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SCREENING OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF VILAZODONE IN RODENTS

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ABSTRACT: The analgesic activity of vilazodone in albino rats and mice was compared with the standard drug pentazocine and diclofenac, and its anti-inflammatory activity in albino rats was compared with the standard drug indomethacin. The doses of vilazodone used were 1.8mg/kg and 3.6mg/kg in albino rats and 2.6mg/kg and 5.2 mg/kg in albino mice, and the methods to screen analgesic activities were eddy's hot plate method, tail flick method using radiant heat and acetic acid induced writhing method and formalin-induced peritonitis was used to screen anti-inflammatory activity. The data was analyzed statistically and results were expressed as numbers and percentages. For vilazodone dose of 1.8mg/kg and 3.6mg/kg using eddy's hot plate method mean reaction time were 6.50± 0.288 and 5.70± 0.285, in tail flick method 6.50±0.288 and 6.45± 0.521. In the writhing method at a dose of 2.6mg/kg and 5.2 mg/kg in albino mice vilazodone showed 30.33±1.15 and 28.16±0.98 analgesic activity compared to control. In formalin induced peritonitis vilazodone 1.8mg/kg and 3.6mg/kg showed 2.2±0.13 and 1.86±0.05 anti-inflammatory activity when compared to control. Statistically significant results were obtained with a higher dose in hot plate and tail flick method and with low dose in writhing method when compared to the standard. Vilazodone, compared to standard in formalin-induced peritonitis, has shown statistically significant results in both doses. Vilazodone has shown significant analgesic and anti-inflammatory activities.

INTRODUCTION: Pain is one of the common health problems with a sensory experience that is multidirectional, intrinsically unpleasant, ill-defined, disabling of many medical conditions. According to the International Association for the Study of Pain (IASP), the updated and recent definition of pain is an unpleasant sensory and emotional experience associated with or resembling that associated with, actual or potential tissue damage [1].

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The principal objective of alleviating pain is to remove the cause of pain, hence analgesics are used for the symptomatic treatment of pain. The most common types of acute and chronic pain based on etiology and clinical presentation include nociceptive, inflammatory and neuropathic syndromes [2]. Pain is one of the cardinal signs of inflammation. There is increased pain mediators, either due to direct damage or an inflammatory response itself [3].

The most common method to control pain is by using analgesic drugs [4]. Analgesic treatment includes non-steroidal anti-inflammatory drugs (NSAIDs), opioids, anticonvulsants and muscle relaxants, but they have not shown outstanding efficacy. Opioids are recommended to be used as second and third-line treatments because of their

adverse effects. Tramadol and FDA-approved tapentadol are used in second-line treatment [5], while the strong opioids, oxycodone, and morphine are used in the third-line treatment [6]. Most of these have their own side effects. Thus, there is a need for more synthetic formulations which have lesser side effects with improved efficacy.

Anxiety and depression are the mental disorders most commonly investigated in patients with chronic pain [7]. The analgesic efficacy of many antidepressant drugs has been proven in experimental acute pain models and in different pain models. Antidepressant medicines produce pain relief faster than antidepressant effects, and analgesia can be achieved with lower plasma concentrations [8].

Antidepressant medications can directly reduce pain by enhancing the activity of descending anti-nociceptive signaling pathways. Accumulating evidence suggests that activation of the 5-HT1A receptor subtype can modulate the processing and control of signals associated with pain [9]. Antidepressants are also studied for anti-inflammatory activity.

Vilazodone is the latest US Food and Drug Administration (FDA) approved antidepressant treatment (ADT). It acts as a selective serotonin reuptake inhibitor (SSRI) with 5-HT1A receptor partial agonist actions or a serotonin partial agonist reuptake inhibitor (SPARI). Use of vilazodone can reduce polypharmacy. Vilazodone has fewer side effects like nausea, diarrhea, headache and taken with food increases its bioavailability and protect from gastrointestinal side effects [10]. So, the present study is undertaken to evaluate the analgesic and anti-inflammatory activity of vilazodone in albino rodents to provide pharmacological basis for its use as an analgesic and anti-inflammatory medication.

