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STUDY OF SYNERGISTIC EFFECT OF VENLAFAXINE AND ASPIRIN IN EXPERIMENTALLY INDUCED PAIN MODELS IN RATS AND MICE

Pharmacology

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ABSTRACT

In this era, there is need for new analgesics. Venlafaxine is an antidepressant and has antinociceptive effect. This study evaluates analgesic property of Venlafaxine and combination of subanalgesic doses of Aspirin and Venlafaxine in experimentally induced pain models in rats and mice and compared with standard drug Aspirin. Albino mice and wistar rats of either sex were included and were divided into 4 groups of six in each. Methods used to produce pain were chemical method (by intraperitoneal injection of 0.6% &0.1% of acetic acid in rats and mice), and radiant heat method (by analgesiometer). Results obtained from tail flick response (thermal method) in rats and mice and Writhing test (chemical method) in mice showed that Venlafaxine and aspirin combination has more antinociceptive effect when compared with individual drugs Aspirin and venlafaxine. Venlafaxine at 10mg/kg dose has shown to produce antinociceptive effect, which is less than the antinociceptive effect of standard drug Aspirin.

KEYWORDS

Analgesiometer, Venlafaxine, Pain, Aspirin, Writhing

Introduction

Pain is an unpleasant sensation, ranging in intensity from slight through severe to indescribable[1]. Pain is experienced as having qualities such as sharp, throbbing, dull, nauseating, burning and shooting. Pain is a subjective experience. Many new targets including receptors, enzymes, transport systems and membrane ion channels will focus drug development towards the peripheral nociceptors or the spinal nociceptive pathway.

Several antidepressants are known to possess intrinsic analgesic activity [2,3]. Venlafaxine is a bicyclic antidepressant used primarily for the treatment of major depression, generalized anxiety disorder, social anxiety disorder and panic disorder in adults [4]. It works by blocking the transporter reuptake proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. The neurotransmitters affected are serotonin (5-hydroxytryptamine) and norepinephrine (noradrenaline) [5]. It is well absorbed with at least 92% of an oral dose being absorbed into systemic circulation. It is extensively metabolized in the liver via the CYP2D6 isoenzyme to desvenlafaxine. Steady state concentrations of venlafaxine and its metabolite are attained in the blood within 3 days. Common side effects of venlafaxine are nausea, headache, apathy, constipation, dizziness, insomnia, vertigo, sweating, dry mouth.

Acetyl Salicylic Acid or Aspirin is analgesic, anti inflammatory and antipyretic and inhibitor of platelet aggregation. It inhibits cyclooxygenase by acetylation of the active site of enzyme and the pharmacological effects of it are due to the inhibition of the formation including prostaglandins, thromboxanes, and prostacyclin [6]. It is rapidly absorbed from gastrointestinal tract. Appreciable quantities are found in plasma in less than 30 minutes. Aspirin causing adverse effects like intolerance, gastric erosions, reduces plasma prothrombin level by interfering with action of vitamin k in the liver, Reyes syndrome, salicylism [7].

The analgesic drugs introduced so far are associated with some considerable side effects namely euphoria, depressant action on vital centers, addiction, tolerance, gastric acidity, gastric erosions and renal problems etc. Therefore search continues for a new, potent safe and nontoxic analgesic. Hence the present study is planned to evaluation of analgesic effect of venlafaxine in both thermally and chemically induced pain models in animals. And also to evaluate the synergistic effect of venlafaxine with standard drug aspirin in thermal and chemical induced pain models in rats and mice and compared with standard drug aspirin.

Materials and Methods:

The study was conducted in the research laboratory, Dept of Pharmacology, Osmania medical college, Hyderabad, Telangana state, India. Twenty four swiss albino mice weighing 25-30 gm and wistar rats weighing 150 – 250g of either sex were included in the study. The animals were procured from the central animal house of Osmania medical college, Hyderabad. They were housed in cages in standard laboratory conditions with natural light and dark cycle and at room temperature. Food and water were given ad libitum. The protocol was approved by Institutional Animal Ethics Committee (IAEC). Venlafaxine and Aspirin were obtained from local pharmacy.

