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Standardization of a Newly Fabricated Multicompartment Model to Evaluate Analgesic, Antipyretic and Anti-inflammatory Action of a Drug

Jacky Dumbwani[*1], Neetesh K. Jain[1]

[1]Oriental College of Pharmacy & Research, Oriental University, Jakhya, Indore, Madhya Pradesh – 453555

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ABSTRACT

In the research work a design of a multiple compartment model was successfully fabricated. The purpose of present study is to standardize the model by comparing the results obtained from the traditional and standard model for analgesic, antipyretic and anti-inflammatory activity with the results obtained from the newly fabricated multicompartment model. Many drugs have more than one action and evaluation of one action of a drug requires one whole screening model whereas for the evaluation of other action of same drug will require one more different screening model. In this study a new model has been fabricated for the evaluation of three different actions of one drug. Aspirin has analgesic, antipyretic, anti-inflammatory, and antiplatelet activity. Evaluation of all the activities of aspirin, we will require four different methods and models but in our study the newly fabricated model alone will be able to evaluate three actions of a single drug. A detailed analysis of data was performed by applying statistics on the data collected from both the ways.

Keywords: *Standardization, Model fabrication, validation, new screening methods, statistics, aspirin, multiple actions, new model design, model.*

1. INTRODUCTION

Despite large investments in drug development, the overall success rate of drugs during clinical development remain slow. One prominent explanation is flawed preclinical research, in which the use and outcome of animal models is pivotal to bridge the translational gap to the clinic. Therefore, the selection of a validated and predictive animal model is essential to address the clinical question (Tinneke Denayer 2014).

Hyphenated techniques combine chromatographic and spectral methods to exploit the advantages of both. A couple of decades ago, Hirschfeld introduced the term “hyphenation” to refer to the on-line combination of a separation technique and one or more spectroscopic detection techniques (Wilson and Brickman 2003). In pharmacological screening methods we can also utilize the concept of hyphenation by combining three models in one model.

It is required to reduce the use of animals during experimentation using the validated animal models. Animals are used in significant numbers for these purposes. In the UK alone, in 2012, drug testing involved the use of more than 277,000 animals (Jarrod Bailey 2014). The sign of inflammation (including pain and fever) usually occurs when any infectious agent or foreign particle enters into human body then human body triggers the immunological response. In such situation the COX (cyclooxygenase) enzymes activates which act on the arachidonic acid and produce prostaglandin which increase the normal temperature of the human body then that infectious agent which enters into our body cannot resist the higher temperature and does not survive into our body but sometimes the temperature becomes too high then we use the antipyretic agents to reduce the temperature of human body (Aronoff and Neilson 2001)

In the present investigation, we have attempted to standardize the use of a newly fabricated model, which employs three compartments in one model for estimation of analgesic, antipyretic and anti-inflammatory action of a single drug. We have taken special care to ensure the validity of one experiment with estimation of three actions of a drug which was one of the major challenges while using this model. For standardization, we have used the comparison of data obtained from the newly fabricated model and from three different traditional models for evaluation of analgesic, antipyretic and anti-inflammatory activity

2. MATERIAL AND METHODS

Chemicals and reagents

The present study determined the antipyretic, analgesic and anti-inflammatory activity of the aspirin and it was purchased as ecosprin 75 from Dava bazaar, Indore, India. All other chemicals used were of analytical grade. Fresh distilled water was used throughout the experiments.

Experimental animals

Male Albino Wistar rats (150-200 g) born and reared in the animal house of Institute of Animal Health and Veterinary Biologicals Rasalpura, Mhow M.P (735/GO/RBi/S/03/CPCSEA) were used for the study. Animals were kept acclimatized to laboratory conditions one week before starting the experiment; they were given free access to water and standard rat diet (Amrut gaw ras pak Trimurti industries Hardia, Maharashtra, India) except during experimentation.

