



ORIGINAL RESEARCH PAPER

Pharmacology

Animal Life Saver Technique To Assess Skill Of 2:2 Four Point Assay Of Histamine Using Animal Experiment Software On Guinea Pig Ileum.

KEY WORDS: bioassay, histamine, ileum, software

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ABSTRACT

Bioassay experiment on live tissue needs animal sacrifice and dissection. As per communication from government, animal dissection should be minimized. During examination animal experiment software use combines with OSPE and mounting assessment. In our method we have searched for the same. We calculated heights of contraction of two known strength solutions S1, S2 and two unknown strength solutions T1, T2. The values were kept in standard formula to calculate relative potency. Using assumption of 10mcg/ml strength of standard, further calculations were done to find out the strength of unknown test solution. In our findings the unknown strength solution was 1.2 times stronger that denotes unknown strength solution to be 20% stronger, further calculation to rectify was done wherein the actual difference between S1 and T1 was 7% ,this was deducted from 20% there by giving corrected strength of unknown to be 11.3 i.e. 13% stronger than that of assumed standard.

Introduction

Bioassay serves as research tool being the quantitative part of screening procedure¹

Subsequent to this the efforts at different centers are being taken to find alternative technique/method to fulfill the criteria of performing bioassay in MD examination under MUHS Nasik, wherein bioassay of acetylcholine and histamine on isolated chick/cock ileum have been demonstrated and recommended as alternative methods.^{2,3}

⁴ point bioassay is an important exercise in final MD Pharmacology examination. Earlier before the instructions by authorities of family welfare ministry, the animals like guinea pig, frog, rabbit, and rat were being sacrifice to get one inch piece of required tissue on which bioassay experiment was conducted. In recent past years animal dissection and sacrifice are to be avoided, particularly for the experiments of least or no benefit to mankind. Bioassay experiment has its value mainly in MD Pharmacology examinations. Mulkalwar S, Rane B, Behera L in 2014 have reported that out of 30 participants, 29 (96.6%) did not use their knowledge of bioassay during their 10 years of post MD career, whether in pharmaceutical industry or in academics. Only 5 of them (16.6%) feel that experiment on bioassay should be continued in the current state. 76.7% of them wish it to be modified to a dose response curve (DRC). 6.71% feel that it should be totally scrapped.(4). As it is shown in a survey that 96% respondents felt that Pharmacology curriculum emphasizes on becoming good undergraduate teachers, and 83% felt that it gives inadequate practical training in research methodology.⁵

In the current situation it has been advised in a letter addressed to directors/principals/deans of medical colleges and pharmacy colleges from the undersecretary in the Ministry of Health and Family Welfare with a subject heading "guidelines for discontinuation of dissection and animal experimentation and introduction of use of alternatives to animal experimentation." Subsequently university grants commission (UGC) has formulated and circulated letter to Colleges teaching zoology and other life sciences.⁶

In the present scenario it appears that a new method is deemed necessary to save the life of Animal, to fulfill the objective of bioassay experimentation during MD pharmacology exam and also to test the basic skill required for bioassay in examinees. In case we do not want to dissect or sacrifice the animal some other methods are to be adopted. Oral examination is for assessing knowledge, other methods like OSPE, directly observed methods

etc., should be used⁷.

A letter from health education beuro [HEB/DGF/ 2016/914 Date: 4/1/2016] indicates that the undergraduate and postgraduate level animal experiments are to be done on computer using computer assisted learning software

We have developed a new innovative method to test the skill of bioassay using animal software⁸. Using combination of OSPE with evaluation of mounting skill followed by evaluation of bioassay skill using software. In this technique we focused on interpolation technique of bioassay. 4 point assay is expected method in MD examination Pharmacology In this article we are reporting the possibility of 4 point bioassay using animal experimental software.

Material Methods:

We have designed the method which is combination of Objective Structured Practical Examination OSPE, mounting and software. Keeping these two stages same in reference 8 whereas stage 3 is different for four point assay There is no need to sacrifice innocent animal. The objective is to fulfill the criteria of long exercise bioassay for the MD examination as mentioned MUHS syllabus.⁹

OSPE for (evaluation of dissection)

Stage 1

Incorporates use of objective structured practical examination (OSPE) using paper pencil technique. There will be six stations i.e. station 1 to 6. Each station will be containing questions regarding sequential steps of dissection of animal. The check list containing correct answer and marks thereon will separately be prepared for purpose of use by examiner. Each question will be carrying 2-6 marks and total assigned marks are 40. The candidates appearing in the exam will be provided the worksheet individually and will be asked to visit each station to write answer of questions. Their answers on the worksheet will be evaluated by the team of examiners and the scores will be recorded for stage 1 of each candidate separately.

Evaluation of mounting skill

Stage 2

The candidates who completed the work in stage 1 will be subjected to tissue mounting exercise. The simulation of 1 inch piece of ileum will be provided to them and they will be asked to mount it in the assembly using pre- requisites. The questions will be asked by examiner on different aspects about assembly,

prerequisites, precautions and other relevant aspects and will be awarded the marks of stage 2 out of 30.

