

Effect of Ondansetron on Pain among rodents by Tail Flick Method and Eddy's Hot Plate Method

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Abstract: Introduction: 5-HT₃ receptors were involved in the regulation of both mood and pain. 5-HT₃ receptors are present in many parts of the body including central nervous system, peripheral neurons, spinal cord, gastrointestinal tract etc. the present study was planned to evaluate its analgesic activity of ondansetron on pain in rodent models by two different models of pain viz tail flick method and eddy's hot plate. **Materials and Methods:** Albino rats (Wistar) weighing 180-250 g and Swiss albino mice weighing between 25-30 gm were procured from the central animal house of Narayana Medical College, Nellore. Control animals (group I) received equal volume of normal saline. Ondansetron 0.25mg/kg, 0.5mg/kg, 1mg/kg and 2mg/kg were given once a day for seven days to group II, III, IV and V rats respectively for all the models. Statistical analysis was performed using Microsoft Excel-2007 and Sigma Graph pad prism version-5 USA. **Results:** In Tail flick method, OND of 1 and 2 mg/kg at 30 and 60 min showed significant ($p < 0.05$) increase in mean reaction time as compared to control, whereas OND 2mg/kg at 90 min also showed significant ($p < 0.05$) increase in mean reaction time as compared to control. DFC 10mg/kg showed significant ($p < 0.001$) increase in mean reaction time as compared to control. In Eddy's hot plate method Mean reaction time of OND in dose of 0.25, 0.5, 1 and 2mg/kg at 90 min was 4.0(1.6), 5.5(1.3), 5.8(1.4) and 7.3(1.9) respectively. OND of 2 mg/kg at 30, 60 and 90 min showed significant ($p < 0.05$) increase in mean reaction time as compared to control. DFC 10mg/kg showed significant ($p < 0.001$) increase in mean reaction time as compared to control. **Conclusion:** The current study demonstrates high doses having significant analgesic activity in thermal procedures. This can be further substantiated with well controlled experimental and clinical studies.

Keywords: Analgesia, Ondansetron, Wistar Rat.

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I. Introduction

Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1]. Pain is the most common reason for physician consultation. It is a major symptom in many medical conditions, and can significantly interfere with a person's quality of life and general functioning [2].

The task of medicine is to preserve and restore health and to relieve suffering. Understanding pain is essential to both these goals. Because pain is universally understood as a signal of disease, it is the most common symptom that brings a patient to a physician's attention. The function of the pain sensory system is to protect the body and maintain homeostasis. It does by detecting, localizing and identifying tissue damaging processes. Since different diseases produce characteristic patterns of tissue, the quality, time course and location of a patient's pain complaint and the location of tenderness.

Ondansetron is widely used anti-emetic which is 5HT₃ antagonist. Glaum et al(1990) described activation of spinal 5HT₃ receptor produce a nociceptive effect, that is reversed by specific 5HT₃ receptor blockade. Mc Cleane et al (2003) showing possible use of 5HT₃ receptor antagonist for treatment of neuropathic pain [3].

5-HT₃ receptors sites are ligand gated ion channels which mediate the release of number of neurotransmitters [4]. Previous studies have suggested the common biological pathways and neurotransmitters (serotonin and nor epinephrine) may be involved in the [5-7] mechanism of pain and depression. In addition, 5-HT₃ receptors were involved in the regulation of both mood and pain. 5-HT₃ receptors are present in many parts of the body including central nervous system, peripheral neurons, spinal cord, gastrointestinal tract etc.

Activation of 5HT₃ on peripheral afferent neurons in animal models lead to acute and persistent nociceptive effect that blocked by 5HT₃ receptor antagonist. Based on the above literature available on

Ondansetron, the present study was planned to evaluate its analgesic activity of ondansetron on pain in rodent models by two different models of pain viz tail flick method and eddy's hot plate.

II. Materials And Methods

All the experiments involved in this work were performed in accordance with "Committee for Purpose of Control and Supervision of Experimental Animals" (CPCSEA) guidelines for the use and care of experimental animals.

All the experimental procedures and protocols used in this study were carried out according to the guidelines of institutional animal ethical committee and Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) with protocol number (16/2011/NMC).

Experimental Design:

Animals: Albino rats (Wistar) weighing 180-250 g and Swiss albino mice weighing between 25-30 gm were procured from the central animal house of Narayana Medical College, Nellore.

