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Review on Drug Evaluation Method

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ABSTRACT-

The pharmacokinetics and pharmacodynamics of drugs are different in adult and paediatric populations, the latter being particularly heterogeneous. These differences in pharmacokinetics and pharmacodynamics justify specific studies but raise a number of ethical and practical issues. The main practical difficulties to circumvent while performing clinical studies in children are the invasiveness of the procedures and the obstacles to patient recruitment. The invasiveness related to pain/anxiety and blood loss precludes the performance of classical pharmacokinetic studies in children in many instances, particularly in neonates and infants. Population approaches, which rely on pharmacokinetic-pharmacodynamic modelling, are particularly appealing in paediatric populations because these models can cope with sparse data. The relevance of population approaches to investigation of the dose-concentration-effect relationships and to qualitative/quantitative assessment of factors that may explain interindividual variability has already been emphasized.

Objectives:

To evaluate medication effectiveness;

- To improve patient safety of drug
- To avoid medication misadventure including adverse drug events
- To standardize therapy to reduce variation
- To optimize therapy
- To meet federal, local, regulatory, professional, or accreditation standards
- To minimize costs.

Module 1: Screening pharmacological activities

1. Cardiovascular activity:

- **Antiarrhythmic activity:** Arrhythmias occur when the electrical signals that coordinate heartbeats are not working correctly, an irregular heartbeat may feel like a racing heart or fluttering many heart arrhythmias are harmless. However, if they are highly irregular or result from a weak or damaged heart.

Classification of drugs:

CLASS	DRUGS
Class I (Membrane stabilizing agents)	**Ia** :Qunidine, Procainamide, Disopyramide **Ib**: Mexiletin, Lidocaine, Phenytoin **Ic**: Flecainide, Moricizine, Propafenone
Class II (B-blocker)	Propranolol, Esmolol, Sotalol
Class III (Prolong duration of action)	Amidarone, Sotalol, Bretylium, Dofetilide, Ibutilide
Class IV (Calcium channel blockers)	Verapamil, Diltiazem

Amidarone: mechanism of action

This unusual iodine containing highly lipophilic long-acting antiarrhythmic drug exerts multiple (Class I, II, III and IV) actions:Prolongs APD and Q-T interval attributable to block of myocardial delayed rectifier K+ channels. This also appears to reduce nonuniformity of refractoriness among different fibers. Preferentially blocks inactivated Na+channels (like lidocaine) with relatively rapid rate of channel recovery: more effective in depressing conduction in cells that are partially depolarized or have longer APD.Partially inhibits myocardial Ca2+ channels. Has noncompetitive β adrenergic

blocking property alters thyroid function. Thus, amiodarone is a multichannel blocker with some additional activities. Conduction is slowed and ectopic automaticity is markedly depressed, but that of SA node is only slightly affected. Effect of oral doses on cardiac contractility and BP are minimal, but i.v. injection frequently causes myocardial depression and hypotension.

- **List of herbal plants showing Antiarrhythmic activity**

Broom, Bugleweed, Motherwort, Ailanthus (Ailanthus altissima), Cactus, Dogbane (Apocynumcannabinum), Adonis (Adonis vernalis), Gelsemium (Gelsemium sempervirens), Hawthorn, Lobelia seed (Lobelia inflata), Pink root (Spigeliamarilandica).

IN VIVO MODELS:

1. The Langendorff-perfused rabbit heart model
2. Models of Atrial Arrhythmia Vagal AF in Anesthetized Closed-Chest Dog
3. Models of Ventricular Arrhythmia Reperfusion-Induced Arrhythmias in Anesthetized Rats
4. Experimental Models of Atrial Arrhythmia Vagal AF in Anesthetized Closed-Chest Dogs
5. Experimental Models of Ventricular Arrhythmia Reperfusion-Induced Arrhythmia in Anesthetized Rats

2. Respiratory activity-

- **Antihistamatic activity**-Antihistamines suppress the histamine-induced wheal response (swelling) and flare response (vasodilation) by blocking the binding of histamine to its receptors or reducing histamine receptor activity on nerves, vascular smooth muscle, glandular cells, endothelium, and mast cells.