MATERIALS AND METHODS: This study was carried out in the pharmacology research laboratory, Belagavi institute of medical sciences, Belagavi. The study protocol was approved by IAEC ref no: BIMS/IAEC/PG/04/2016. Albino wistar rats weighing 120-150 grams and albino mice weighing between 15-30 grams of either sex were procured and maintained under good laboratory conditions and acclimatized to 12: 12 hour light-dark cycle for 10 days prior to the day of experimentation. They were provided with a normal standard food pellet diet with water *ad libitum*. In our study, three pain models- eddy´s hot plate method, tail flick method in rats and acetic acid induced writhing method in mice and one inflammatory model- formalin-induced peritonitis in rats were used based on simplicity, feasibility and reproducibility. Rats and mice were divided into 4 groups of 6 each.

Eddy's Hot Plate Method: The method described by Woolfe *et al.* [11] to study the pain reaction induced by electrically heated hot plate surface and used to evaluate centrally acting analgesics. Screening of rats was done and selected based on their reaction time of 3-5 sec. Rats were placed on the hot plate, and the cut-off time was 15 seconds to avoid damage to the paws.

Then one hour later respective compounds were administered: Group 1- Control group 1ml Normal saline orally, Group 2-Standard group: pentazocine 10mg/kg intraperitoneally (i.p.), Group 3-Test groups drug vilazodone 1.8mg/kg orally and Group 4 vilazodone 3.6mg/kg orally. The latency period was recorded at 20, 60 and 90 minutes. Mean reaction time for various groups was determined and expressed in seconds.

Tail Flick Method using Radiant Heat: The method described by the Howes *et al.* [12] was adopted to study the pain reaction using analgesiometer to evaluate centrally acting analgesics. In the pretest session, the normal reaction time was determined within 5 seconds when the animal flicks the tail. The responsive rats were administered: Control group: 1ml normal saline orally, Standard group: pentazocine 10mg/kg i.p, Test groups: vilazodone (1.8mg/kg and 3.6mg/kg) orally and same testing procedures were done after 30, 60 & 120 min. Mean reaction time for various groups was determined and expressed in seconds.

Acetic Acid Induced Writhing Method: The method described by Hendershot *et al.* [13] was used to study the pain reaction induced by i.p. injection of 0.6% acetic acid in mice to evaluate peripherally acting analgesics. Albino mice were divided and

administered orally 1ml normal saline in control, Standard group: diclofenac 10mg/kg, test groups 1 and 2: vilazodone (2.6mg/kg and 5.2mg/kg). One hour after the administration of drugs, acetic acid 0.6% was administered 10 mg/kg i.p [14] to induce the pain. The mice were then placed in plexiglass chamber and number of writhes was recorded for next 10 minutes in each animal. A mean number of writhing for various groups was determined to calculate the percentage of protection using the following formula:

% of protection = Control mean-treated mean / control mean × 100

Formalin-Induced Peritonitis in Rats: The method described by Teotino *et al* [15] with slight modifications was adopted, to study the acute inflammatory reaction induced by i.p. injection of formalin in rats. Albino rats were fasted overnight, divided into groups, and administered orally 1ml normal saline in the control group, indomethacin 10mg/kg, test groups 1 and 2: vilazodone (1.8mg/kg and 3.6mg/kg). After one-hour acute inflammation peritonitis was induced by 1ml 1% formalin i.p. After four hours, animals were sacrificed the peritoneal exudate volume was collected by opening the ventral abdominal wall. The mean peritoneal exudate volume for various groups was calculated and expressed in ml. The percentage inhibition of peritoneal exudates was calculated using the following formula: % inhibition of peritoneal exudates = (1-Vt /Vc) x100, where Vc - Mean volume of exudates in the control group, Vt - Mean volume of exudates in the treated group.

RESULTS AND DISCUSSION: In this study, vilazodone was investigated for its possible analgesic and anti-inflammatory effect, and results were analyzed using the Mann-Whitney U test (SPSS software version 22).

Eddy's Hot Plate Method: The mean reaction time in seconds for the control group, standard (pentazocine), and test groups is shown in **Table 1**. A percentage increase in reaction time indicated statistically significant analgesic activity of test groups (P = 0.01) compared to the control. But when compared with that of the pentazocine group, there was no statistically significant difference in the mean reaction time of vilazodone 1.8mg/kg.