They were housed in standard polypropylene cages with paddy husk as bedding and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12h light –dark cycle. Animals were given a standard laboratory diet and water ad libitum. All experiments were run between 09:00 AM and 13:00 PM in order to minimize the effect of circadian rhythms. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.

For both the models groups are divided as follows.

Group I - Control group (normal saline NS)(i.p).

Group II - Ondansetron at a dose of 0.25 mg/kg(i.p).

Group III - Ondansetron at a dose of 0.5 mg/kg(i.p).

Group IV - Ondansetron at a dose of 1 mg/kg(i.p).

Group V - Ondansetron at a dose of 2 mg/kg(i.p).

Group VI - Standard drug(i.p).

Drugs & Chemicals:

Ondansetron (sigmaaldrich), fluoxetine(sunpharma),diclofenac(pfizer), diazepam (Nitin life science Ltd).

Instruments:

Glass chamber, Analgesiometer and stop watch.

Treatment Schedule:

Control animals (group I) received equal volume of normal saline.

Ondansetron 0.25mg/kg,0.5mg/kg,1mg/kg and 2mg/kg were given once a day for seven days to group II, III , IV and V rats respectively for all the models.

Standard drugs like Fluoxetine 20 mg/kg (i.p),Diclofenac sodium 10mg/kg (i.p) and diazepam 2mg/kg(i.p) were given once a day for seven days to Group VI (standard drugs) animals respectively for all the models.

TAIL FLICK METHOD [8]:

Principle:

Analgesia is defined as a state of reduced awareness to pain and analgesics are substance which decrease pain sensation by increasing threshold to painful stimuli .painful reaction in experimental animals can be produced by applying noxious stimuli such as thermal(radiant heat),chemical(irritants),physical(tail compression). In the laboratory commonly used procedures are tail flick (tail withdrawal from the radiant heat) using analgesiometer.

Procedure:

Six groups of animals were taken .Basal reaction time of animals to radiant heat was recorded by radiant heat source. Group I was treated with NS (0.1ml/10g) Group II, III, IV and V were given OND in the doses of 0.25, 0.5, 1.0 and 2.0 mg/kg (i.p.) respectively, while Group VI received Diclofenac 10 mg/kg (i.p.) for 7 day. The tail withdrawal from the heat placing the tip (last 1- 2 cm) of the tail on the (flicking response) is taken as the end point. The animals, which showed flicking response within 3-5 seconds, were selected for the study. A cut off period of 15 seconds is observed to avoid damage to the tail. The measurement of withdrawal time using the tail flick apparatus was conducted at 30min, 60 min, 90 min after of drugs administration Increase in mean reaction time was taken as index of analgesic activity.

EDDY'S HOT PLATE METHOD [8]:

Principle:

In this method heat is used as a source of pain .Animals are individually placed on a hot plate maintained at constant temperature (55 °c) and the reaction of animals such as paw liking or jump response was taken as the end point .Analgesics increase the reaction time. The method was first described by Eddys and Leimbach (1953)

Procedure:

Albino rats were screened by placing them on a hot plate maintained at 55 + 2⁰C with the help of thermostat. Group I was treated with NS (5ml/kg), Group II, III, IV and V were given OND in the doses of 0.25, 0.5, 1.0 and 2.0 mg/kg (i.p.) respectively, while Group VI received Diclofenac 10 mg/kg (i.p.) for 7 days. After placing the rats on hot plate, responses such as jumping, withdrawal of paws and licking of paws were seen. The time period (latency period) when rats are placed and until responses occur was recorded by stopwatch. A cut-off period of 15 seconds was maintained to avoid damage to the paw. Reaction time will be observed again at 30, 60, 90 minutes. Increase in mean reaction time is taken as index of analgesic effect.

STATISTICAL ANALYSIS:

The data was collected in case record forms. Then they were entered into excel spreadsheet 2007. Statistical analysis was performed using Microsoft Excel-2007 and Sigma Graph pad prism version-5 USA. Data was described as Mean (Standard deviation)[9]. One way ANOVA followed by Tukeys Multiple Comparison Test was used for analysis of data between inter individual groups. For all inferential statistical tests a two tailed P < 0.05 was considered significant. All the results of test drug [Ondansetran 0.25mg/kg, 0.5mg/kg, 1mg/kg, 2mg/kg] were compared with control.

