Comparative evaluation of various analgesic drugs in Wistar rats used as a teaching tool to teach animal experiments to post graduate MD Pharmacology students

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INTRODUCTION

The rat formalin test which causes local tissue injury of the paw, has been used as a model for tonic pain and localized inflammatory pain. Formalin induced pain is caused by peripheral tissue inflammation. Acute inflammation lasts for a shorter duration only for minutes, several hours or a few days and its main characteristic is exudation of fluid and plasma proteins and the emigration of leukocytes predominantly neutrophils. Neutrophils also cause increased vascular permeability and produces edema. Several studies have shown that injection of formalin causes edema of paw to develop rapidly.

Thus, anti-inflammatory effects can be assessed among different drugs and compared. This short study was designed to sensitize postgraduate students to animal experiments, animal ethics, routes of drug administration and animal handling. Knowledge gained by postgraduate students was assessed by using a pre and post study questionnaire.

ABSTRACT

Background: Postgraduates when join Pharmacology department, they don’t have much idea about guidelines and methods to do animal experiments. This study was designed to sensitize postgraduate students to animal experiments by evaluating the anti-inflammatory effects and the efficacy of diclofenac, tramadol and etoricoxib in reducing paw edema in wistar rats.

Methods: The wistar rats of 225-250gram were divided into four groups (n=10 each). In all the groups the paw edema on right paw was created using 0.1ml of 1% formalin subcutaneously. Group 1 rats (control), group 2 were given diclofenac, group 3 were given tramadol and group 4 were given etoricoxib orally. The parameters assessed were water displacement in plethysmography after 2, 4, 24, 48 hours of giving the drug. The circumference of edematous paws after 4 hours, licking of paw was observed over 30minutes of giving the drug and was compared. Pre and post study feedback were obtained from the students by using a preformed questionnaire to assess the knowledge gained by the students.

Results: The test showed that all the drugs were effective in reducing inflammation, circumference of edema and the licking duration significantly (p<0.05). The post study answering of questions by postgraduate was 100% in comparison to pre-study answering which was 33%.

Conclusions: A short animal study conducted as a teaching tool to postgraduate students was able to teach the guidelines of animal experiments and handling of animals effectively.

Keywords: Paw edema, Paw licking, Plethysmograph
METHODS

With the approval of the institutional animal ethics committee, rats were divided into four groups (n-40). Rats weighing 100-250 grams were used and 10 animals per group were taken.

The formalin 0.1 ml of 1% was injected in all the rats subcutaneously and In control group 1 (n-10) rats were not given the test drug given normal saline and in group 2 (n-10) rats were given the diclofenac (50mg/10ml) and group 3 (n-10) rats were given tramadol (50mg/10ml) and group 4 (n-10) were on etoricoxib (90mg/10ml) orally. The dose was calculated as per the weight of the rat individually. The rats were assessed for licking of edematous paw, and plethysmography and measurement of circumference of edematous paw. The duration of licking was assessed over 30 minutes duration by observing the animals.

The circumference of edema created was measured 4 hours later of giving the drug and comparison was made between all the groups. The edematous paw was put into plethysmograph and the water shift or displacement was assessed at 1, 4, 24, 48, 72 hours.

All experiments were conducted in accordance with the guidelines on the welfare of experimental animals. The circumference of edema was measured using a thread that was tied around the ankle joint of hind limb and compared with the normal hind limb. The edema was measured using water displacement in plethysmograph. Pre and post study feedback were obtained from the students by using a preformed questionnaire (annexure 1) to assess the knowledge gained by the students. The intergroup comparison was done using unpaired t test.

RESULTS

The mean duration of licking of edematous paw site was reduced among rats given the anti-inflammatory drugs than the control group. After injecting formalin s.c. the mean licking duration was assessed 1st and then assessed after giving drugs orally over the interval of 30 minutes. The mean duration of licking was reduced significantly in all the study groups in comparison to the control group (p<0.05).

In the control group the mean duration of licking was 51±8.73 seconds and in group 2, 3 and 4 was 14.7±6.69, 21.85±6.84 and 26.15±5.82 seconds respectively (Table 1).

The comparative evaluation for circumference of edematous paw was done after 4 hrs and 24 hrs later of giving the test drug. The mean circumference without edema was 23.66±1.79. The mean circumference of edematous paw was 31±1.62 in control group. The mean circumference was reduced to 26.5±0.51, 26.2±0.38 and 23.4±0.38 in group 2, 3 and 4 respectively (p<0.05, Table 2). The mean water displacement without edema was 2.33±0.55. In control group the water displacement due to edema lasted for 72 hrs. In group 2, 3 the water

### Table 1: Comparative evaluation of licking period of edematous paw (seconds) over the 30minutes observation in study group 1, 2, 3 and 4.

| Licking | Group 1 | Group 2 | Group 3 | Group 4 |
|---------|---------|---------|---------|---------|
| Over the 30 mins | 51±8.73 | 14.7±6.69 | 21.85±6.84 | 26.15±5.82 |
| vs 2 | 1 vs 3 <0.05 | 1 vs 4 <0.05 |

