at the site of required and sufficient amount of enzymes or along the course of drug transit. For example, enalapril is prodrug, after oral administration it is bioactivated by hydrolysis to its active form that is enalaprilat.

There are many reasons for using prodrug110, which are summarized as:

1. Solubility: Solubility is one of the greatest challenges in drug discovery process. Many drugs are less soluble in body plasma and take much time in absorption and circulation process inside the body. So less soluble drugs show therapeutic effects after a long period of time than required or assumed. Due to this a high concentration of dosage of such drugs are required for patients. To overcome this problem, prodrugs are designed which have good or
increased aqueous solubility with required or small dosage forms than active drugs. Some ionizable polar, neutral functional groups like thiol, carboxylic acid, hydroxyl, amino acid, amides, phosphates, sugar moieties like glucose, galactose, esters etc. are attached to prodrugs. These functional groups are detached by various metabolic processes and release the active drugs inside the body. This type of prodrugs are orally taken or injected inside the patient's body.

2. Absorption: The drug has to pass through several lipid membranes during the transportation processes to its site of action. So permeability of membranes play important role on drugs efficacy. Majority of drugs are orally taken and this mode of absorption follows passive transport mechanism. Sometime drugs have poor permeability. Their water or lipid permeability can be increased by changing the hydrocarbon moieties or alkyl chain length and then required or desired lipophilicity can be obtained. After absorption prodrugs are converted into its parent drug via rapid conversion.

3. Biostability: After administration, a drug has to cross many pharmaceutical barriers before reaching their selective site and showing desired effects. During this period, drug molecules may be destroyed by various enzymes and chemicals present inside the body. Use of such prodrugs which have safer profiles, increased efficacy and specific to their actions and target sites, may overcome this situation.

4. Prolonged release: Some drugs are rapidly metabolized in gastrointestinal tract. Then liver may reduce the total amount of active drugs. Due to this small amount of active form of drugs is reached in circulation and to its target site. This problem has been overcome by use of prodrugs having slow metabolism and then retain the drug action for long time and show slower rate of releasing.

Kidney is the main organ of execration and eliminate water soluble substances from body. Some drugs are rapidly eliminate from body before showing their complete therapeutic effects. Use of lipophilic drugs promoieties in prodrugs decreases the solubility and prolong the duration of action of very soluble drugs. We can also used the other routes of administration such as buccal, renal and allow the drugs to be absorbed in the systematic circulation through blood vessels other than hepatic. Many anticancer, antiviral prodrugs are efficiently used.
5. Overcoming toxicity problems: Some time drugs show adverse reactions inside the body and alter the structures, functions, physiology of cells, tissues and organs and cause toxicity that may lead to death also.

So in drug designing process, drug safety is the main concern. Due to this, it is very important to find out the metabolic products of drugs that cause toxicity and nontoxicity in animals and human beings. Toxicity problems of drugs may be overcome to large extent as by designing a site selective and function selective prodrugs which are safely, selectively and quantitatively transformed into active drugs and show desired therapeutic effects. Exogenous enzymes in prodrugs are widely used to prevent toxicity at other sites in the body and at target places. Today many anticancer prodrugs are widely used.

Individual response to drugs: Different individual even of same species respond differently to same drug with same dose at same time. Same patient may also differently respond to same drug at different stages. This is known as individual response to drugs\textsuperscript{111}. This individual response to drugs also depends on physiological factors as:

1. Age: Age is main factor as different age groups patients respond differently to same drug. Small children are very sensitive as their organs are not fully developed. They have less weight so small amount of drug is given to them. Older patients react differently to drugs than younger ones. Due to ageing, weight of body decreases but storage of fats increases, hepatic blood pressure reduced, liver & kidney size changes, malnutrition may change the blood pressure and sugar level in body. So in different age groups, pharmacodynamics and pharmacokinetics to drugs is different due to difference in absorption, metabolism, distribution and excretion etc. process also at different stages of age.

2. Gender difference: The physiology of men and women is different to each other and this cause the variations in metabolism of some drugs under the influence of sex hormones also. Comparatively female has less weight and more percentage of body fat than male. In some cases women show more drug induced reactions, toxic effects, allergy, skin rashes etc. than male. During pregnancy treatment of women should be done by doctors very carefully so that any unwanted effect of drugs may not cause adverse effect to fetus and women also. So gender difference must be taken in account in the clinical treatment of men and women.
3. Body weight of patient should be considered by doctors at time of prescribing a dose. Concentration of dose that attained in body can be calculated by ratio between body weight and amount of drug given. Fatty patient needs more amount of dose than thinner patient.