Methodology:

1)Tail flick method:

This experiment was carried out on rats and mice by means of analgesiometer[8] devised by M.L.Gujral (Techno).Wistar rats weighing between 150 to 250gms and swiss albino mice weighing 25-30 gm were chosen for preliminary screening. All the animals were tested for noting the latent period of the withdrawal of tail after exposure to the radiant heat from the red hot wire of the analgesiometer. The current was adjusted so that tail withdrawal by all the animals on exposure to the red hot wire was within 3-5 seconds. If the reaction time exceeded more than 10 seconds, it was assumed that complete analgesia has been produced. Further delay might cause tissue injury influencing the sensation.

Rats and mice were divided in to four groups of six in each. Groups were named as control, standard, test and combination group. The measured quantities of drugs were given according to the body weight of animals. Control groups were treated with distilled water equal in volume to that of test groups and Standard groups were treated with Aspirin in dose of 20mg/kg for rats and 25mg/kg for mice. Test group in rats and mice was treated with Venlafaxine in the dose of 10mg/kg and combination group in rats and mice was treated with combination of aspirin in dose of 12.5 mg/kg and Venlafaxine in dose of 7.5mg/kg. All drugs administered intraperitoneally 30 minutes before applying the radiant heat. Animals were tested and reaction times were noted at 0, 30, 60 and 90 minutes time intervals and results were tabulated.

2) Acetic acid Induced Writhing Test:

Chemical Method (Intra Peritoneal Injection of 3% Acetic Acid to rats and 0.6% Acetic acid to mice): Writhing response[9] induced with acetic acid was described by WITKIN et al (1961).Writhing is described as a stretch, torsion to one side, drawing of a hind leg, retraction of abdomen and opisthotonos, so that the belly of the rat or mouse touches the floor.

Rats and mice were divided in to four groups of six in each. Groups were named as control, standard, test and combination group. The measured quantities of drugs were given according to the body weight of animals. Control groups were treated with distilled water equal in volume to that of test groups and Standard groups were treated with Aspirin in dose of 20mg/kg for rats and 25mg/kg for mice. Test group in rats and mice was treated with Venlafaxine in the dose of 10mg/kg and combination group in rats and mice was treated with combination of aspirin in dose of 12.5 mg/kg and Venlafaxine in dose of 7.5mg/kg. 45 minutes after drug administration all groups of rats and mice were injected with 1ml/100mg body weight of 3% acetic acid and 0.6% acetic acid intraperitoneally respectively. The number of writhings produced by each animal was recorded over a period of 20 minutes. The observations were tabulated. The percentage inhibition in the writhing was also calculated.

Statistical analysis :

All the observations and results are presented in the form of mean ± Standard error of mean (SEM) and the percentage increase in reaction time of means in test and standard, synergistic effect of standard and test drugs in comparison to control calculated. Statistical analysis of data was performed using Student's 't' test to study the differences among the means [10]. p value < 0.05 is considered significant whereas a p valve < 0.01 is considered highly significant [11].

Results

Analgesic activity was based on increase in the mean reaction time of pain responses to thermal stimulation and reduction in number of writhings to chemical stimulation in rats and mice.

statistical analysis of data was performed using unpaired t test.

Table-I :Tail flick response in rats in different groups at different time intervals.

Group	Dose mg/kg	Reaction Time in Seconds at time intervals (Min.)			
		0 Min.	30 Min.	60 Min.	90 Min.
Control	-	3.50+0.43	4.17+0.31	4.83+0.40	4.50+0.67
Test (Venlafaxine)	10	3.33+0.42	4.67+0.21	6.33+0.21	5.83+0.31
Standard (Aspirin)	25	3.83+0.48	7.83+0.65	8.50+0.50	9.00+0.37
Combination (Venlafaxine + Aspirin)	7.5+12.5	4.00+0.37	8.17+0.48	9.00+0.37	8.33+0.67

Number of Animals in each group (n)=6. Values are mean + SEM

Figure : I : Tail flick response in rats in different groups at different time intervals.