Classification-

Class	**Drugs**
1) H1 First-Generation	Meclizine, Clemastine, Hydroxyzine, Brompheniramine, Dimetindene, Doxylamine, etc.
2) H1 Second-Generation	Meclizine, Clemastine, Hydroxyzine, Brompheniramine, Dimetindene, Doxylamine, etc....
3) H2	Ranitidine, Cimetidine, Famotidine, etc.

Meclizine- Mechanism of action

Vomiting is a centrally regulated reflex mechanism that initiates from the vomiting center and the chemoreceptor trigger zone (CTZ) located in the medulla. Motion sickness is also regulated by CTZ. The blood-brain barrier near the CTZ is relatively permeable to circulating mediators and CTZ can transmit impulses to vomiting center located in the brainstem. Different receptors responding to different factors, including histamine, 5-HT, enkephalins, substance P, and dopamine, is expressed along the brainstem to activate respective pathways and contribute to the control of vomiting. Histamine H1 receptors are expressed on the vestibular nuclei and nucleus of the solitary tract (NTS) that are activated by motion sickness and stimuli from the pharynx and stomach. When activated, H1 receptor signaling from these nuclei is transmitted to the CTZ and vomiting centre.

Through its antagonistic action on the H1 receptors, meclizine primarily works by inhibiting signaling pathway transduction through histaminergic neurotransmission from the vestibular nuclei and NTS to the CTZ and medullary vomiting center.1 Meclizine may also decrease the labyrinth excitability and vestibular stimulation.

List of plants showing antihitamatic activity-

1) Aerva lanta Linn (Amaranthaceae)
2) Ageratum conyzoides
3) Argemone mexicana (A. mexicana)
4) Asystasia gangetica T. Adams (Acanthaceae)
5) Bacopa monnieri L. (Scrophulariaceae)
6) Cassia sophera (caesalpiniaceae)

In vivo models-

1) Histamine-induced bronchoconstriction in guinea pigs/mice/rats.
2) Passive paw anaphylaxis in rats/guinea pig/mice

3) Milk-induced leukocytosis in mice.
4) Milk-induced eosinophilia in mice.
5) Clonidine-induced catalepsy in mice
6) Haloperidol-induced catalepsy in mice.In-vitro model/tissue model

3. Psychotropic and neurotropic activity-

Antiepileptic activity-It means drugs design to control or prevent seizures with epilepsy.

Classification-

Class	**Drugs**
Barbiturates	Phenobarbitone
Hydantoin	Phenytoin,Fosphenytoin
Succinimide	Ethosuximide
Benzodiazepines	Clonazepam, diazepam, lorazepam, clobazam
Deoxybarbiturate	Primidone
Iministilbene	Carbamazepine,oxcarbamazepine
Aliphatic carboxylic acid	Valproate sod, divalproex
Phenyltriazine	Lamotrigine
Cyclic GABA analogues	Gabapentin, pregabali
Newer drugs	Topiramate, zonisamide, vigabatrin tiagabine, lacosamide.

Phenytoin-

Mechanism of action

Although phenytoin first appeared in the literature in 1946, it has taken decades for the mechanism of action to be more specifically elucidated. Although several scientists were convinced that phenytoin altered sodium permeability, it wasn't until the 1980's that this phenomenon was linked to voltage-gated sodium channels.

Phenytoin is often described as a non-specific sodium channel blocker and targets almost all voltage-gated sodium channel subtypes. More specifically, phenytoin prevents seizures by inhibiting the positive feedback loop that results in neuronal propagation of high frequency action potentials.

