DEVELOPMENT OF SIMULATED EXPERIMENTAL DEVICES TO MEET LEARNING NEEDS OF EXPERIMENTAL PHARMACOLOGY.

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ARTICLE INFO	ABSTRACT
Article history Received 30/03/2017 Available online 30/04/2017 **Keywords** Mydriatics, Calmodulin, Microcontroller, Sensor Box.	The limitation of use of animals in Pharmacology practical classes lead to develop innovative simulated experiments to understand the pharmacological actions produced by various drugs. Laboratory based practical classes, have been the corner stone of undergraduate Pharmacology learning. Ethical issues with the use of animals and rapid development of information technology has led to newer trends in teaching and learning such as computer assisted learning and other methods.

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INTRODUCTION

Pharmacology is the branch of biomedicine concerned with the study of drug action [2] or physiological effect on the cell, tissue, organ, or organism. More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function[2].The effect of drug on the subject which maybe cells, tissues is known as pharmacokinetics, like absorption, distribution, metabolism and excretion. Similarly the effect of the counter reaction of subject towards the drug is known as pharmacodynamics.

Practical knowledge is an important part of pharmacology curriculum of various undergraduate courses of pharmacy, nursing, science and medicine. In vitro and in vivo animal experiments have been widely used to help students gain hands-on experience of pharmacological experiments, and also to reinforce their knowledge learned from theory and lectures [1]. Traditional live animal experiments are invaluable, they do have shortcomings, and their cost effectiveness has been questioned. Apart from being time consuming, animal experiments can only test a limited number of drugs and on limited lab animals at a given period of time. Furthermore, animal experiments, in particular whole animal studies are often labor-intensive [1] .In the recent years, the undergraduate program in pharmacy has been turned around with the adoption of computer assisted learning (CAL), use of audio-visual aids, clinical and community pharmacology studies [3].The usage of lab animals is restricted and limited which is a profound loss of practical knowledge to students. Though the current simulating software available teaches various pharmacokinetic and pharmacodynamics factors but, they lack the realistic effect which is seen in live animal experiments.

Witnessing and experiencing the above facts, we have created software and various working models of tissues and organs; required drug is analyzed and its activity is expressed in the respective tissues. This software also shows required information about the pharmacokinetics and pharmacodynamics on the connected device. In this simulating model the realistic effect of drug action is preserved. Currently, simulated models of heart, eye, brain and smooth muscle are functioning with very smooth self-designed software.
This uprising simulated model is very much helpful for school, pre-universities, and undergraduate courses.

Theory involved in the Mydriasis[4]-

The simulated experimental models are developed to understand the mechanisms of mydriatics and miotics. The mydriatics are the drugs that induce dilatation of the pupil. The stimulation of sympathetic nerves or administration of atropine and atropine like drugs causes mydriasis. Normally in dim light mydriasis takes place to increase the entry of light into the lens. The adrenaline acts by stimulating $\alpha 1$ adrenoreceptor (G-protein coupled receptor; Gq type) located on the radial muscles of the iris. The α GTP binds to membrane bound phospholipase C (PLC) and stimulate it. The stimulated PLC converts PIP_2 into IP_3 (inositol triphosphate) and DAG (diacylglycerol) which are second messengers. The IP_3 is water soluble, and diffuses into cytosol and binds to the second messenger gated calcium channel located on the membrane of sarcoplasmic reticulum (SR). This opens the channel and calcium concentration in the cytoplasm increases. The DAG is lipid soluble and retains in the membrane and it causes the opening of calcium channel present on the membrane. This causes influx of calcium concentration into the cytoplasm. The calcium binds to calmodulin protein to form calcium-calmodulin complex. This complex activates the myosin light chain kinase (MLCK). The activated MLCK bring about phosphorylation of myosin light chain (MLC) to MLC-P. This causes contraction of radial muscle and mydriasis takes place.

Theory involved in the Miosis[4] –

The miotics are the drugs that produce constriction of pupil. The stimulation of parasympathetic nerves or administration of cholinergic drugs causes miosis. Normally in bright light, miosis takes place to restrict the entry of light into the lens. Acetylcholine combines with muscarinic (M3) receptor present on the endothelial cells. Through activation of PLC, converts PIP2 to IP_3 (Inositol triphosphate) and DAG (Diacylglycerol).IP_3 triggers the Calcium (Ca^{2+}) within the cytoplasm. The Ca^{2+} combines with calmodulin (CaM). The resultant Ca^{2+} -CaM complex stimulates the endothelial nitric oxide synthase (eNOS). The activated eNOS catalyzes the formation of NO (nitric oxide) from L Arginine. The NO diffuses to the subjacent vascular smooth muscle cells, where it activates guanylylcyclase. The activated guanylylcyclase catalyses the conversion of GTP to cGMP, which dephosphorylates Myosin-Light Chain (MLC). This prevents actin-myosin interaction. This leads to the relaxation of smooth muscle. The NO also acts on K^+ channel to cause relaxation of the smooth muscle.