Evaluation of bioassay skill using software

Stage 3

Graded dose response using the software of bioassay of histamine on guinea pig ileum is first depicted. 2 doses of the standard in mcg on the basis of second showing near double contraction height were selected on log of 4. These were labeled as S1 and S2 respectively. Subsequently in continuity unknown strength solution doses in ml were used in matching bracketing method of bioassay. 2 doses of unknown strength solution which show near double response were labeled as T1 and T2 with log of 4. As it is computerized software generated experimental graph the point of slight variation of contractile response seen in actual tissue is not possible. Therefore 16 responses in Latin square were not taken.

The values of parameters like S1 S2 T1 T2 and there contractile height in cm were calculated and put in the formula. Calculation for M i.e. relative potency and calculation for x i.e. strength of unknown concentration which is stronger than that of standard were obtained, assuming the strength of standard use as 10microgram/ml.

Result and Observation

Figure1 standard

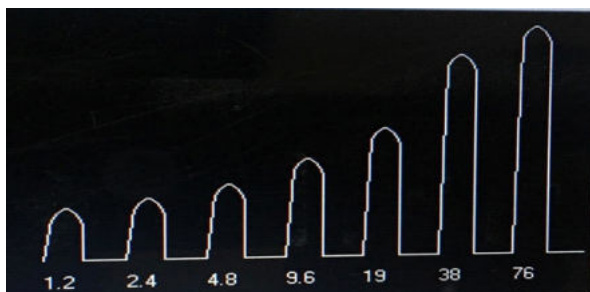
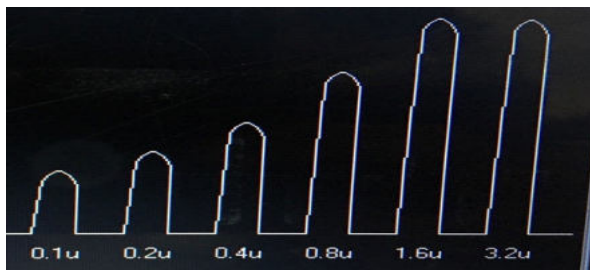


Figure 2 Unknown

s1= 9.6 mcg s2= 38mcg
 t1= 0.4ml t2= 1.6 ml
 S1= 3.5 cm S2= 7.25cm
 T1= 3.75cm
 T2= 7.75cm

Height of contraction measured using software measuring scale.
 $M = (S2-T2) + (S1-T1) / (S2-S1) + (T2-T1) \times \log (s2/s1)$
 $= (7.25 - 7.75) + (3.5 - 3.75) / (7.25 - 3.5) + (7.75 - 3.75) \times \log (38/9.6)$
 $= (-0.5) + (-0.25) / (3.75 + 4.00) \times \log 3.95$
 $= (-0.75) / 7.75 \times 0.5966$
 $M = (-0.05)$
 $x = s1 \times \text{antilog } M / t1$
 $x = 9.6 \times (-0.05) / 0.4$
 $x = 24 \times (-0.05)$
 $x = (-1.2)$

Unknown is 1.2 times stronger than that of standard Minus (-) sign in the value of 'x' so arrived denotes that unknown is stronger than that of standard

Now based on assumption standard was of 10 mcg/cc accordingly x value will be converted to 12 mcg/cc i.e. by calculation unknown is 20% stronger than that of standard. Wherein the difference in the height of contraction of S1 and T1 is 7% in favor of T1 similarly difference between s2nad t2 is 6.8% i.e. approximately 7%

Now the corrected strength of unknown will be (20%-7%= 13%) 11.3 mcg/ml

However it has been mentioned in some books that the percentage error should not be more than 10% in bioassay.

Limitation:

Error margin is > 10% (actual 13%) 75% — 25% response to be taken in ideal situation where as in this study s2, t2 dose response > 75% log of 4 is used (instead of log 2) for the purpose of gaining double contractile height with larger dose standard strength solution was assumed to be of 10mcg/ml

Discussion:

4 point assay carried out in this manner not only saves life of animal but also saves much of the time to conduct the experiment to do the calculations there is no need of taking 16 responses following the cycle of 3 min each time. These entire doses do not produce minor variations in the responses since it is computer simulation experiment. Similarly there is no need for calculation of averages of 4 responses each of s1 s2 t1 and t2. Using the sophisticated software's there will be no need of assuming concentration of standard, and there will be possibility of taking 16 responses in Latin square design, if situation demands. The ultimate aim of testing the skill of bioassay by 2:2 four point method can very well be served in this manner using 3 stage technique which includes OSPE, mounting skill testing and animal experiment software use as mentioned above.

However the scientists may advocate use of animals for research to facilitate new discoveries, although animal handling and use is not uniformly possible in different laboratories and institutions.¹⁰

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