List of plants showing antiepileptic activity-

1) Annona senegalensis
2) Citrus sinenis
3) Daniellia oliveri
4) Datura stramonium
5) Detarium microcarpum

In vivo models-

1) Electroshock in mice
2) Bicuculline test in rats
3) Epilepsy induced by focal lesions
4) Kindled rat seizure model
5) Posthypoxic myoclonus in rats

4. Diuretic or antidiuretic activity-

Diuretic/antidiuretic activity was determined as the ratio of urinary output of treated to control group; diuretic potency as ratio of urinary out of treated to reference group; urinary excretion as the ratio of total urinary output to total volume of liquid administered; saline excreted as the ratio of urine volume.

Classification-

Class	Drugs
1) antidiuretic hormone	(ADH, vasopressin), demopressin, lypressin, terlipressin
2) thiazide diuretics	Amiloride
3) miscellaneous	Indomethacin, cloropropamide, carbamazepine

Carbamazepine- Mechanism of action

Carbamazepine's mechanism of action is not fully elucidated and is widely debated.5 one major hypothesis is that carbamazepine inhibits sodium channel firing, treating seizure activity. Animal research studies have demonstrated that carbamazepine exerts its effects by lowering polysynaptic nerve response and inhibiting post-tetanic potentiation. In both cats and rats, carbamazepine was shown to decrease pain caused by infraorbital nerve stimulation. A decrease in the action potential in the nucleus ventralis of the thalamus in the brain and inhibition of the lingual mandibular reflex were observed in other studies after carbamazepine use. Carbamazepine causes the above effects by binding to voltage-dependent sodium channels and preventing action potentials, which normally lead to stimulatory effects on nerves.8, 15 in bipolar disorder, carbamazepine is thought to increase dopamine turnover and increase GABA transmission, treating manic and depressive symptoms.19

A common issue that has arisen is resistance to this drug in up to 30% of epileptic patients, which may occur to altered metabolism in patients with variant genotypes.13 A potential

therapeutic target to combat carbamazepine resistance has recently been identified as the EPHX1 gene promoter, potentially conferring resistance to carbamazepine through methylation.

Plant shows diuretic action-

Drugs affecting learning and memory-It should be noted that aside from alcohol, numerous drugs have been associated with cognitive memory deficits. Exposure to morphine, heroin, methamphetamine, MDMA (ecstasy), or chronic cocaine similarly produces hippocampus- dependent spatial memory impairments across a variety of tasks

In vivo models-

1) Diuretic activity in rats (LIPSCHITZ test)
2) Saluretic activity in rats
3) Diuretic and saluretic activity in dogs
4) Chronic renal failure in the rat
5) Chronic renal failure after subtotal (five-sixths) nephrectomy in rats

5. Immunomodulary activity-

A substance that stimulates or suppresses the immune system and may help the body fight cancer, infection, or other diseases. Specific immunomodulating agents, such as monoclonal antibodies, cytokines, and vaccines, affect specific parts of the immune system.

Classification- Immunostimulants-

1. Vaccines-poliomyelitis vaccine, rota virus vaccine
2. Adjuvants
3. Immunoglobulins
4. Miscellaneous agents used as stimulans- levamisole, thalidomide, Isoprinosine, immunocynin.

Immuno sup presents-

Class	**Drugs**
1) Calcineurin inhibitors	Cyclosporine, tacrolimus
2) m-TOR inhibitors	Sirolimus, everolimus

3) Antiproliferative drugs	Azathioprine, methotrexate, cyclophosphamide, clorambucil, mycophinolate, mofetil (MMF)
4) Glucocorticoids	Prednisolone

Prednisolone- Mechanism of action

The short term effects of corticosteroids are decreased vasodilation and permeability of capillaries, as well as decreased leukocyte migration to sites of inflammation.4 Corticosteroids binding to the glucocorticoid receptor mediates changes in gene expression that lead to multiple downstream effects over hours to days. Glucocorticoids inhibit neutrophil apoptosis and demargination; they inhibit phospholipase A2, which decreases the formation of arachidonic acid derivatives; they inhibit NF-Kappa B and other inflammatory transcription factors; they promote anti-inflammatory genes like interleukin- Lower doses of corticosteroids provide an anti- inflammatory effect, while higher doses are immunosuppressive.4 High doses of glucocorticoids for an extended period bind to the mineral corticoid receptor, raising sodium levels and decreasing potassium levels.