4. Time of administration of drugs also affects the individual response to drugs. Some drugs are absorbed rapidly when stomach is empty while some others are effective if taken after meal. So we should follow the prescription given by doctors about the time of administration to avoid any side effects.

5. Tolerance: Prolong habit of taking same drugs may make the patients tolerable to that particular type of drug. So amount of dose is increased to get required response. All this occurs mainly in case of taking narcotics drugs by a person.

6. Drug combination: When more than two drugs are given to patients at same time and their combination effect is more than the individual drug, then amount of effective dose is decreased by doctors and vice versa.

7. Rate of excretion of drugs from body must be considered by doctors at time of prescribing a dose to patients. Prolong intake of any medicine and its quickly absorption and small rate of excretion may increase its concentration in the body. All this may cause toxic effects or unwanted reactions. To get desired or required therapeutic effect an even concentration of dose must be maintained in the body of patients.

Physical properties and chemical structure of compounds also impart important role in drug action. So many efforts have been put to find the relationships between them. Chemist work hard to predict the physical properties of an organic compound and assign the right structure of the compound. Various spectroscopic methods have been used to determine the structures of the compounds. The aim of structural investigation is to find out how the atoms are arranged with respect to one another and how they are bonded together. Even when a structure has been determined it is often desirable to infer by analogy more about the bonding. The justification for this structure is so important in understanding properties that one wants to be able to visualize the structure in order to think about the properties and relate them to general patterns. A great deal of interest in organic chemistry is centered around trying to get a better understanding of bonding in molecules and crystals and so in turn to understand better properties of chemical substances. Chemist is particularly interested in the properties that a substance shows in reacting with other substances.
In the early work, the structure of an organic compound was solved by chemical analysis as by analyzing qualitative, quantitative properties, empirical formula and molecular formula of substance.

If the molecule is relatively simple, the various possible structures are written down. Then the reactions of the compound are studied and the structure which best fits is chosen. In other cases where the molecules are not relatively simple, then particular test are performed to find out the different groups present in the compounds. The compounds are breakdown in to smaller fragments and then it is easy to determine the best possible structures.

The last step for determine the structure of compound is the synthesis. If the same compound is formed by different routes then more reliable structure will be assigned to that compound.

Heterocyclic reactions are carried out in solution. If we understand organic reactions, we must learn something about role played by the component of the reaction system that seldom appears in our equations but that is nearly always present: the solvent.

The role played by the solvent is not minor one. The presence of a solvent can speed up or slow down a reaction by a factor of $10^{20}$, a change from one solvent to another can bring about a million fold change in reaction rate. Solvent effects can be more powerful than the effects exerted by any other factor: vastly more powerful than polar or steric effects; sometimes more powerful, even than the symphoric effects.

Choice of the particular solvent can be major factor to determine how fast a reaction occurs and even whether it take place at all; It can determine which of several alternative pathways a reaction actually follows. The solvent is intimately involved in any reaction that take place in it and it is important for us to find how much it is involved and in what ways. Clinging to each dissolved particle is a cluster of solvent molecules. These are held by bonds. It was formation of these bonds that provided energy necessary to break the bonds that held solute particles to each other. In the solution all participants of chemical reaction are solvated: the reactant and the products and the transition state. We do this by examining mentally, by use models, physically by the structure involved. This examination must include any solvent clusters that help make up those structures and help determine their stabilities.

Thus solvent adds a new dimension to our study of organic chemistry. It offers us most practical way to control what happens in a chemical reaction. The effect exerted by a solvent is one kind of medium effect-environmental effect and in that sense is just the beginning of a
trail that leads all way to the ultimate organic reaction, the action of an enzyme. This vital action is possible only because the substrate is dissolved in the enzyme, held to it by the same kinds of forces that a solvent uses.

Now a days, Physical methods are considered to be necessary tools for the elucidation of structures. They are used on the compound itself and also used in the examination of the fragments obtained by degradative work. These physical methods like X-ray analysis make synthesis as a means of structure determination. There are various criteria for purity. The most common one for solids is melting point; for liquids, boiling point, density and refractive index are used. The examination of IR, UV, NMR, Mass fragmentation of a compound are also used as a test for purity. In other cases, process of purification is repeated until the physical constant or spectrum remain unchanged. Furthermore, it is best to use at least two methods of purification; a very good combination is the recrystallisation and chromatography. Other physical properties that may be used for characterization are specific rotation, optical rotatory dispersion, X-ray powder photographs, cracking pattern in mass spectrometer etc. It is usually essential for research purpose to confirm the purity and identity of the compound by spectroscopic investigation. In a research situation, the best combination of isolation and purification techniques requires the application of the mature judgment acquired by the competent practical worker.