Simulated device to study Miotic and Mydriatic activity-
Materials required-

Iris diaphragm valve, a plastic sphere, servo motor, a microcontroller (arduino), photo sensor, vials and power source.

Methodology-
Setup-

Iris diaphragm is placed on a servo motor in such a way that the propeller coincides with the diaphragm controller of the iris diaphragm; this setup is enclosed in a hemisphere (preferred white color, resembling the cornea). A color sensor is placed in box with opening for the glass vial. Sensor and the servo motor are connected to the microcontroller. The microcontroller is in turn connected with a personal computer for better interface.

Working-

Adrenergic or cholinergic drugs with their respective color are placed in a glass vial in the opening of the sensor box. A command from the computer is given to analyze the drug. This activates the light source on the sensor. The sensor then sends an array of red, green and blue waves and interprets the reading received from it. The readings checked with the stored values and the respective drug is determined. Important information about the drug, its action, kinetics and dynamics are displayed on the computer screen and commands are sent to the micro controller to change servo motor position. The servo motor has the ability to move to precisely. If the eye in normal state is said to be at 45° position of the servo motor then when an adrenergic drug is introduced, the iris valve have to move to cause dilation. This is enacted by the motor by moving to 90°, thereby causing opening of iris diaphragm to its maximum size thus depicting the dilation. Similarly when a cholinergic drug is analyzed, the servo motor moves to near 0° position, displaying the constricting action of the pupil.

Theory involved in Epilepsy[4]**:**

Epilepsy is a condition in which a person has recurrent seizures. Epileptic seizures are sudden, transient episodes of loss or disturbed consciousness +there is an abnormality of EEG. In epilepsy there are neurological disturbances resulting from synchronized firing of neurons. When inhibitory mechanism fails (e.g.GABA), a focus maybe formed which may or may not spread. Mutations in several genes have been linked to some types of epilepsy. One speculated mechanism for some forms of inherited epilepsy are mutation of the gene which code for sodium channel proteins; these defective sodium channels stay open for too long thus making the neuron hyper-excitable. Glutamate, an excitatory neurotransmitter, may thereby be released from these neurons in large amounts which act by binding with nearby glumtamanergic neurons triggers excessive Ca^{2+} release in these post-synaptic cells. Such excessive calcium release can be neurotoxic to the affected cell. Another possible mechanism involves mutations leading to ineffective GABA action.

Simulated device to study antiepileptic activity-
Materials required –
Led, silicone or plastic hollow brain mold,a microcontroller (arduino), photo sensor, vials and power source.

Methodology-
Setup-

The required amount of led bulbs are attached to the brain model internally, the wires are connected to the microcontroller via a bread board. A color sensor diode is placed in such a way that the contents of the vial can be scanned. The analyzer and microcontroller are connected to personal computer for better interface.

Working-

The required colored drug in vial is placed for analysis. A command from the computer is given to analyze the drug. This activates the light source on the sensor. The sensor then sends an array of red, green and blue waves and interprets the reading received from it. The readings checked with the stored values and the respective drug is determined. Important information about the drug, its action, kinetics and dynamics are displayed on the computer screen and commands are sent to the micro controller to change the intensity of the flickering light of the LEDs based on the scanned drug. for example if the identifies drug is an epileptogenic, then the intensity and the rate of flickering of LEDs increase , illustrating epilepsy ; similarly if the drug identifies as an anti-epileptic, the intensity of the brightness decrease showing the treatment and effect of the antiepileptic.