III. Observation And Results

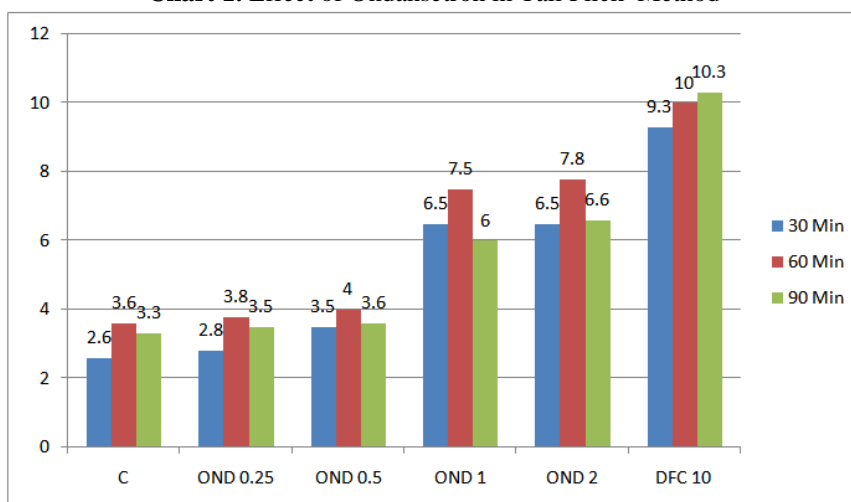
The mean reaction time of control group I at 30 min was 2.6(1.2), at 60 min was 3.6(1.6), at 90 min was 3.3(1.2) respectively. For DFC 10mg/kg at 30 min, 60min, 90min was 9.3(1.5), 10(1.7) and 10.3(1.3) respectively .The mean reaction time of OND in dose of 0.25,0.5,1 and 2 mg/kg was 2.8(1.8), 3.5(1.3), 6.5(2.9), 6.5(2.0) and 9.3(1.5) respectively. Mean reaction time of OND in dose of at 60 min was 8(1.8), 4(1.4), 37.5(2.2) and 7.8(3.3).respectively. Mean reaction time of OND in dose of 0.25, 0.5, 1and 2mg/kg at 90 min 3.5(1.5), 3.6(1.6), 6(1.7) and 6.6(2.8) respectively. OND of 1 and 2 mg/kg at 30 and 60 min showed significant (p<0.05) increase in mean reaction time as compared to control, whereas OND 2mg/kg at 90 min also showed significant (p<0.05) increase in mean reaction time as compared to control. DFC 10mg/kg showed significant (p<0.001) increase in mean reaction time as compared to control (Table 1).

Table 1: Effect of Ondansetron on Mean Reaction Time in Tail Flick Method

Groups and doses(mg/kg)	Mean Reaction Time (sec)		
	30Min	60Min	90Min
1.GroupI control	2.6(1.2)	3.6(1.6)	3.3(1.2)
2.Group II Ondansetron0.25mg/kg	2.8(1.8)	3.8(1.8)	3.5(1.5)
3.Group III Ondansetron0.5mg/kg	3.5(1.3)	4(1.4)	3.6(1.6)
4.Group IV Ondansetron1mg/kg	6.5(2.9)*	7.5(2.2)*	6(1.7)
5.Group V Ondansetron 2mg/kg	6.5(2.0)*	7.8(3.3)*	6.6(2.8)*
6.Group VI Diclofenac 10mg/kg	9.3(1.5)***	10(1.7)***	10.3(1.3)***

ns p>0.05,*p<0.05,**p<0.01,***p<0.001.

Chart 1. Effect of Ondansetron in Tail Flick Method



Bars represent the mean reaction time in seconds (SD) in TFM as compared to control (C). ns- not significant (P>0.05), *P<0.05, **P<0.01, ***P<0.001 one way ANOVA followed by Post hoc Tukey's multiple comparison tests.