The newly fabricated multicompartment model

The design of the model to be standardize and used for the present study is depicted in Fig. 1

The model is consisting of three compartments: Compartment A (Anti-pyretic Compartment) – It consist of wooden walls with thermometer observation space for observing the temperature of animal.

Compartment B (Analgesic Compartment) – It consists of wooden walls with pointed pins at the bottom for observing analgesic effect of drug.

Compartment C (Anti-inflammatory Compartment) - It consists of wooden walls with a steel slab at the bottom whose temperature is raised with the use of candle for observing anti-inflammatory effect of drug.

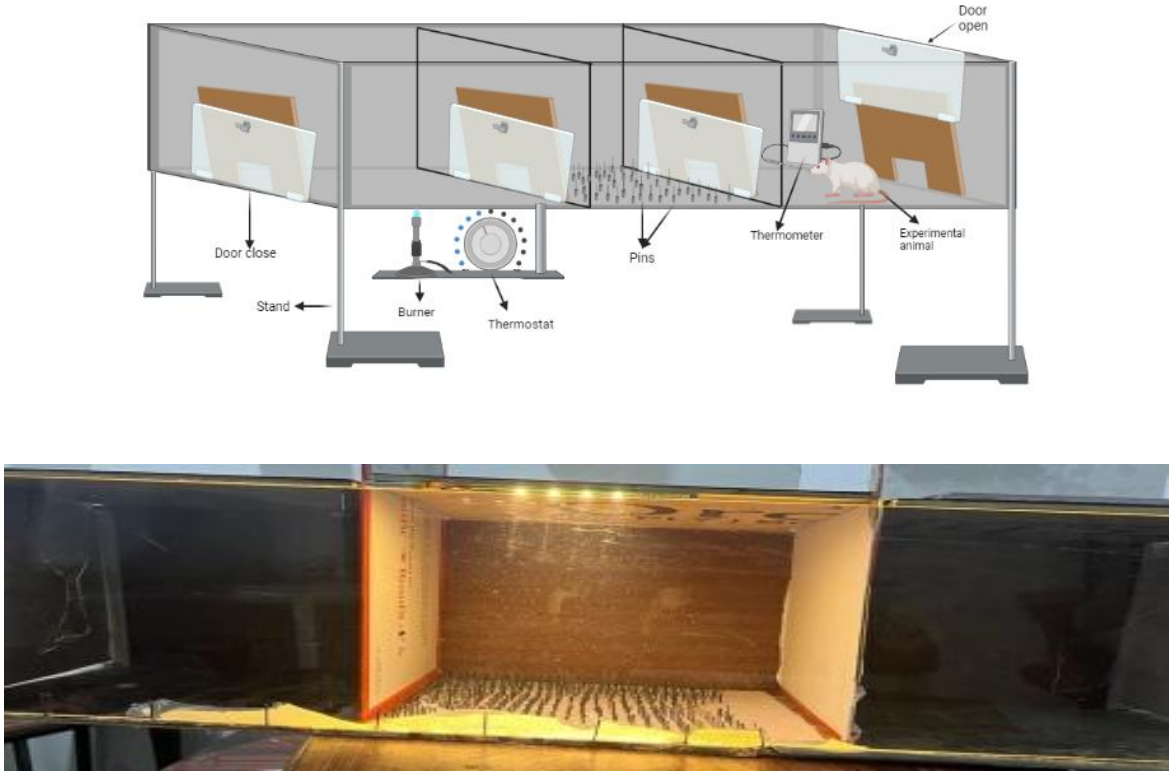

Fig. 1 (Design and a view of multicompartment model)

3. METHODS

Observation of antipyretic activity

For determination of antipyretic activity the experimental animal was allowed to enter in the antipyretic compartment of the model. Before entering to the compartment the temperature t_1 was observed using the clinical mercury thermometer via rectal route. Then, rat was allowed to enter in the next two compartments. After observation of activity in all three compartments,

again the temperature was observed by clinical mercury thermometer. In antipyretic activity, rise in body temperature was induced by inflammation and fear factors involved in analgesic and anti-inflammatory activity. The sign of inflammation are fever, redness, pain, and swelling. So, after completion of analgesic and anti-inflammatory observation, the temperature was noted again as t_2. In this activity we also replace the use of pyrogen to induce the increased body temperature. The difference between initial and final temperature was observed. This procedure was performed for all the animals of control and treated group.