Theory involved in Heart contraction[4] –

The simulated experimental models are developed to understand the mechanisms of chronotrophy and inotrophy. The adrenergics are the drugs that produce increase in heart contraction and increase in rate of heart beat (i.e. positive chronotrophy and positive inotrophy). Normally in fight or flight response, increase in heart contraction and heart rate is observed. The adrenaline acts by stimulating $\alpha 1$ and β1 adrenoreceptor (G-protein coupled receptor; Gq type and Gs type respectively) located on the myocardium and sino-atrial node (SAN). The α GTP binds to membrane bound phospholipase C (PLC) and stimulate it. The stimulated PLC converts PIP_2 into IP_3 (inositol triphosphate) and DAG (diacylglycerol) which are second messengers. The IP_3 is water soluble, and diffuses into cytosol and binds to the second messenger gated calcium channel located on the membrane of sarcoplasmic reticulum (SR). This opens the channel and calcium concentration in the cytoplasm increases. The DAG is lipid soluble and retains in the membrane and it causes the opening of calcium channel present on the membrane. This causes influx of calcium concentration into the cytoplasm. The calcium binds to troponin; this leads interaction of actin-myosin. This causes contraction.

Simulated device to study heart contractions-
Materials required-
Silicon model of heart, fibers, two servo motors, a microcontroller (arduino), photo sensor, vials and power source.

Methodology-
Setup-

The auricles and ventricles are separately connected to a servo motor by means of fibers. A color sensor is placed in box with an opening for the glass vial. Sensor and the servo motor are connected to a microcontroller. The microcontroller is in turn connected with a personal computer.

Working-

Adrenergic or cholinergic drugs with their respective color are placed in a glass vial in the opening of the sensor box. A command from the computer is given to analyze the drug. This activates the light source on the sensor. The sensor then sends an array of red, green and blue waves and interprets the reading received from it. The readings checked with the stored values and the respective drug is determined. Important information about the drug, its action, kinetics and dynamics are displayed on the computer screen and commands are sent to the micro controller to change servo motor position. When no drug is administered, the heart beats at 72 beats per minute with normal contraction. This can be modulated by speed of movement of servo motor and the extent to which it rotates or pulls the fibers and releases them. When an adrenergic is administered, a command is given to micro-controller. This in turn sends command to servo motor and increases speed along with slight increase in pulling of fibers. When a cholinergic drug is administered, command is given to decrease the speed of servo motor and also the force of pulling is reduced thereby depicting decreased heart contraction and heart rate (i.e. negative chronotrophy and inotrophy).
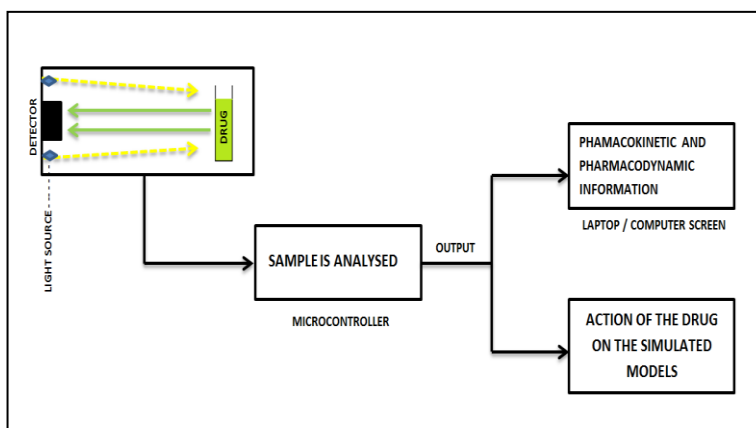

Fig 1 - Flowchart on working of simulated device.

Once students understand the concepts, a test mode is available to check their understanding. In test mode, visual changes are made on normal working of heart showing improper functioning of heart (i.e. arrhythmia, tachycardia, bradycardia, etc.) along with this; key diagnostic values are displayed on the screen. Now a student has to select appropriate drug with its dosage to save the patient. If student chooses the correct option, heart begins to work in normal state otherwise heart stops beating.

CONCLUSION

This approach of using simulated models for experimental purpose in pharmacology is not only ethical , but also gives a similar perception of that of traditional experimentation on live animals. Our approach provides better understanding of pharmacokinetic and pharmacodynamics aspects of a drug by visual experience and has an interactive approach. Our Vision is to provide a good alternative to animal experiments aligned to the curriculum of medical, pharmacy and pre university colleges, ensuring homogenous learning experience in a cost effective manner with round the clock availability.

Abbreviations

CAL   – Computer Assisted Learning
PLC   – Phospholipase C
PIP_2 – Phosphatidylinositol biphosphate
IP_3 – Ionisitol TriPhosphate
DAG   – Diacyl Glycerol
MLCK   – Myosin Light Chain Kinase
CaM   – Calmudulin
GTP   – Guanosine Triphosphate
cGMP   – cyclic Guanosine Triphosphate

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