There are various techniques for isolation of the products from a selection of relatively simple organic reactions provides the beginner with the necessary experience in the standard procedures and purifying compounds. These are distillation, sublimation, recrystallisation from suitable solvents. Counter-current distribution, electrophoresis, chromatography in its various forms are mostly used today. Zone melting and this is gaining ground are as a means of preparing substances in a state of ultra purity. These long established methods are frequently adequate for isolation of product in desired degree of purity.

Conformational and stereo chemical factors also impart important role in drug action. The elucidation of the steric features of known pharmacological agents hold much promise for progress in drug design and this should enable medicinal chemists to synthesize new drugs with not only the essential functional groupings, but also the capability of adopting the optimal strike arrangements.

Functions that drug serve:
It is useful for some purpose to characterize the main motivational thrust that underlies the use of many drugs from clinical point of view. It is more important to recognize that drugs serve a variety of functions for different individuals and these functions often shift at different points in drug career. The phases in a typical drug career might be characterize as initiation, maintenance, escalation and dependence or heavy involvement as inability or unwillingness to stop dangerous or excessive use. The concept of stages is to introduce the underline point that drug is not monolithic behavior and there are various points along the way where different types of intervention may be indicated. The situation will be somewhat different for certain individuals and certain drugs; but the general approach should have heuristic value for planning prevention, treatment and rehabilitation programs.

Functions associated with direct pharmacologic properties of drugs: The person uses drugs specifically to produce positive feelings, to relieve anxiety, to relieve depression, to avoid emotional pain etc. in a therapeutic fashion. This usually follows previous exposure to the drug but may be present at the outset, if the person is aware of claims for the drug or its general indications. This function is probably important in the early phases of addiction and demands of dependency notwithstanding, remains so as times goes on. Curiosity is one most commonly reported for almost all drugs. A significant number of persons continue on with the drug or become heavily involved with it because it fulfills an immediate psychological need. For many young people, the drug is a form of self-treatment that enables them to avoid the passive dependency of the sick role and at the same time maintain a positive public image. The implications for treatment and medical care are obvious, but it also obvious that in this group of young ones we should anticipate resistance to medically oriented treatment offerings.

Licensing or masking functions:

Some person use drugs and well known effects as an occasion and semi-acceptable rationalization for openly acting out in ways that would otherwise be unacceptable to the person or society. For some person, drug may mask underlying fears or inadequacies, prevent impulses from surfacing or permit the person to avoid unpleasant responsibilities or situations. The particular drug or class of drugs is important but mainly in the sense that it must have general properties or secondary effects that fit the intent of the user or could be reasonably associated with the observed behavior.

Instrumental function: For many people there is secondary gain associated with taking drugs. The use of drug may simply be the means whereby the person obtains and maintains
companionship or achieves intimacy or he may achieve status or identity. He may like the ritual or ceremonial aspects of taking drugs in group fashion. He may get heavily involved with the drugs because of secondary reinforcements.

Effects of drugs:\textsuperscript{112}: Many drugs are analgesic agents and produce major effects on the central nervous system (CNS) and gastrointestinal system. The outstanding effect of morphine on human is the relief of pain. The action of drugs is very selective; other senses are altered very little or not at all. Morphine is effective for all types of pain, it is more so against continuous dull pain than against sharp intermittent pain. In human body, pain is separated into two components: (1) as a specific sensation, which can feel; (2) as suffering or how human react to the specific sensation. Drugs like morphine decreases the perception of pain and also alter the individual's reaction to it.

Therapeutic uses:

Natural and synthetic drugs are effective pain relievers. They are among the most valuable drugs available to the physicians and are widely used for short term acute pain resulting from surgery, burns and to reduce suffering in the later stages of terminal illnesses, such as cancer.

Analgesia: The major therapeutic indication of drugs and narcotics is the reduction of pain. The effects of narcotics are relatively specific to pain. Fewer effects on mental and motor ability accompany analgesic and depressant drugs.

Antitussive actions: The narcotics drugs also have effect on decreasing coughing. Codeine has been widely used for its antitussive properties and is still available in some prescription cough remedies. Nonprescription cough remedies contain dextromethorphan, a narcotic analogue that is more selective in its antitussive effects.

Gastrointestinal: Regarding diarrheal disorders, synthetic opioids and proving effective agents for decrease of bowel motility. The opiates remain at the forefront of treatment for these disorders.