List of plants showing immunomodulatory activity-

Some of the plants with established immunomodulatory activity are Viscum album, Panax ginseng, Asparagus racemos, Tinospora cordifolia etc.

In vivo models-

1. Radiant heat method
2. HAFFNER's tail clip method
3. Hot plate method
4. Tail immersion test
5. Monkey shock titration test
6. Formalin test in rats
7. Experimental neuropathy

Eddy's hot plate- Consisting of heating surface with perpex enclosure with micro-controller based digital temperature indicator controller to set surface temp. Between 30°C to 80°C.piece.

MODULE NO 2: ANIMAL CELL CULTURE TECHNIQUES INTRODUCTION –

Cell culture technique was first developed in the early 20th century as a method of studying animal cell behaviour *in vitro*. The principle of cell culture was established when Roux, an embryologist used warm saline to maintain chicken embryo for several days, thereby, coming up with tissue culture principle. Cell culture has therefore, been defined as the is removal of animal cells and its propagation and cultivation *in vitro* in an artificial environment that is suitable for its growth. This usually begins with a primary culture aiming at achieving confluence, that is formation of monolayer of cell in a culture flask supplemented the required nutrients and growth factors. With achievement of confluence, the cells are then passaged or sub cultured from the primary to secondary and subsequence to tertiary, until a continuous cell line is established. The isolation of virus in a cell culture is labour-intensive, and consumes time. Many clinically important viruses are still either difficult to grow or don't grow at all in tissues culture while others may require a sophisticated culture system which may either not be suitable for diagnostic laboratory use or not available at all. These might reduce the impact of tissue culture in clinical diagnosis, thereby making it less attractive in diagnosing human diseases; while, some scientist found tissue culture as a relatively unbiased, whose limitation is only by the ability of the virus to grow on the selected cell lines. However, Vero E6 cells were considered as the most

permissive of all cell lines by providing a versatile medium for recovery of unknown pathogens, together with Electron Microscopy (EM) to the detection and classification of unknown agent.

Recent advances in metagenomics with deep sequencing techniques have made it possible to analyse the genome of microorganism without isolating the virus via cell culture. This is done via high-throughput sequencing using random amplified DNA product and comparison of sequences with available extensive bank of sequences for the final identification of the detected agent. This is possible because random primers can specifically amplify the template for sequencing without having a prior knowledge of the suspected agent. This technique is readily advancing in the aspect of pathogen discovery. It has been used forever to discover viruses such as Lioviu virus, Schmallenberg virus and Bas –Congo virus. In the cases of severely ill patients or infectious diseases outbreak, it is important to identify the causative agent of infection. As such this review is aimed at describing some of the events in which viruses are isolated for identifying the causative agent and recognition of emerging diseases, by additional laboratory diagnosis assay such as Electron Microscope (EM), serological and molecular techniques.

Equipments:

Laminar flow hood, incubator, inverted microscope, centrifuge, water bath, fridge and freezer, liquid nitrogen storage, hemocytometer, pipettes, cell culture vessels, consumables

Aseptic techniques

Bacterial infections, like Mycoplasma and fungal infections, commonly occur in cell culture creating a problem to identify and eliminate. Thus, all cell culture work is done in a sterile environment with proper aseptic techniques. Work should be done in laminar flow with the constant unidirectional flow of HEPA filtered air over the work area. All the material, solutions and the whole atmosphere should be of contamination-free

Sterilization-

Sterilization is the complete elimination or destruction of all forms of microbial life. The most obvious form of sterilization is incineration. However, incineration is not practical because there are many instruments in the medical field that must be recycled. Therefore, the second best option is use of the autoclave.