Photograph 1: Tail Flick Method



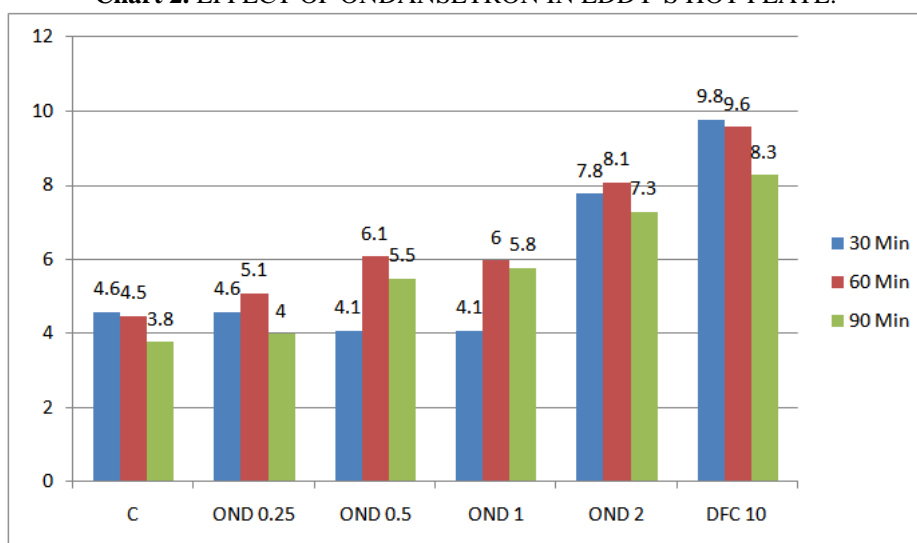
Mean reaction time of control group I at 30 min was 4.6(1.3), at 60 min was 4.5(1.3), at 90 min was 3.8(1.9) respectively. For DFC 10mg/kg at 30 min, 60min, 90min was 9.8(1.8), 9.6(1.3) and 8.3(2.4) respectively. The mean reaction time of OND in dose of 0.25,0.5,1 and 2 mg/kg at 30 min was 4.6(1.6), 4.1(1.1), 4.1(1.8) and 7.8(1.6) respectively. Mean reaction time of OND in dose of at 60 min was 5.1(2.0), 6.1(2.0), 6.4(1.8), 8.1(1.8) and 9.6(1.3) respectively. Mean reaction time of OND in dose of 0.25, 0.5, 1 and 2mg/kg at 90 min was 4.0(1.6), 5.5(1.3), 5.8(1.4) and 7.3(1.9) respectively. OND of 2 mg/kg at 30, 60and 90 min showed significant (p<0.05) increase in mean reaction time as compared to control. DFC 10mg/kg showed significant (p<0.001) increase in mean reaction time as compared to control (Table 2).

TABLE 2: Effect of Ondansetron on Mean Reaction Time in Eddy's Hot Plate

Groups and Doses(mg/kg)	Mean Reaction Time in Min		
	30Min	60Min	90Min
1.Group I control	4.6(1.3)	4.5(1.3)	3.8(1.9)
2.Group II Ondansetron 0.25mg/kg	4.6(1.6)	5.1(2.0)	4.0(1.6)
3.Group III Ondansetron 0.5mg/kg	4.1(1.1)	6.1(2.0)	5.5(1.3)
4.Group IV Ondansetron 1mg/kg	4.1(1.8)	6.0(1.8)	5.8(1.4)
5.Group V Ondansetron 2mg/kg	7.8(1.6)*	8.1(1.8)*	7.3(1.9)*
6.Group VI Diclofenac 10 mg/kg	9.8(1.8)***	9.6(1.3)***	8.3(2.4)**

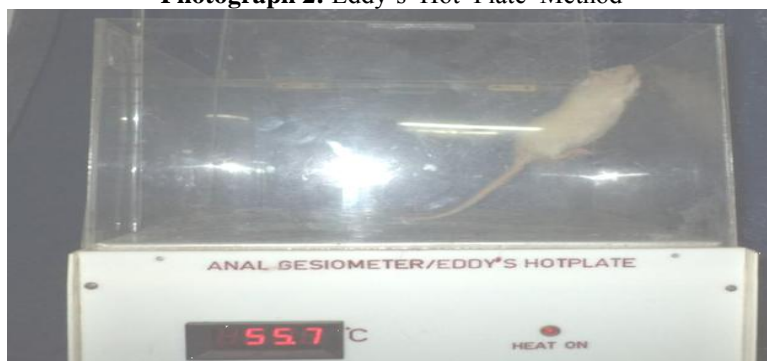
ns p>0.05, *p<0.05, **p<0.01, ***p<0.001.

Chart 2. EFFECT OF ONDANSETRON IN EDDY'S HOT PLATE.