Drugs produce sedation, sleep, stupor etc. Drugs act selectively at specific synapses in certain location rather depress every central nervous system neurons. They produce true general anesthesia at high dose, however at low to moderate doses they have sedative-hypnotic
Drugs like benodiazepines dilate blood vessels of the heart with intravenous use and at very high doses, blockage of the nerves to the muscles.

Drugs have muscle-relaxing properties. They are powerful and selective effect. The muscle relaxation is not peripheral but central mediated although the exact sites of action are unknown. Human develop tolerance to this effect.

Some drugs like benzodiazepines affect the reticular formation at moderate dosages, blocking arousal and causing sleep. For this reason they are commonly prescribed sleeping pills. They produce drowsiness however diminishes over the course of a few days as tolerance develops. This is ultimately an advantage since it means that drugs like benzodiazepines can soon calm a person without sedation and therefore they are more selective. Tolerance for the anxiety relieving effects have not been shown. Sensitivity of these drugs increases with age and in the presence of liver disease and it decreases with alcohol use, cross tolerant drugs, or recent use of other drugs like benzodiazepines.

Drugs like cocaine has local anesthetic and vasoconstrictor properties and hence valuable in several medical applications. They are also used as pain killer. Many drugs are used as local anesthetic in surgery in eye, nose, throat and upper respiratory passages and also have ability to anesthetize the mucous membranes involving in surgery. Many drugs like cocaine acts to constrict the blood vessels. It prevents massive blood loss during surgery that would otherwise occur in membranes richly supplied with blood vessels; in addition to minimizing blood loss. It allows for more accurate surgery by keeping the area free of vision-obstructing blood and surgical field clear. In medical area some drugs has excellent record for safety. Many drugs has been used on an experiment in the treatment of depression and other mental or psychotic disorders.

Many drugs are also used as minor stimulants. Xanthines are the oldest stimulants known to man. The three xanthines of primary importance are coffee, tea and cocoa. It is added to many carbonated soft drinks and over the counter medications as well. The cured coffee bean contain about 1% caffeine and dried tea leaves have 5% caffeine plus theophylline. Cocoa has little caffeine and about 2% theobromine. These minor stimulants have proven medicinal value. They stimulate the central nervous system, act on the kidney as a diuretic, stimulate cardiac muscle and relax smooth muscle. Person who have bronchial spasms or asthma probably have noticed that they can breathe a bit easier after drinking a cup of tea.
These three chemicals are methylated xanthines and are closely related alkaloids. Most alkaloids are insoluble in water but these are unique because they are slightly water soluble. Caffeine has greatest effect on CNS and skeletal muscles. Theophylline is the most potent and caffeine is the least potent agent on the cardiovascular system.

Acute toxicity: In acute drug poisoning, the individual is comatose and cyanotic with slow respiration and pinpoint size pupils. The major development in the treatment of acute morphine poisoning has been discovery of the antidotal of nalorphine or naltrexone.

Causes for concern: Most opiate dependent individuals take heroin. However, heroine by the time it reaches the user, is grossly contaminated with such things as starch, quinine, baking soda and mannitol. Its strength is seldom known and thus it is not surprising that heroin overdose may cause deaths of heroin user. Nonsterile drugs and equipment and faulty techniques cause much damage. The practice of sharing the same needle to inject drugs into the veins can result in the spread of blood-borne diseases as AIDS, serum hepatitis. Bacterial infection of the heart quite often. Acute pulmonary congestion and edema are also frequent findings.

Chronic toxicity: Prolonged misuse of opiates usually results in physical dependence, withdrawal and tolerance to the actions of the narcotic being abused.

Drug use is a part of human behavior. One basic behavior principle is that behavior persists when it either increases the individual's pleasure or reduces his or her discomfort. People do not take just any drug, they take those affect them pleasurably or make a situation less intolerable. People turn to use drugs in the hope of finding peace, oblivion, expended consciousness or euphoria, stress release. Drug use is the result of complex interaction of past experience and present environment. Many drugs are such powerful, immediate reinforcers that if all the population were allowed to try every major psychoactive drug and then were permitted free access to the drug for their choice, the effect on society would be disastrous. The effect of drug dependence on the particular person is related to the nature of the psychoactive substance, the quantity used, characteristics of the person and of his or her environment.¹¹³

Drug harmful is not inherent in its chemistry but rather in the contest in which it is used. This simple but fundamental truth about drug taking has yet to penetrate public opinion. Drug is associated with substances about which they are concerned and to lesser degree with the
antidepressants and tranquillizers physicians prescribe and are not popularly regarded as such and therefore are less likely to arouse public concern.