TABLE 1: ANALGESIC ACTIVITY WITH VARIOUS TREATMENTS SHOWN BY USING EDDY'S HOT PLATE METHOD IN ALBINO RATS

Group	Dose	Reaction time in seconds at				Mean reaction time Seconds ± SE
		0 min	20 min	60 min	90 min	
Control (Normal saline)	1ml	4.33	4.66	5.33	5.5	5.00 ±0.21703
Standard (Pentazocine)	10 mg/kg	4.16	6.83	11	11.33	8.33±0.68542
Test 1 (Vilazodone)	1.8mg/kg	5.16	6.33	7.33	7.16	6.50±0.28868
Test 2 (Vilazodone)	3.6mg/kg	3.83	5.5	6.5	7	5.70±0.28539

Tail Flick Method: The mean reaction time in seconds for control, standard pentazocine and test groups is shown in **Table 2**. The percentage increase in reaction time indicated significant analgesic activity of pentazocine from 30 min compared to the control. Whereas test groups also showed statistically significant analgesic activity compared to the control. But there was not much statistically significant difference with the pentazocine group.

TABLE 2: ANALGESIC ACTIVITY SHOWN BY USING TAIL FLICK METHOD

Group	Dose	Reaction time in seconds at				Mean reaction time Seconds ± SE
		0 min	30 min	60 min	120min	
Control (Normal saline)	1ml	4.5	5	5.66	6.16	5.33±0.17720
Standard (Pentazocine)	10 mg/kg	4.66	7.66	8.33	10.83	7.87±0.51429
Test 1 (Vilazodone)	1.8mg/kg	5.33	6.5	8	8.83	7.04±0.33872
Test 2 (Vilazodone)	3.6mg/kg	4	4.83	7.83	9.16	6.45±0.52121

Acetic Acid-Induced Writhing Method: The mean number of writhes and percentage of protection for control group, standard and test groups are shown in **Table 3**.

Diclofenac and test-treated groups showed a statistically significantly increased percentage of protection of number of writhes compared to the control.

When, the vilazodone (2.6 mg/kg) group was compared with diclofenac, there was a statistically significant.

TABLE 3: ANALGESIC ACTIVITY OF STANDARD DRUG AND TEST COMPOUNDS IN THE ACETIC ACID-INDUCED WRITHING METHOD

Groups	Dose(mg/kg)	Writhing ± SEM	% of protection
Control (NS)	1ml	46.5±0.84676	-
Standard (Diclofenac)	10mg/kg	26.83±0.79232	42.30%
Test 1 (Vilazodone)	2.6 mg/kg	30.33±1.15	34.77%
Test 2 (Vilazodone)	5.2mg /kg	28.16±0.98	39.44%

Formalin-Induced Peritonitis in Rats: The mean peritoneal exudate volume in milliliters (ml) for control group after 4 hour interval was 3.433±0.11. The corresponding mean peritoneal exudate volume in indomethacin treated group was 1.46±0.06 with the calculated percentage inhibition of 57.47 % is shown in **Fig. 1.**

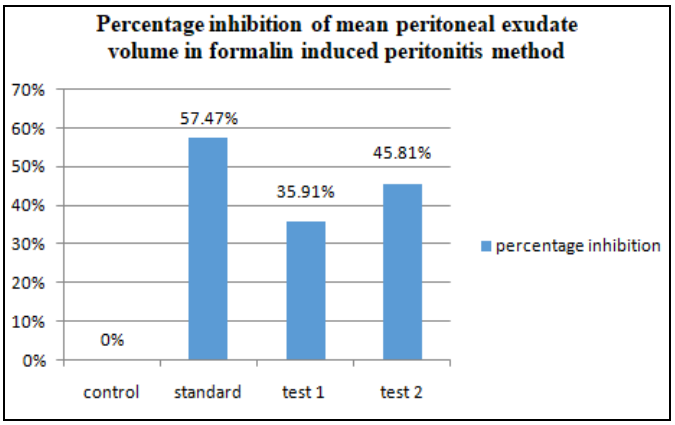

FIG. 1: ANTI-INFLAMMATORY ACTIVITY OF STANDARD DRUG AND TEST COMPOUNDS IN THE FORMALIN-INDUCED PERITONITIS

The above results clearly emphasize the anti-inflammatory effect of vilazodone in the acute model of inflammation when compared to control. Further anti-inflammatory effect of vilazodone group was compared with that of indomethacin group P = 0.004 and P = 0.05, respectively.

This study evaluated the analgesic and anti-inflammatory effects of vilazodone in albino rats and mice. Vilazodone is an antidepressant agent that belongs to SSRIs. Clinically used antidepressants like fluoxetine, duloxetine, amitriptyline, sertraline etc., have shown analgesic and anti-inflammatory activity which involves the role of serotonin. Hence, this study was chosen to evaluate the role of vilazodone in pain and inflammation management.