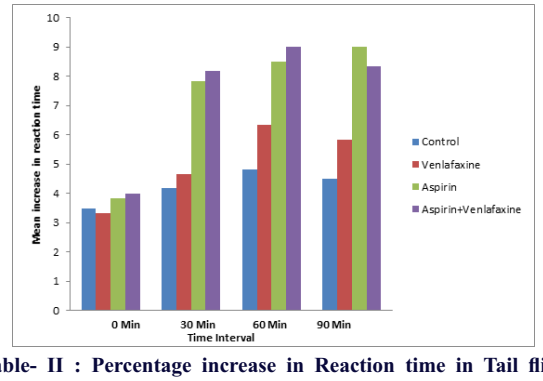
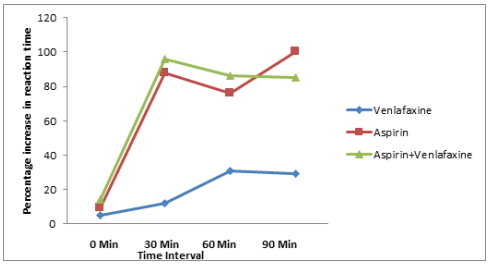


Table- II : Percentage increase in Reaction time in Tail flick method in Rats at different time intervals

Group	0 Min.	30 Min.	60 Min.	90 Min.
Control	-	-	-	-
Test (Venlafaxine- 10 mg/kg)	4.85	11.99	31.05	29.55
Standard (Aspirin-25mg/kg)	9.42	87.76	75.98	100.00
Combination (Venlafaxine +Aspirin) (7.5 mg/kg +12.5 mg/kg)	14.28	95.92	86.33	85.11

Figure : II Percentage increase in Reaction time in Tail flick method in Rats at different time intervals



In Rats : (as per table I and II):- At 0,30,60 and 90 min aspirin is showing increase in mean reaction time when compared to venlafaxine. The increase in mean reaction time of venlafaxine was much less when compared to aspirin and is more when compared to control. An increase in mean reaction time of combined effect of aspirin and venlafaxine were seen when compared to aspirin and control at 30, 60 and 90 min, p value is 0.0001.

Table- III : Tail flick response in Mice in different groups at different time intervals

Group	Dose mg/kg	Reaction Time in Seconds at time intervals (Min.)			
		0 Min.	30 Min.	60 Min.	90 Min.
Control	-	2.83+0.4	3.33+0.49	3.83+0.48	3.33+0.42
Test (Venlafaxine)	10	3.00+0.37	3.83+0.48	5.17+0.31	4.50+0.67
Standard (Aspirin)	25	3.33+0.49	4.17+0.6	5.83+0.31	5.17+0.31
Combination (Venlafaxine + Aspirin)	7.5+12.5	4.00+0.52	4.83+0.40	6.33+0.33	5.33+0.61

Number of Animals in each group (n)=6. Values are mean + SEM

Figure : III Tail flick response in Mice in different groups at different time intervals

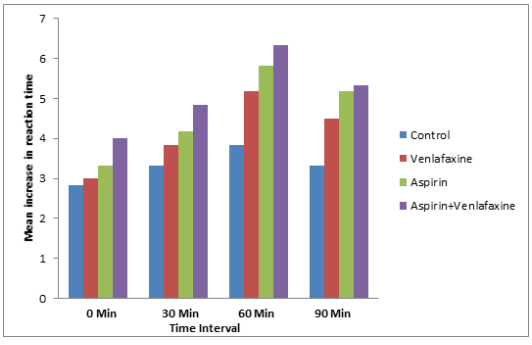
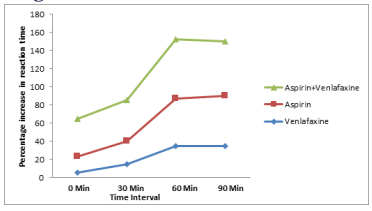


Table- IV: Percentage increase in Reaction time in Tail flick method in Mice at different time intervals

Group	0 Min.	30 Min.	60 Min.	90 Min.
Control	-	-	-	-
Test Venlafaxine (10 mg/kg)	6.007	15.01	34.96	35.13
Standard Aspirin(25 mg/kg)	17.66	25.22	52.21	55.25
Combination (Venlafaxine +Aspirin) (7.5 mg/kg +12.5 mg/kg)	41.34	45.04	65.27	60.06

Figure : IV: Percentage increase in Reaction time on Tail flick method in Mice



In Mice : (as per table III and IV):- At 0,30,60 and 90 min aspirin has shown an increase in mean reaction time when compared to venlafaxine and control. The increase in mean reaction time of venlafaxine was less when compared to aspirin. An increase in mean reaction time has observed with combined effect of aspirin and venlafaxine when compared to control and standard at 60 min (p value = 0.0016) at 30 min (p value = 0.04) and at 90 min (p value = 0.005).