Pharmacologists distinguish between psychological and physical dependence on drugs. One of them has to do with drawing the boundaries between the two sets of dependencies. Use of some drugs may lead to one dependency and not the others, whereas use of other drugs may lead to both physical and psychological dependence. Opiate drugs such as heroin as well as alcohol is generally agreed and have the potential for leading to both physical and psychological dependence.

Drug use inside and outside the medical system differ in several important respects, but there are many similarities among the various types of drug use. These similarities can be cast in the form of a set of assumptions or hypothesis which could be of great theoretical and practical importance in the area of drug use.

Basic assumptions:

Coping: The use of psychoactive drugs can be seen as but one alternative among many for coping with the subjective needs, desires and problems of life.

Choice: Most of the drugs used can be viewed as a matter of choice whether inside or outside the medical system, for seldom is the person so ill or in such distress that is no other recourse. This implies that there are wide individual differences in personal criteria of illness and tolerance for discomfort. Decisions to visit the physician or seek drugs directly need not be rational or deliberate but the action suggests that at some level a choice among available alternatives has been made.

Interaction of drugs and needs: Individuals take drugs that fit their needs or that are deemed desirable by their important references group. In the physician's office, the patient is prescribed a drug that fits his complaints; if he does not like it he rejects it and gets another. This approach is generally in keeping with what we know about the psychedelic and drugs, although the fit is far from perfect. He argues for an approach to the problem which emphasizes a thorough understanding of both drugs and subcultures.

Benefit-Risk ratio: There is benefit-risk ratio associated with use of all drugs whether inside or outside the medical system, and illicit drug users are making choices similar to those made inside medical system buy much less precision and with much less reliable knowledge at their
disposal. A drug that are widely used and has a moderate risk ratio but is low rated on severity of adverse consequences will get a fairly low priority and may be ignored whereas a drug that is much less widely used but has a high risk ratio and severe adverse consequences may get a high priority and demand immediate action.

Cost-Benefit ratio: There is also cross-benefit ratio associated with all drugs use in terms of personal and social consequences. The cost-benefit question applies to the use of drugs versus other coping mechanism, the use of one drug versus another, the use of drugs in an otherwise hopeless situation. The use of some drugs may cause immediate relief in pain but its use may cause bad personal and social consequences.

Compensatory behavior: The model anticipates the occurrence of compensatory behavior and assumes that if an individual—for personal, social, economic or cultural reasons does not appear in the medical system, it may appear in some other system where he gets the drugs that meet his needs. He has alternative coping mechanisms or other social pathway readily available.

**Effects of drugs:** Drugs can affect the functioning of the body through side effects, secondary effect and toxic effects.

1. Side effects: These effects are unwanted but often unavoidable that occur at therapeutic doses. For example: some of the anti-allergic drugs may produce sedation.

2. Secondary effects: These effects are indirect consequences of a primary action of the drug. For example: antibiotics kill the friendly bacteria along with harmful bacteria.

3. Toxic effects: These effects are the result of excessive pharmacological action of the drug due to over dosage or prolonged use. For example: over dosage of paracetamol causes liver damage. The toxicity of the drugs is expressed in terms of LD$_{50}$, which stands for lethal Dose-50. It is defined as the dose required to kill 50% of the population of the test animals and expressed in mg/kg of the body weight of the animal. Smaller the LD$_{50}$ value, the more toxic is the drug.

**Evaluation of antimicrobial agents:**

**Factor affecting antimicrobial activity:**

Among the many factors that affect antimicrobial activity in vitro, the following must be considered because they significantly influence the results of tests.
1. **pH of environment**: Some drugs are more active at acidic pH (nitrofurantoin) others at alkaline pH (aminoglycosides and sulfonamides).

2. **Components of medium**: Sodium polyanetholsulfonate and other anionic detergents inhibit aminoglycosides. Addition of NaCl to the medium enhances the detection of methicillin resistance in S. aureus.

3. **Stability of drug**

   At incubator temperature, several antimicrobial agents lose their activity. Chlortetracycline is deactivated rapidly and penicillins more slowly whereas aminoglycosides, chloramphenicol and ciprofloxacin are quite stable for long periods.

4. **Size of inoculum**: In general larger bacterial inoculum lowers the apparent “susceptibility” of the organism. Large bacterial populations are less promptly and completely inhibited than small ones. In addition a resistant mutant is much more likely to emerge in large populations.

5. **Length of incubation**

   In many instances microorganisms are not killed but only inhibited upon short exposure to antimicrobial agents. The longer incubation continues, greater is the chance for resistant mutants to emerge or for the least susceptible numbers of the antimicrobial population to being multiplying as the drug deteriorates.