Fig. 2 (Observation of antipyretic activity)

Observation of analgesic activity

For determination of analgesic activity the experimental animal was allowed to enter in the analgesic (the middle one) compartment of the model. After observing the rectal temperature in antipyretic compartment of model, rat was allowed to enter in the analgesic compartment. When rat is in analgesic compartment then the doors of antipyretic and anti-inflammatory compartment were closed so that rat can spent time in analgesic compartment only. Here in analgesic compartment, a base of nails pinned at a uniform distance was there. Rat has to walk on this bed of inverted nails. The time in seconds spent by a rat on the nailed bed was observed and the behavior to avoid the nails was also taken into the consideration. In the animals of control and treated groups the difference in time spent and change in protective behavior was observed and noted. In this analgesic compartment the pain stimulus and discomfort was provided by the inverted nailed bed. The heat as a pain stimulus was replaced with the pan with inverted nailed bed.

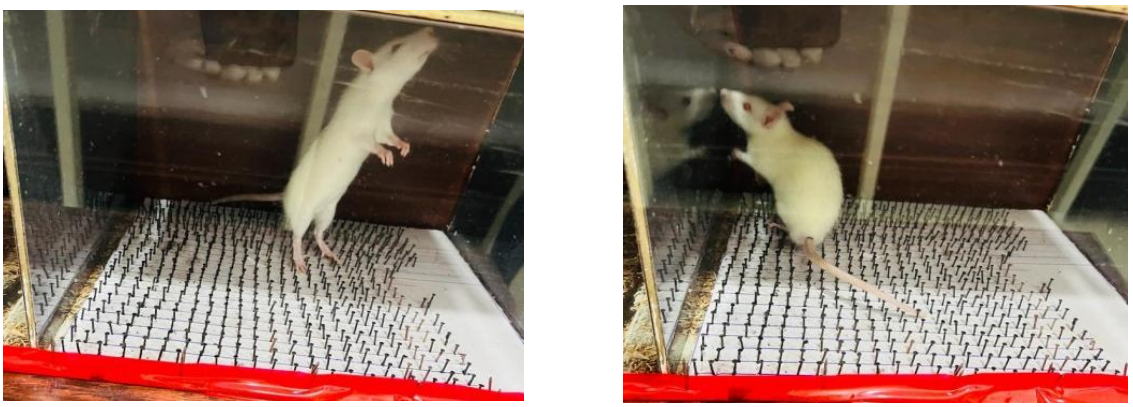

Fig. 3 (Observation of analgesic activity)

Observation of anti-inflammatory activity

For determination of anti-inflammatory activity the experimental animal was allowed to enter in the anti-inflammatory (the third one) compartment of the model. After observing the analgesic activity in the analgesic compartment of model, rat was allowed to enter in the anti-inflammatory compartment. When rat is in anti-inflammatory compartment then the doors of antipyretic and analgesic compartment were closed so that rat can spent time in anti-inflammatory compartment only. Here in anti-inflammatory compartment, a base of stain less steel like a heating pan was installed. As the rat entered in this

compartment, a candle was lighted just below the steel pan to heat it up to 60 ºC. The response (Jump response, tail movement response and avoidance response) time in seconds spent by a rat on the heated pan was observed and the behavior to avoid the heated pan was also taken into the consideration. The change in color of paw skin was taken as a sign of inflammation. The cut off time was 15 seconds. In the animals of control and treated groups the difference in time spent and change in protective behavior was observed and noted. In this anti-inflammatory compartment the inflammatory stimulus and discomfort was provided by the heat. The carrageenan as an inflammatory stimulus was replaced with the heat stimulus and change in color of rat's paw as a sign of inflammation.