Table V : P values in Tail flick Response in Rats and Mice

Time intervals	Group	P value	
		Rats	Mice
0 min	Standard to test	0.4506	0.5995
	Standard to Combined	0.7872	0.3730
	Test to Combined	0.2596	0.1449
	Control to test	0.7872	0.7650
	Control to standard	0.6145	0.4506
	Control to combined	0.3951	0.1040
30 min	Standard to test	0.001	0.6732
	Standard to Combined	0.6892	0.3780
	Test to Combined	0.0001	0.1399
	Control to test	0.2094	0.4835
	Control to standard	0.0005	0.3094
	Control to combined	0.0001	0.0403
60 min	Standard to test	0.0025	0.1560
	Standard to Combined	0.4381	0.2950
	Test to Combined	0.0001	0.0277
	Control to test	0.0079	0.0407
	Control to standard	0.0002	0.0055
	Control to combined	0.0001	0.0016
90 min	Standard to test	0.0001	0.2447
	Standard to Combined	0.4010	0.8133
	Test to Combined	0.0067	0.3813
	Control to test	0.1009	0.1717
	Control to standard	0.0002	0.0056
	Control to combined	0.0023	0.0230

$p<0.05$ – significant ; $p<0.01$ – highly significant

Table- VI: Results of Writhing Response in Mice

Group	Dose mg/kg		
		No. of writhings (in 20 min.)	% Decrease in the no.of wriths
		Mean+ SEM	
Control(Distilled water)	-	46.00+1.98	-
Test (Venlafaxine)	10	25.00+0.37	45.65
Standard (Aspirin)	25	11.33+0.71	75.43
Combination (Venlafaxine + Aspirin)	7.5 +12.5	7.17+0.60	85.41

Number of Animals in each group (n)=6. Values are mean + SEM

Figure V: Writhing Response in Mice

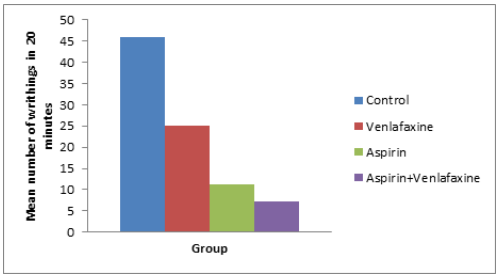
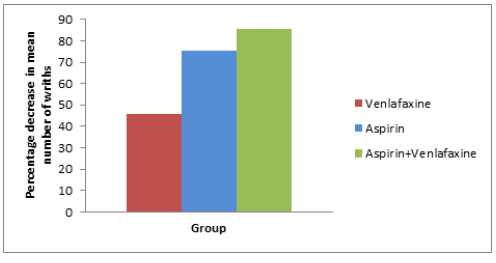


Figure VI: Writhing Response percentage decrease in Mice



Analysis of Writhing reflex in Mice: As per table VI: Mean percentage inhibition of writhing reflex is 75.43% with aspirin when compared to control with significant p value of 0.0001. Mean percentage inhibition of writhing reflex is 45.65% with venlafaxine when compared to control with significant p value of 0.0001. Mean percentage inhibition of writhing reflex is 84.41% with combined effect of aspirin and venlafaxine when compared to control with significant p value of 0.0001 and also with standard with a p value of 0.0012 (significant).

Table- VII : Results of Writhing Response in Rats

Group	Dose mg/ kg	No. of writhings (in 20 min.)	% Decrease in the no.of wriths
		Mean+ SEM	
Control (Distilled water)	-	44.83+1.70	-
Test (Venlafaxine)	10	19.50+0.89	56.50
Standard (Aspirin)	25	15.67+1.05	65.04
Combination (Venlafaxine + Aspirin)	7.5 +12.5	10.50+0.76	76.57

Number of Animals in each group (n)=6.