**Methods:**

(a) **Dilution method**: Antimicrobial substances are poured into liquid or solid bacteriological media in graded amounts. The media are subsequently added with test bacteria and incubated. The amount of antimicrobial substance required to inhibit the growth of the test bacteria (MIC) is taken as the end point. Two types of dilution methods are employed:

(i) **Agar dilution test (Cup-plate method)**

   In this method the solution of the antimicrobial agent is placed in contact with agar media, which is already inoculated with the test organism and after incubation zones of inhibition are observed. The solution is placed in a small cup made by cutting the agar layer with a sterile cork-borer.

(ii) **Broth dilution method (Turbidity method)**
In this method graded doses of the test substance are incorporated into nutrient broth dispensed in test tubes and the tubes are inoculated with the test organism and incubated.

**Diffusion method:** The most widely used method is disc diffusion test. A filter paper disc containing a measured quantity of a drug that is put on the surface of a solid medium. Surface of the solid medium has been incorporated with the test organism. Now measure the diameter of the clear zone of inhibition that surrounds the disc. It is taken as the inhibitory power of the drug against the particular test organism.

4.2 **Experimental studies of biologically screened derivatives:** The in-vitro antibacterial and antifungal activities of the newly synthesized pyrazoline and pyrazole derivatives were carried out by microdilution susceptibility test using cup-plate (cylinder plate) technique\(^9\). The minimal inhibitory concentrations (MICs \(\mu\text{g}\text{mL}^{-1}\)) of all the compounds were determined.

The MIC is considered to be the lowest drug concentration for which there is no microbial growth\(^9\).

**Antibacterial activity:**

**Materials and Methods:**

**Receptacles:**

- Sterilized plastic Petridishes (100 mm)
- Sterile stainless steel borer, outside diameter 8.0 ± 0.1 mm and inside diameter 6.0 ± 0.1 mm.

**Test Organisms:**

Staphylococcus areus (ATCC – 29737)

Escherchia coli (ATCC – 8739)

These strains obtained from the Department of Microbiology, Majeedia Hospital, New Delhi.

**Preparation of inoculum:** A loop full of culture from frozen agar slant was introduced into 10 ml of sterilized nutrient broth and incubated at 30° to 35 °C for 24 hours to obtain the stock culture. The stock solution of the culture was diluted by serial dilution method to a dilution factor which has given 25% light transmission at 530 nm. The suspension was stored under refrigerator.
**Media: (Nutrient Agar, Himedia M001)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gm/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.5</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Final pH (at 25 °C) 7.4 ± 0.2

**Procedure:**

28 gm of nutrient agar was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Standard Drug:** Ofloxacin

**Test Compounds**

6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml in Dimethyl Formamide (DMF)

**Method**

- Sterilized liquefied agar medium was inoculated by the suspension of the micro-organism (0.1 in 100ml medium) at a temperature between 40°- 50°C.
- The inoculated medium was immediately poured into the sterilized petridishes in such a way to obtain uniform thickness.
- After setting of the medium four holes were made in plate and various concentrations (6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml) of the test compounds (0.1 ml) were placed in the cavities.
- The plates were kept at room temperature for 1 hour and incubated at 35°C for 24 hours.
- The MIC of the compounds was calculated from the zone of inhibition formed around the cup after incubation.
Antifungal activity:

Materials and Methods: Receptacles:

- Sterilized plastic petridishes (100 mm)
- Sterile stainless steel borer, outside diameter 8.0 ± 0.1 mm and inside diameter 6.0 ± 0.1 mm.

Test Organism:

Aspergillus niger (ATCC 16404)

Preparation of inoculum:

The recently grown stock culture of the test organism was subculture on the nutrient agar medium. The culture was then incubated at 20° to 25 °C for 48 hours and then harvested with sterile normal saline containing 0.05% w/v of polysorbate 80. The spore count was adjusted to 1x 10⁸ per ml with sterile normal saline.

Media: (Sabouraud’s Dextrose Agar Medium)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gm/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>40.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>to 1000 ml</td>
</tr>
</tbody>
</table>

Final pH (at 25 °C) 5.6 ± 0.1

Procedure:

The ingredients were dissolved completely in distilled water by warming and cooled to room temperature. The pH of the solution was adjusted to 7.3 ± 0.2 with 1M NaOH solution and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

Standard Drug: Ketoconazole

Test Compounds
6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml in Dimethyl Formamide (DMF)

Method

- Sterilized liquefied agar medium was inoculated by the suspension of the micro-organism (0.1ml in 100ml medium) at a temperature between 40˚- 50˚C.
- The inoculated medium was immediately poured into the sterilized petridishes in such a way to obtain uniform thickness.
- After setting of the medium four holes were made in plate and various concentrations (6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml) of the test compounds (0.1 ml) were placed in the cavities.
- The plates were kept at room temperature for 1 hour and incubated at 28˚C for 48 hours.
- The MIC of the compounds was calculated from the zone of inhibition formed around the cup after incubation.