1. Autoclave-

The etymology of autoclave is auto (self) and clave (closing with a clanking sound).

To understand the autoclave, the concepts of vaporization pressure and boiling point should be comprehended first: If liquid is placed in a container in an enclosed space, it is evaporated and then returns, thus repeating condensation into water. Then, evaporation and condensation reach dynamic equilibrium. At this point, the pressure inside at which vapor is formed is the vapor pressure. When heat is applied to water, the temperature rises, the vapor pressure rises, and then the vapor pressure becomes equal to the atmospheric pressure. At that moment, the water surface evaporates, and vaporization occurs inside the water. The temperature at this moment is called the boiling point, at which the atmospheric pressure and the vapor pressure of the liquid are the same.

At normal atmospheric pressure (760 mmHg), boiling temperature is 100°C. However, the temperature must exceed 100°C to kill endospores. To overcome this, the boiling point should be increased by artificially increasing the pressure. This is the principle of the autoclave. By increasing the pressure, the autoclave reaches a boiling point of 100°C or higher (121°C) and kills endospores. Geobacillus stearothermophilus is used as an indicator to confirm whether sterilization has successfully occurred. The autoclave is mainly used for glass, surgical instruments, and pre-treatment of wastes. Of course, it cannot be used for heat-labile instruments (e.g., plastic, rubber, etc.), and it other methods are performed for low temperature sterilization (e.g., ethylene oxide, hydrogen peroxide vapour or plasma).

2. Ethylene oxide-

Ethylene oxide is cyclic ether, with a three-membered ring, like a snowman. As it is formed in an equilateral triangle shape around the oxygen, the two opposing sides of the triangle strongly pull against each other. Therefore, when provided an opportunity to reacts, it reacts more strongly than the other ethers. Ethylene oxide is used for sterilization of critical items such as plastics, which cannot withstand high temperatures.

Owing to its nature as a gas, ethylene oxide penetrates well into the cell, reaching the DNA of the microorganism and killing it by alkylation. It should be carefully handled because may explode easily, and it should usually be maintained frozen. In terms of disadvantages, ethylene oxide can be harmful to the human body, and adequate time should be allowed for its function (6–12 hours). It is also a pollutant to the ecosystem

3. **Hydrogen peroxide-**

Plasma is the fourth phase of matter, comprising substance that are not in the water, solid, or gas state. It is produced by the application of microwave energy to hydrogen peroxide gas molecules. Hydrogen peroxide plasma contains numerous anions, cations, and hydroxyl and hydroperoxyl radicals. It penetrates the instruments well and sterilizes them. Unlike ethylene oxide, it can complete sterilization within a short time (about 50 minutes) and does not leave any toxic residue. However, it is expensive.

Culture media-

The influence of cell culture technology on human society has been immeasurable. Progress in biology in recent years, for example, has depended heavily on cell culture technology.[1] In addition, cell culture-based practical technologies have been developed in various areas, including the assessment of the efficacy and toxicity of new drugs, manufacture of vaccines and biopharmaceuticals, and assisted reproductive technology. As the reprogramming of somatic cells became technically feasible recently, researchers around the world are fiercely competing for leadership in the advances of regenerative medicine. In this area likewise, cell culture technology is regarded as a foundation for further development and popularization.

No one probably would argue against the claim that a culture medium is the most important factor in cell culture technology. A medium supports cell survival and proliferation, as well as cellular functions, meaning that the quality of the medium directly affects the research results, the biopharmaceutical production rate, and treatment outcomes of assisted reproductive technology. It is essential, therefore, for investigators who are working with cell cultures to select an appropriate medium that is suitable for their aims. In some cases, researchers should modify a medium themselves. In addition, when facing problems, researchers have to know the properties of the medium in order to identify the cause of any problem with their experiments.