Fig. 4 (Observation of anti-inflammatory activity)

Experiment using old models

Evaluation of anti-inflammatory effect of drug was performed as per the method given by Winter et al. (1962), evaluation of antipyretic effect of drug was performed as per the method given by Baker's Yeast Induced Pyrexia Method and evaluation of analgesic effect of drug was performed as per the method given by Eddy as Eddy's hot plate method.

4. RESULTS

Antipyretic activity: The experiment for antipyretic activity of ecosprin was performed in the first chamber of multicompartment model at various time intervals (Up to 3 hours) for all the experimental animal groups. The readings were taken in ℃ in triplicate for each rat of each group. The readings were taken in ℃ in triplicate for each rat of each group. One reading requires minimum 60 seconds of exposure time for the rectal temperature of measurement of a rat with the help of a mercury thermometer.

Table 1: Mean temperature of control group animals at different time interval

Control Group					
Animal	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**
Mean Temperature (In ℃) at time 0 minute (n=3)	37.36	37.26	37.53	37.53	37.36
Mean Temperature (In ℃) at time 30 minute (n=3)	37.30	37.36	36.90	37.2	37.06
Mean Temperature					

(In ℃) at time 60 minute (n=3)	37.23	37.33	37.53	37.16	37.23
Mean Temperature (In ℃) at time 120 minute (n=3)	37.60	37.26	37.23	37.23	37.10
Mean Temperature (In ℃) at time 180 minute (n=3)	37.63	37.20	37.46	37.13	37.43

Table 2: Mean temperature of treated group animals at different time interval

Treated Group					
Animal	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**
Mean Temperature (In ℃) at time 0 minute (n=3)	37.43	37.23	37.43	37.33	37.26
Mean Temperature (In ℃) at time 30 minute (n=3)	37.06	36.56	37.03	37.06	37.03
Mean Temperature (In ℃) at time 60 minute (n=3)	36.83	37.03	37.00	37.03	36.90
Mean Temperature (In ℃) at time 120 minute (n=3)	37.03	37.00	36.66	37.06	36.90
Mean Temperature (In ℃) at time 180 minute (n=3)	36.70	36.60	36.46	36.90	36.80

Analgesic activity: After observing the rectal temperature the rat was allowed to enter into the second compartment (The first and third compartment remain closed at this time) for evaluation of analgesic activity of ecosprin in the multicompartment model at various time intervals, (Up to 3 hours). The readings were taken as paw licking response (how many times paw licking in 2 minutes) and time spent in blank floor area (in second) in triplicate for each rat of each group. One reading requires minimum 120 seconds of exposure time.

Table 3: Analgesic activity reading (Paw licking and time spent in blank area) of a rats of control group at different time intervals

Control Group	**Number of Paw licking response (Up to 120 seconds)**					**Time spent in blank floor area (In seconds)**				
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 1**	**Rat 1**	**Rat 3**	**Rat 4**	**Rat 5**

Mean reading at time 0 minute (n=3)	19.33	17.66	16.00	20.33	19.33	57.33	56.66	52.66	55.00	63.00
Mean reading at time 30 minute (n=3)	18.00	16.66	15.00	17.00	18.00	70.00	61.00	59.00	62.00	61.66
Mean reading at time 60 minute (n=3)	15.00	13.00	14.33	14.00	15.00	69.00	73.33	69.33	71.33	79.33
Mean reading at time 120 minute (n=3)	10.00	9.00	10.00	10.66	10.33	94.66	97.33	98.00	95.00	101.66
Mean reading at time 180 minute (n=3)	9.66	8.33	7.00	8.00	7.66	99.00	96.66	102.00	94.66	106.00

Table 4: Analgesic activity reading (Paw licking and time spent in blank area) of a rats of treated group at different time intervals