4.2 (a) Experimental studies of biologically screened pyrazoline derivatives:
In vitro antibacterial activities of newly synthesized pyrazoline derivatives was carried by microdilution suspectibility test using cup-plate technique. Staphylococcus areus (ATCC – 29737) and Escherchia coli (ATCC – 8739) were taken as test organisms. Nutrient Agar, Himedia M001 was used as medium. Ofloxacin was used as standard drug. 6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml in Dimethyl Formamide (DMF) were taken as test compounds. Results were shown in table-4 and table-5.

4.2 (b) Antifungal activity of newly synthesized pyrazole derivatives:
In vitro antifungal activities of newly synthesized pyrazoline derivatives was carried by microdilution suspectibility test using cup-plate technique. Aspergillus niger (ATCC 16404) was taken as test organism. Sabouraud’s Dextrose Agar was used as medium. Ketoconazole was used as standard drug. 6.5, 10, 12.5, 25, 50, 100 and 200 µg/ml in Dimethyl Formamide (DMF) were taken as test compounds. Results were shown in table- 6.
### Table-4  Antimicrobial Activities of Quinolinoxyacetyl Pyrazoline Derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>MIC (µg/ml)</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>10.0</td>
<td>12.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>-</td>
<td>-</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>100</td>
<td>&gt;200</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>100</td>
<td>200</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>4d</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>4e</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4f</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4g</td>
<td>100</td>
<td>200</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>4h</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4i</td>
<td>&gt;200</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4j</td>
<td>200</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

### Table-5  Antimicrobial Activities of 6-Chlorobenzothiazolyl Pyrazoline Derivatives
<table>
<thead>
<tr>
<th>Compound No.</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>10.0</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>-</td>
</tr>
<tr>
<td>7a</td>
<td>50</td>
</tr>
<tr>
<td>7b</td>
<td>25</td>
</tr>
<tr>
<td>7c</td>
<td>200</td>
</tr>
<tr>
<td>7d</td>
<td>100</td>
</tr>
<tr>
<td>7e</td>
<td>100</td>
</tr>
<tr>
<td>7f</td>
<td>200</td>
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<tr>
<td>7g</td>
<td>50</td>
</tr>
<tr>
<td>7h</td>
<td>50</td>
</tr>
<tr>
<td>7i</td>
<td>200</td>
</tr>
<tr>
<td>7j</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table-6  Antimicrobial Activities of Quinolinoxyacetyl Pyrazole Derivative**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
</table>
### Table-7: Anti-inflammatory Activity of 1,3,4-Oxadiazole Derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Mean Paw Volume ± SEM</th>
<th>% Inhibition ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>E. coli</td>
<td>A. niger</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>10.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9a</td>
<td>50</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9b</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>9c</td>
<td>&gt;200</td>
<td>100</td>
</tr>
<tr>
<td>9d</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>9e</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>9f</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>9g</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>9h</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>9i</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>9j</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

### 4.2(c) Experimental studies of biologically screened 1,3,4 oxadiazole derivatives:

1,3,4-Oxadiazole derivatives were screened for their in vitro anti-inflammatory activity by carrageenin-induced rat paw edema method of Winter et al\textsuperscript{180}. Ibuprofen was taken as the standard drug. Results were shown in table-7, table-8.

### 4.2 (d) Experimental studies of biologically screened 1,2,4 triazole derivatives: The anti-inflammatory activity of 1,2,4-triazole derivatives was carried out by the method of Winter et al\textsuperscript{180}. Ibuprofen was taken as the standard drug. Results were shown in table-9.