At present, synthetic media can be classified into several groups, based on the type of supplements added; for example, serum-containing media, serum-free media, protein-free media, and chemically defined media Serum-containing media naturally contain various serum-derived substances, which make the medium composition unclear and whose concentrations can fluctuate from batch to batch. This situation makes the culture results less reproducible and poses a risk of microbial contamination. Serum-containing media, however, can be designed easily and be used effectively for a variety of cell types because serum includes a lot of active substances that are necessary for the survival and growth of animal cells.[2] Serum-free media, in contrast, have a defined composition, resulting in a high reproducibility of results, and the cultivation process can be validated. In addition, target cells can be grown selectively in an intermingled cell population if the culture conditions are configured to benefit them. Among the serum-free media, subgroups of protein-free media (which do not contain any protein at all) and chemically defined media (which do not contain any undefined ingredient) provide additional stability and reproducibility for culture systems, facilitating the identification of the cellular secretions and reducing the risk 1of microbial contamination. However, the serum-free media are difficult to design: only specific cell types have been cultivated this way to date.

Serum free media –Insulin was discovered earlier by Frederick Banting and Charles Best (1921), but full-scale research into this peptide as a supplement for culture media began in the 1960s. Initially, the effectiveness of insulin alone was found to be inferior to that of serum, but the use of insulin in combination with low-concentration serum yielded a higher level of efficacy of baby hamster kidney (BHK)21 cell growth. This finding led researchers to conclude that insulin acts in a coordinated manner with serum components. Growth factors were discovered one after another during this era: nerve growth factor, epidermal growth factor, insulin-like growth factor, fibroblast growth factor (FGF), platelet-derived growth factor, and transforming growth factor (TGF). The addition of these growth factors to a culture medium increased cellular proliferation. Nevertheless, their effect on cell proliferation, as with insulin, was found to be almost always inferior to the effect of serum.

Contamination- Foreign substances from unidentified sources can contaminate a culture medium, thus possibly affecting the empirical results. Typically, those contaminants include viruses, bacteria, mycoplasma, and endotoxins. There are, however, other types of contaminants, like plasticizers that might be eluted from plastic instruments· Or trace elements, even in water. These substances also can affect the cells in culture. It also was reported that some toxic substances are eluted from the microfilters that are used for sterilization. Some of this contamination seems to be even inevitable, but care must be taken to minimize it in order to make culture experiments reliable and highly reproducible. Thus, researchers may consider practicing the sterile technique strictly and selecting culture instruments carefully. Washing the instruments with the culture medium immediately before use is recommended in some special cases.

Cryopreservative

It is the storage of biological material at low temperatures. Since ancient times, it has been known that biological material can be preserved longer at low temperatures. Indeed, archaeological findings indicate that as early as 2000 BC, icehouses were used throughout Mesopotamia to store foods [1]. The preservative effect of cold was also a topic of interest to the early experimentalists of the seventeenth century, most notably Boyle [2], who commented on the ability of ice to preserve human bodies and made several attempts to freeze and revive live animals, discovering species of frogs and fish that could survive encasement in ice.

Whereas Boyle could only speculate on the nature of cold, the intervening centuries have revealed its preservative effect lies in depriving biological systems of the thermal energy required for normal molecular motion and metabolism, in turn slowing cellular processes and decomposition. The ability to reliably generate the extremely low temperatures required for long- term preservation, typically below − 100 °C, came with the development of cryogenic technologies at the turn of the twentieth century. The modern cryopreservation of living systems, in the sense of biotechnology, can be traced to the discovery of the first effective cryoprotective agents (CPAs), otherwise known as “cryoprotectants”, in the 1940s [3]. Notably, Lovelock provided crucial early insights into the origins of cryoinjury and the action of CPAs [4, 5]. Later work by Mazur pioneered the application of quantitative models to describing cell changes during cooling [6] and paved the way towards theoretical approaches to studying cryopreservation.