Treated Group	**Number of Paw licking response (Up to 120 seconds)**					**Time spent in blank floor area (In seconds)**				
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 1**	**Rat 1**	**Rat 3**	**Rat 4**	**Rat 5**
Mean reading at time 0 minute (n=3)	20.33	23.00	18.66	20.33	19.33	57.33	48.66	49.33	55.00	65.00
Mean reading at time 30 minute (n=3)	12.33	15.66	11.33	15.66	16.00	43.33	43.66	45.00	54.66	62.00
Mean reading at time 60 minute (n=3)	14.00	10.33	12.66	8.66	8.33	53.66	53.66	51.00	57.66	71.33
Mean reading at time 120 minute (n=3)	7.33	8.00	8.00	8.66	8.00	65.00	79	76.66	68.33	71.33
Mean reading at time 180 minute (n=3)	8.33	6.33	7.00	7.00	7.33	89.00	90.33	84.00	83.00	80.33

Anti-inflammatory activity: After observing the analgesic response for paw licking and time spent in the bank area the rat was now allowed to enter into the third compartment (The first and second compartment remain closed at this time) for evaluation of anti-inflammatory activity of ecosprin in the multicompartment model at various time intervals, (Up to 3 hours, each reading not more than 15 seconds). The readings were taken as tail flicking and jump response, and change external paw anatomy. One reading requires minimum 15 seconds of cut off time.

Table 5: Anti-inflammatory response activity reading (Tail flicking and Jump response) of rats of control group at different time interval

Control Group	Time in seconds for tail flicking (In seconds)					Time in seconds for jump response (In seconds)				
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 1	Rat 1	Rat 3	Rat 4	Rat 5
Mean reading at time 0 minute (n=3)	4.33	3.66	4.33	4.00	4.33	8.33	9.66	9.33	8.33	9.33
Mean reading at time 30 minute (n=3)	3.33	4.33	4.00	4.00	3.66	8.00	8.33	8.00	8.33	8.66
Mean reading at time 60 minute (n=3)	3.00	3.33	3.66	3.66	3.33	7.33	7.66	7.33	7.33	7.66
Mean reading at time 120 minute (n=3)	2.66	3.00	3.33	3.33	2.66	7.33	7.33	6.66	7.00	7.33
Mean reading at time 180 minute (n=3)	4.33	3.00	3.00	4.00	3.33	6.33	5.33	5.66	6.33	6.33

Table 6: Anti-inflammatory response activity reading (Tail flicking and Jump response) of rats of treated group at different time interval

Treated Group	Time in seconds for tail flicking (In seconds)					Time in seconds for jump response (In seconds)				
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 1	Rat 1	Rat 3	Rat 4	Rat 5
Mean reading at time 0 minute (n=3)	4.33	4.33	4.66	5.00	5.00	8.66	9.00	8.33	8.66	9.33
Mean reading at time 30 minute (n=3)	5.66	5.33	5.33	4.66	4.00	9.00	10.00	8.66	10.33	8.66
Mean reading at time 60 minute (n=3)	6.33	5.66	6.00	4.66	6.33	10.00	11.00	11.00	9.33	9.66
Mean reading at time 120 minute (n=3)	6.00	5.00	5.66	5.33	5.33	10.66	12.00	11.33	11.33	12.00

Mean reading at time 180 minute (n=3)	6.33	6.00	6.00	6.33	5.33	13.33	12.33	12.33	13.66	13.33

After observing and analyzing the data obtained from newly fabricated multicompartment model, the experiments were performed using the old models for estimation of antipyretic, analgesic and anti-inflammatory activity and data were analyzed and compared.

Analgesic activity using Eddy's hot plate: After observing analgesic, antipyretic and anti-inflammatory activities using multicompartment model now, the same activities were observed separately using previously available models. In Eddy's hot plate method for the observation of analgesic activity the jump response of rat was taking into consideration. The readings for jump response were observed for control and aspirin treated groups. Each group consists of 5 albino wistar rats (either sex) of 150 gm to 200 gm.