Table-7: Anti-inflammatory Activity of 1,3,4-Oxadiazole Derivatives
<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Mean Paw Volume ± SEM</th>
<th>% Inhibition ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>0.030 ± 0.0044</td>
<td>86.35 ± 2.033*</td>
</tr>
<tr>
<td>3a</td>
<td>0.093 ± 0.0042</td>
<td>57.57 ± 1.916*</td>
</tr>
<tr>
<td>3b</td>
<td>0.090 ± 0.0044</td>
<td>59.08 ± 2.033*</td>
</tr>
<tr>
<td>3c</td>
<td>0.083 ± 0.0061</td>
<td>62.11 ± 2.794*</td>
</tr>
<tr>
<td>3d</td>
<td>0.060 ± 0.0073</td>
<td>72.72 ± 3.319*</td>
</tr>
<tr>
<td>3e</td>
<td>0.073 ± 0.0042</td>
<td>66.66 ± 1.916*</td>
</tr>
<tr>
<td>3f</td>
<td>0.106 ± 0.0066</td>
<td>51.51 ± 3.030*</td>
</tr>
<tr>
<td>3g</td>
<td>0.073 ± 0.0042</td>
<td>66.66 ± 1.916*</td>
</tr>
<tr>
<td>3h</td>
<td>0.110 ± 0.0044</td>
<td>49.99 ± 2.033*</td>
</tr>
<tr>
<td>3i</td>
<td>0.073 ± 0.0066</td>
<td>66.66 ± 3.030*</td>
</tr>
<tr>
<td>3j</td>
<td>0.060 ± 0.073</td>
<td>72.72 ± 3.319*</td>
</tr>
<tr>
<td>3k</td>
<td>0.070 ± 0.0044</td>
<td>68.17 ± 3.033*</td>
</tr>
<tr>
<td>3l</td>
<td>0.067 ± 0.0042</td>
<td>69.69 ± 1.916*</td>
</tr>
<tr>
<td>3m</td>
<td>0.087 ± 0.0042</td>
<td>60.60 ± 1.916*</td>
</tr>
<tr>
<td>3n</td>
<td>0.080 ± 0.0073</td>
<td>63.63 ± 3.319*</td>
</tr>
<tr>
<td>3q</td>
<td>0.067 ± 0.0042</td>
<td>69.69 ± 1.916*</td>
</tr>
</tbody>
</table>

Table-8: Anti-inflammatory Activity of 1,3,4-Oxadiazole Derivatives
<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Mean Paw Volume ± SEM</th>
<th>% Inhibition ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>7c</td>
<td>0.146 ± 0.0084</td>
<td>55.55 ± 2.555*</td>
</tr>
<tr>
<td>7d</td>
<td>0.113 ± 0.0066</td>
<td>65.65 ± 2.020*</td>
</tr>
<tr>
<td>7e</td>
<td>0.186 ± 0.006</td>
<td>43.43 ± 2.020*</td>
</tr>
<tr>
<td>7f</td>
<td>0.123 ± 0.0080</td>
<td>62.62 ± 2.432*</td>
</tr>
<tr>
<td>7g</td>
<td>0.150 ± 0.0085</td>
<td>54.54 ± 2.595*</td>
</tr>
<tr>
<td>7h</td>
<td>0.110 ± 0.0044</td>
<td>66.66 ± 1.355*</td>
</tr>
<tr>
<td>7i</td>
<td>0.153 ± 0.0066</td>
<td>53.53 ± 2.020*</td>
</tr>
<tr>
<td>7j</td>
<td>0.117 ± 0.0061</td>
<td>46.96 ± 2.794*</td>
</tr>
</tbody>
</table>

*P<0.0001, compared w.r.t. control. Data were analyzed by student’s t-test for n= 6

Table-9: Anti-inflammatory Activity of 1,2,4-Triazole Derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Mean Paw Volume ± SEM</th>
<th>% Inhibition ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>0.107 ± 0.0042</td>
<td>51.51 ± 1.916*</td>
</tr>
<tr>
<td>8b</td>
<td>0.077 ± 0.0061</td>
<td>65.15 ± 2.794*</td>
</tr>
<tr>
<td>8c</td>
<td>0.090 ± 0.0044</td>
<td>59.08 ± 2.033*</td>
</tr>
<tr>
<td>8d</td>
<td>0.106 ± 0.0066</td>
<td>51.51 ± 3.030*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>8e</td>
<td>0.100</td>
<td>54.54± 2.347*</td>
</tr>
<tr>
<td>8f</td>
<td>0.120</td>
<td>45.45± 2.347*</td>
</tr>
<tr>
<td>8g</td>
<td>0.110</td>
<td>49.99± 2.033*</td>
</tr>
<tr>
<td>8h</td>
<td>0.090</td>
<td>59.08± 2.033*</td>
</tr>
<tr>
<td>8i</td>
<td>0.070</td>
<td>68.17± 3.105*</td>
</tr>
</tbody>
</table>

*P<0.0001, compared w.r.t. control. Data were analyzed by student’s t-test for n= 6