The discipline of cryopreservation is now well established as a practical means of storing living cells and tissues and has grown to find applications throughout biology and medicine. As this review will highlight, cryopreservation now holds the potential to strongly benefit several areas of medicine by increasing the ease with which therapeutic cells, tissues, and organs can be stored.

Animal cell culture – A. Primary cell culture

This is the cell culture obtained straight from the cells of a host tissue. The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture. Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents. Depending on their origin, primary cells grow either as an adherent monolayer or in a suspension.

Adherent cells-These cells are anchorage dependent and propagate as a monolayer. These cells need to be attached to a solid or semi-solid substrate for proliferation. These adhere to the culture vessel with the use of an extracellular matrix which is generally derived from tissues of organs that are immobile and embedded in a network of connective tissue. Fibroblasts and epithelial cells are of such types.When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, these are known as monolayer cultures. Majority of continuous call lines grow as monolayers. As being single layers, such cells can be transferred directly to a cover slip to examine under a microscope.

Suspension cells-Suspension cells do not attach to the surface of the culture vessels. These cells are also called anchorage independent or non-adherent cells which can be grown floating in the culture medium. Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in suspension. These cells grow much faster which do not require the frequent replacement of the medium and can be easily maintained. These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly these cultures have short lag period.

B. Secondary cell culture and cell line-When a primary culture is sub-cultured, it is known as secondary culture or cell line or sub-clone. The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).Sub-culturing of primary cells to different divisions leads to the generation of cell lines. During the passage, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population. However, as they are sub-cultured serially, they become different from the original cell.

Cytotoxicity The toxic chemicals in the culture medium affect the basic functions of cells. The cytotoxicity effect can lead to the death of the cells or alterations in their metabolism. Methods to access viable cell number and cell proliferation rapidly and accurately is the important requirement in many experimental situations that involve in vitro and in vivo studies. The cell number determination can be useful for determining the growth factor activity, concentration of toxic compound, drug screening, and duration of exposure, change in colony size, carcinogenic effects of chemical compounds, and effects of solvents

Organ culture Whole organs from embryos or partial adult organs are used to initiate organ culture in vitro. These cells in the organ culture maintain their differentiated character, their functional activity, and also retain their in vivo architecture. They do not grow rapidly, and cell proliferation is limited to the periphery of the explants. As these cultures cannot be propagated for long periods, a fresh explanation is required for every experiment that leads to inter experimental variation in terms of reproducibility and homogeneity. Organ culture is useful for studying functional properties of cells (production of hormones) and for examining the effects of external agents (such as drugs and other micro or macro molecules) and products on other organs that are anatomically placed apart in vivo.

Module 3-Animal cell culture techniques:

Bladder cancer is any of several types of *cancer* arising from the *tissues* of the *urinary bladder*. Symptoms include *blood in the urine*, *pain with urination*, and low back pain. It is caused when epithelial cells that line the bladder become malignant.

Risk factors for bladder cancer include *smoking*, family history, prior *radiation therapy*, frequent *bladder infections*, and exposure to certain chemicals. The most common type is carcinoma. Other types include *squamous cell carcinoma* and *adenocarcinoma*. Diagnosis is typically by *cystoscopy* with *tissue biopsies*. Staging of the cancer is determined by *transurethral resection* and *medical imaging*.

HTB-4

Product category	Human cells
Organism	Homo sapiens, human
Morphology	Epithelial
Tissue	Urinary bladder
Disease	Transitional Cell Carcinoma
Applications	3D cell culture; Cancer research
Product format	Frozen

Outcomes:

After the completion of report on DRUG EVALUATION METHOD I understood

1. Patient safety of drug is improved.

2. Medication misadventure including adverse drug events are avoided.
3. We reduced variation standardize therapy
4. Therapy is optimsed

Conclusion-

1. Basic pharmacological knowledge can be applied for easier understanding
2. By using different pre-clinical methods we can discover the drug