Table 7: Analgesic activity using Eddy's hot plate method for control and treated groups of rats at different time interval

Control Group	**Time in seconds for jump response (In seconds)**				
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**
Mean reading at time 0 minute (n=3)	5.33	4.66	5.33	5.66	5.66
Treated Group	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**
Mean reading at time 0 minute (n=3)	5.66	5.33	5.66	6.00	5.00
Mean reading at time 30 minute (n=3)	9.00	7.00	7.00	6.6	7.00
Mean reading at time 60 minute (n=3)	8.33	8.33	8.66	9.00	9.66
Mean reading at time 120 minute (n=3)	9.00	10.00	9.33	9.33	9.66
Mean reading at time 180 minute (n=3)	9.00	10.66	10.00	8.66	11.00

Antipyretic activity: Evaluation of antipyretic effect of drug was performed as per the method given by Baker's Yeast Induced Pyrexia Method, pyrexia was induced in rats by administering freeze-dried baker's yeast as 20% suspension in 0.9% saline (1g/kg s.c.) in the nape of neck. The temperature was estimated by inserting a 3 cm digital thermometer into rectum. The test group was treated with drug, 4 h after injection of baker's yeast. Rectal temperature for control and tested groups was measured at 0, 3, 4, and 6 h using a thermometer.

Table 8: Rectal temperature reading of control and treated groups of rats at different tine interval

Control Group	**Temperature (In ℃)**					
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Mean**
At 0 hr	36.4	37.0	37.1	37.1	36.9	36.90

At 3 hr	37.8	37.4	37.5	37.5	37.4	37.52
At 4 hr	38.2	38.2	37.9	38.1	37.8	38.04
At 6 hr	38.6	38.3	38.2	38.1	38.0	38.24
Treated Group	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Mean**
At 0 hr	37.1	37.0	36.9	37.0	37.2	37.04
At 3 hr	37.5	37.2	37.1	36.9	37.1	37.16
At 4 hr	37.3	37.3	37.0	37.5	37.2	37.26
At 6 hr	37.5	37.1	37.3	37.1	37.3	37.26

Anti- inflammatory activity: Evaluation of anti-inflammatory effect of drug was performed as per the method given by Winter et al. (1962). Edema in the left hind paw of rat was induced by injection of 0.1 ml of 1% (w/v) carrageenan (Sigma, Mumbai) in saline into the footpad, subcutaneously. The perimeter of the paw was measured before injection and then at 1, 2, 4, 6 h, and the edema value was expressed with the difference between the perimeter of paw at certain time point after injection and the one before injection.

Table: Paw edema value (In mm) for rats of control and treated groups

Control Group	**Perimeter of the paw (In mm)**					
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Average**
At 0 hr	4	3.5	4	4	3.5	3.8
At 3 hr	5	5	8	7	6	6.2
At 4 hr	7	6	7	7	7	6.8
At 6 hr	7	6	7.5	7.5	7	7.0
Treated Group	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Average**
At 0 hr	4.5	4	4.5	4.5	4	4.3
At 3 hr	4.5	4.5	6.5	5.5	5	5.2
At 4 hr	6	6.5	6.5	6	5.5	6.1
At 6 hr	6.5	6	7	7	6	6.5

5. DISCUSSION

In this study we present new and relatively simple model standardization for studying antipyretic, analgesic and anti-inflammatory activity using the small animal like rat. In this paper we have described the use of a model that has capabilities to perform the work of three different models. In our laboratory, the traditional and old models were in use to determine the antipyretic, analgesic and anti-inflammatory activity. However, the need for validation of newly fabricated multicompartment model was constantly felt, as we wanted to increase the use of this model for pharmacological screening work. The observed results and data were satisfying to validate the model because the data from old models and newly fabricated models were matching to the requirements of experiments. The stimulus for increasing the body temperature of rat and for inflammation were introduced in a different way and it worked efficiently to induce the sign of inflammation

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