Group 1: control, Group 2: diclofenac Group 3: tramadol Group 4: etoricoxib; Intergroup comparison was non-significant (p >0.05) in 2 vs 3, 3 vs 4 and 2 vs 4

### Table 2: Comparative evaluation of circumference of edematous paw (Mean±SEM) at 0, 1, 4 and 24 hrs after giving the study drug in all the study groups.

| Circumference | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------|---------|---------|---------|---------|
| At 0 hours    | 22.7    | 24.1    | 24.2    | 23.6    |
| 1 hr later    | 24.8    | 26      | 26.1    | 24.4    |
| 4 hrs later   | 31±1.62 | 26.5±0.51 | 26.2±0.38 | 26.4    |
| 24hrs later   | 32.6    | 24.6    | 25.4    | 24.3    |

Intergroup comparison with control
- 1 vs 2 p <0.05 1 vs 3 p <0.05

Group 1: control, Group 2: diclofenac, Group 3: tramadol, Group 4: etoricoxib

The dose then assessed after giving drugs orally over the interval of 30 minutes. The mean duration of licking was reduced significantly in all the study groups in comparison to the control group (p<0.05).
displacement due to edema lasted for 48 hrs. In group 4 the water displacement due to edematous paw was only for 24 hrs (Table 3).

Feedback from the students were taken before and after the completion of the study to assess their knowledge. The post study answering of questions was 100% in comparison to pre-study answering which was 33%.

**Table 3: Comparative evaluation of mean water displacement (mms) in plethysmograph in group 1, 2, 3 and 4.**

|                | Group 1 | Group 2 | Group 3 | Group 4 |
|----------------|---------|---------|---------|---------|
| After 1 hr (mm)| 3.6     | 2.6     | 2.7     | 2.5     |
| 4 hrs (mm)     | 6.4     | 3.1     | 2.9     | 2.65    |
| 24 hrs (mm)    | 4.6     | 1.8     | 1.75    | 1.45    |
| 48 hrs (mm)    | 2.8     | 1.3     | 1.2     | 1.1     |

Group 1: control, Group 2: diclofenac, Group 3: tramadol, Group 4: etoricoxib

**DISCUSSION**

As with passage of time animal experiments have decreased in MD pharmacology curriculum because of animal rights organizations, Supreme court and CPCSEA. But there is a need to train post graduate students in basic animal experiments which involves drug testing. This can be achieved by conducting short animal studies to train postgraduates about all the aspects of animal experiments like animal handling, care and including alternatives to animals. This will also incorporate planning and execution of animal study protocols in postgraduate teaching.

Normally the fine afferent fibres C and Aδ activated by brief, high density stimuli, which induce little or no tissue damage and however inflammation induced by tissue damage or infection, the afferent fibres can be stimulated by low density stimuli with formalin.

Diclofenac being preferential COX-2 inhibitor used as anti-inflammatory agent affords quick relief of pain in wound edema. Etoricoxib being selective COX-2 inhibitor has been found to be effective. Tramadol is atypical opioid which inhibits reuptake of noradrenaline and serotonin thus activates monoaminergic spinal inhibition of pain and reduces the level of prostaglandin E2 concentration in inflammatory exudates. Thus, the present study was designed to compare the efficacy of diclofenac, etoricoxib and tramadol in reducing paw edema induced by formalin and the comparison was with the control group. In the present study the formalin induced changes were comparable to the other study by IO L et al. All the drugs were screened for their anti-inflammatory effects on rat paw edema and the etoricoxib the COX-2 inhibitor was more effective than all other drugs as in the study by Azoubel MC et al, the etoricoxib was found to be effective in reducing the inflammation of periodontitis in rats.

In the present study the drug etoricoxib the selective COX-2 inhibitor has shown significant results over diclofenac and tramadol in reducing licking duration, circumference of edematous paw and water displacement in plethysmograph.

It has been found in the previous study by Khandale M et al, the paw edema induced by Carrageenan has shown improvement more with ibuprofen than with buspirone.

In another study the anti-inflammatory activity of newer macrolides compared with Cox-2 Inhibitor and the newer macrolides found to be more significant in reducing paw edema than Etoricoxib.

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**Ethical approval:** The study was approved by the Institutional Ethics Committee

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## ANNEXURE I

### Pre and Post study questionnaire

| S. No. | Questions                                      | Yes | No |
|--------|------------------------------------------------|-----|----|
| 1.     | Do you know about how to handle animals used in experiments? |     |    |
| 2.     | Do you know about how to administer drugs to animals? |     |    |
| 3.     | Do you know about how to restrain animals?        |     |    |
| 4.     | Do you know about four ‘R’ in animal experiments? |     |    |
| 5.     | Do you know about Animal Ethics Committee?        |     |    |
| 6.     | Do you know about CPCSEA?                         |     |    |