Bars represent the mean reaction time in seconds (SD) in Eddy's hot plate as compared to control (C). ns- not significant ($P>0.05$), * $P<0.05$, ** $P<0.01$, *** $P<0.001$ one way ANOVA followed by Post hoc Tukey's multiple comparison tests.

Photograph 2: Eddy's Hot Plate Method



IV. Discussion

A stimulus that causes tissue damage usually elicits a sensation of pain. Receptors for such stimulus are known as nociceptors. They respond to intense mechanical stress, heat and many chemicals including neuropeptide, transmitters, bradykinin, histamines, cytokines and prostaglandins, many of which are released by damaged cells. The International Association for Study of Pain has defined Pain as “an unpleasant sensory and experience associated with actual or potential tissue damage”. Pain has been classified into two different major types – Fast pain and slow pain [10].

Nociceptors respond to stimuli that produce damage to the organs. The two major classes of cutaneous nociceptors are the A δ and C polymodal nociceptors. The A δ mechanical nociceptors respond to strong mechanical stimuli, like pricking the skin with needle or crushing the skin with forceps. They won't respond to noxious thermal or chemical stimuli unless they have been previously sensitized. C polymodal nociceptors on the other hand, respond to several types of noxious stimuli like mechanical, thermal, and chemical stimuli [11].

Pain is a subjective phenomenon, and clinicians cannot reliably detect its existence or quantify its severity without asking the patient directly. A useful means of assessing pain and evaluating the effectiveness of analgesia is to ask the patient to rate the degree of pain along a numeric or visual pain scale [12] and also using Mc Gill pain Questionnaires [13].

Animal tests used in the search for new analgesics are designed as models for the treatment of pathological pain in many, but they usually differ from the original in that the test drug is given before the noxious stimulus (mechanical, thermal, chemical and electrical types of stimuli) in the animal models. Hence, these tests only measure the power of a drug to increase the threshold of minimal stimulus required to elicit pain or nociceptive response [8].

In general three types of stimuli-physical (Haffner's Tail-Clip Method), thermal (Tail-Flick response, Tail-Immersion Test, Hot-Plate Method) and chemical (Glacial Acetic acid induced writhing, Writhing induced by 4% NaCl solution, Writhing Induced by 4% NaCl Solution) are employed in mice or rats for evaluation of analgesic property of a compound [8].

Effect of ondansetron on Thermal nociception:

In our study, ondansetron showed analgesic effect both in tail flick method and eddy's hot plate method. In the tail flick test, a thermal stimulus is focused on the skin of the animal's tail, activating nociceptors in the surface layers of the skin. The tail flick latency was significantly increased by ondansetron [OND 1 and 2mg/kg vs control ($p<0.05$)]. Whereas, in eddy's hot plate ondansetron only at high dose [OND 2mg/kg vs control ($p<0.05$)] showed significant analgesic effect. From the results it could be concluded that OND exhibits anti-nociceptive activity, the results of our study are consistent with the findings of previous studies which highlight , that activation of spinal 5-HT receptors produce a nociceptive effect which is reversed by specific 5-HT₃ receptor blockade.

Our study is supported by McCleane et al [3] (2003) who postulated that, the 5-HT₃ receptor antagonists can be utilized to alleviate neuropathic pain.

Wojciech Rocznik et al [14] did an analgesic study of ondansetron on adult wistar rats by writhing test, observed analgesic effect was weak and short. Bharathi uppu et al [15] stated that there is no analgesic activity with 2.5 mg/kg of ondansetron in mice by tail clip method.

Activation of 5-HT₃ receptors on peripheral afferent neurons in animal models of tissue injury leads to acute and persistent nociceptive effects that can be blocked by 5-HT₃ receptors antagonists. Therefore we concluded that OND shows anti-nociceptive effect in models of thermal nociception.

Hence from above studies we conclude that OND depicts analgesic effect. neuropsychopharmacological profile of OND may be of value in treating patients suffering with cancer as they frequently suffer from chemotherapy induced vomiting and because of their debilitating illness they are in constant agony due to pain, anxiety and depression as these conditions are inter related to each other. Therefore use of drugs like OND in such patients may be helpful in improving the quality of life.

V Conclusion

Nociception (pain) being one of the most common condition where mankind suffer a lot in daily life. In our study we evaluated the Analgesic activity of ondansetron by using two experimental models namely tail flick method and eddy's hot plate method. The current study demonstrates high doses having significant analgesic activity in thermal procedures. This can be further substantiated with well controlled experimental and clinical studies.

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