Figure VII: Writhing Response in Rats

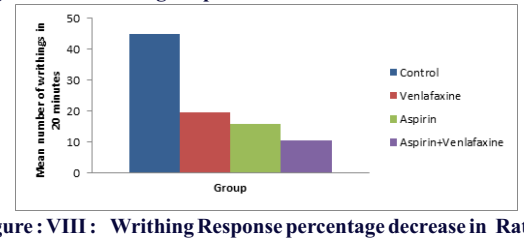
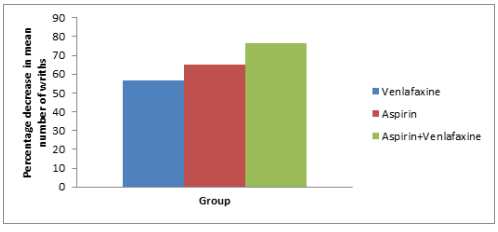


Figure : VIII : Writhing Response percentage decrease in Rats



Analysis of Writhing reflex in Rats: As per table VII: Mean percentage inhibition of writhing reflex is 65.04% with aspirin when compared to control with significant p value of 0.0001. Mean percentage inhibition of writhing reflex is 56.50% with venlafaxine when compared to control with significant p value of 0.0001. Mean percentage inhibition of writhing reflex is 76.57% with combined effect of aspirin and venlafaxine when compared to control with significant p value of 0.0001 and when compared to aspirin p value is 0.0026 (significant).

Table- VIII : P values of Rats & Mice in Chemical method

Group	P value (Rats)	P value (Mice)
Control to standard	0.0001	0.0001
Control to test	0.0001	0.0001
Control to combined	0.0001	0.0001
Standard to test	0.0193	0.0001
Standard to Combined	0.0026	0.0012
Test to Combined	0.0001	0.0001

$p<0.05$ – Significant ; $p<0.01$ – highly Significant

Discussion:

The present study is based on evaluation of analgesic effect of an antidepressant drug Venlafaxine and the combined analgesic effect of subanalgesic doses of venlafaxine and aspirin in thermally and chemically induced pain models in rats and mice. The results are compared with standard drug aspirin.

Pain is an unpleasant sensation with a large subjective component. It is often accompanied by depression and a feeling of hopelessness. Several antidepressants are known to possess intrinsic analgesic activity. Venlafaxine, a widely used newer generation antidepressant, has been cited as a promising drug for neuropathic pain control. We

also found that venlafaxine showed an analgesic effect in both the tail flick and writhing tests. There is ample evidence to suggest the involvement of monoamines such as NA and 5-HT in descending pain pathways [5]. Venlafaxine mainly block reuptake of the above neurotransmitters. This could be the Probable mechanism of action for its antinociceptive effect.

In Tail flick test an increase in mean reaction time at 0, 30, 60, and 90 minutes to thermal stimulus and in acetic acid induced Writhing test decrease in number of writhings at 0, 30, 60, and 90 min was observed with venlafaxine, venlafaxine and aspirin combination and aspirin in both rats and mice. On comparison venlafaxine and aspirin combination was more effective than aspirin and venlafaxine. Aspirin is a known standard analgesic drug has shown better analgesic effect than venlafaxine.

Centrally acting drugs that act at spinal cord level gives best results with tail flick method where as peripherally acting drugs that act by inhibiting the prostaglandins gives good results with acetic acid induced writhings test [6]. Venlafaxine is an antidepressant drug acts by inhibiting the reuptake of serotonin and Norepinephrine, probably by same mechanism it acts as a centrally acting analgesic. Aspirin is a peripherally acting analgesic by inhibiting the prostaglandin synthesis. Venlafaxine and Aspirin combination has an advantage of acting at different levels may be the reason for its synergistic effect and better analgesic response than Venlafaxine and Aspirin in both tail flick and writhing test.

Evidence of analgesic activity as indicated by increase in tail flick latency and decrease in number of writhing movements following venlafaxine treatment suggests that it could possibly have central as well as peripheral action. The finding indicates that the potential use of venlafaxine in antidepressant dose could produce marked pain relief. Thus patients of depression, who are on venlafaxine may be able to tolerate mild to moderate pain without any additional analgesic.

It was observed that combination of subanalgesic doses of aspirin and venlafaxine possessed more analgesic effect than aspirin alone in both the methods. So the combination resulted in a significant synergistic anti nociceptive effect.

From this study we conclude that venlafaxine a SNRI can produce dose dependent antinociceptive action per se, and additive (synergism) anti nociceptive effect when combined with aspirin.

Observations from these animal studies need to be tested in clinical trials.

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