Sara *et al* [16] in their study, mention the advantage of the hot plate test is that, it can be applied repeatedly in the same animals over a short period of time (2–3 h) without causing tissue injury. A study done by Patil *et al.* [17] showed that the test compound fluoxetine, compared with control using the hot plate method, had a statistically significant analgesic activity with p values of < 0.01 and < 0.001 respectively. Pentazocine has also showed the same statistically significant analgesic activity. Our study also shows significant analgesic activity with low-dose vilazodone and high-dose vilazodone compared to the control (P <0.05). Vilazodone low dose using an analgesic model was compared with pentazocine 10mg/kg there was not much statistical difference. Hence the efficacy of low-dose vilazodone was almost equal to pentazocine but more efficacious when compared to control. In our study, it has In a study conducted by Paudel *et al* [18] hot plate latency period at the end of 90 min for the test drug amitriptyline 10 mg/kg was 12.5 sec, whereas with low dose and high dose of vilazodone it was 7.16 sec and 7 sec respectively suggesting its analgesic activity.

In the tail flick method, a spinally integrated response in the form of withdrawal of the tail was observed on the application of thermal radiation to the tail. Kesim M *et al.* [19] in their study with milnacipran (10, 30, 50 mg/kg) and sertraline (10, 20, 50 mg/kg) have shown statistically significant analgesic activity in both with a p-value of < 0.05 when compared to control.

In our study, it has been shown that there is a statistically significant analgesic activity with doses of vilazodone when compared to control and standard (P<0.05). The efficacy of low-dose vilazodone was almost equal to pentazocine. A

study conducted by Sikka *et al* [20] shows that the tail flick latency period at the end of 120 min for test drug fluoxetine 5mg/kg and 10mg/kg was 10sec and 8.75 sec respectively and that with mirtazapine 5mg/kg and 7 mg/kg was 9.83 sec and 9.91 sec.when compared to our study with 1.8 mg/kg and 3.6 mg/kg of vilazodone it was 8.83 sec and 9.16 sec at the end of 120 min.

The acetic acid-induced writhing method using albino mice was selected because of its ability to mimic human clinical pain conditions and sensitivity to mild analgesics. In a study done with the test compound paroxetine [21] the results showed statistically significant analgesic activity with P< 0.05 compared to the control. In our study, both doses of vilazodone have shown it's statistically significant analgesic activity compared to the control and the P value <0.05. In contrast, the percentage protection of the standard drug diclofenac was 42.30% compared to that of low and high doses vilazodone was 34.77% and 39.44%. Hence, a higher dose of vilazodone showed analgesic activity almost similar to the standard drug diclofenac. In a study conducted by Pawar *et al.* [22] with sertraline the mean number of writhes in just 5 min was 19.6, and our study test compounds of lower and higher dose it was 30.33 and 28.16 respectively in 10 min showing better analgesic activity compared to sertraline.

A study for anti-inflammatory activity was conducted by Sadeghi *et al.* [23] with amitriptyline 40 mg/kg, and 80 mg/kg in paw edema model have shown statistically significant anti-inflammatory activity when compared to control with p-value < 0.05 and < 0.01, respectively. And in the same study, the standard drug indomethacin 10mg/kg showed statistically significant inhibition of paw edema with p value < 0.001. In our study formalin-induced peritonitis model of inflammation was used there was statistically significant anti-inflammatory activity with both doses when compared to the control with P value of 0.004 but with standard indomethacin showed more effective results. In a study conducted by Dhawale *et al* [24] with Duloxetine 5mg/kg and 10 mg /kg the mean paw edema volume was 1.55±0.19 and 1.79±0.10 (mean ±SE) respectively when correlated with results of formalin induced peritonitis model of inflammation in our study, the mean peritoneal exudates volume of low dose vilazodone was 2.2±0.13 (mean ± SE) and high dose vilazodone was 1.86 ± 0.05(mean ± SE) showing significant anti-inflammatory activity (P value < 0.05 and 0.05 respectively). As per our knowledge, it is the first study conducted to screen the analgesic and anti-inflammatory effect of vilazodone.

CONCLUSION: Vilazodone has a unique mechanism of action; a single drug acts both as SSRI and 5HT1A partial agonist approved for treating major depressive disorder. Most studies done using other antidepressant have proven their role in neuropathic pain and have anti-inflammatory properties. Based on these findings, vilazodone was screened for analgesic and anti-inflammatory activity using animal models. Results of the present experimental study show that vilazodone an antidepressant is also an effective analgesic and anti-inflammatory drug. Future clinical studies are needed to confirm the analgesic and anti-inflammatory effects of vilazodone.

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