Research Article

Evaluation of the Analgesic Effect of Antidepressants with Nicotinic Receptor Antagonist

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ABSTRACT

Antidepressants such as SSRI and SNRI show the analgesic effect in neuropathic pain. The main reason for the analgesic effect is thought to be the increased level of biogenic amine such as noradrenalin, serotonin, dopamine etc. The nicotinic receptor antagonist has role in increasing the activity of these biogenic amines. This study is mainly focusing to evaluate the combined effect of antidepressants with nicotinic receptor antagonist on relieving pain. Duloxetine and venlafaxine at their median effective dose that is 30mg/kg, 8mg/kg i.p. respectively, were evaluated in combination with dextromethorphan 30mg/kg intraperitoneally for the synergistic potential for ameliorating pain in Swiss albino mice. The effect of these drugs on pain was checked by Eddy's hot plate and tail immersion methods. Studies indicated that antidepressant venlafaxine and duloxetine with dextromethorphan resulted in significant reduction in pain time compared to the vehicle control. All the data were evaluated using the one-way analysis of variance followed by individual comparisons using Tukey's *post-hoc* test. Analgesic study reveals that the combination has slight synergestic effect on pain. The present results suggest the concoction of dextromethorphan with venlafaxine and duloxetine for enhanced synergistic analgesic effects with the reduction of dose.

Keywords: Antidepressants, Dextromethorphan, Duloxetine, Eddy's hot plate, Nicotinic receptor antagonist, Tail immersion test, venlafaxine.

INTRODUCTION

Generally, antidepressants are not originally intended to act as analgesics. But in certain studies they are reported to have analgesic effects for chronic pain. Antidepressants have nearly no analgesic effects, but are considered first-line drugs of choice for neuropathic pain and treatment of fibromyalgia. Specific antidepressants with analgesic effects include tricyclic antidepressants (TCA), which have long been used, and serotonin noradrenaline reuptake inhibitors (SNRI), which are comparatively new antidepressants. Selective serotonin reuptake inhibitors (SSRI), which are frequently used to treat depression. Psychological problems play an important role in chronic pain[1]. Protracted pain causes anxiety accompanied by a progressive depressive state and enhanced pain sensations. Therefore, antidepressant medications may be effective against chronic pain by their effects to improve the depressive state. Antidepressants inhibit neuropathic pain, however, even when the patient is not in a depressive state. In addition, the effects of antidepressants on depression characteristically take approximately two to four weeks to be observed from the time the drug is first taken, whereas the analgesic effects on chronic pain manifest in as little as few days to one week. Therefore, the analgesic effects of antidepressants on chronic pain likely involve a mechanism different from that mediating their antidepressive effects. The pharmacologic effects of antidepressants involve binding to noradrenaline and serotonin (5-HT) transporters. Reuptake of these neurotransmitters is inhibited, leading to increased levels of noradrenaline and 5-HT in the synaptic cleft. Noradrenaline reuptake inhibition enhances analgesic effects, mainly through α2-adrenergic receptors in the dorsal horn of the spinal cord. The α2-adrenergic receptors are coupled to the inhibitory G protein (Gi/o), which inhibits the presynaptic voltage-gated Ca2+ channels in the dorsal horn of the spinal cord that inhibits the release of excitatory neurotransmitters from primary afferent fibers[2]. At the same time, G protein-coupled inwardly rectifying K+ channels are opened on the post-synaptic spinal cord dorsal horn cells, the cell membranes are hyperpolarized, and excitability is decreased. While activation of the α2-adrenergic receptors of the spinal cord dorsal horn has weak antinociceptive effects against noxious stimuli, extensive research indicates that it is extremely effective against allodynia and hyperalgesia associated with neuropathic pain. The reason for the increasing efficacy for hypersensitivity of spinal α2-adrenergic receptors stimulation is that nerve injury changes the function of α2-adrenergic receptors in the dorsal horn of the spinal cord while at the same time the interaction with the cholinergic interneurons strengthens. Our findings support the importance of α2-adrenergic receptors in the spinal cord dorsal horn for the inhibition of neuropathic pain[3]. Neuronal nicotinic ACh receptors (nAChRs) belong to the family of ligand-gated channels. These receptors constitute both the ligand-binding site and the ionic pore through which ions can flow when the receptor is stabilized in the open conformation. Historically, the existence of such receptors was first revealed in 1857 by Bernard, who showed that the poison

curare blocks transmission at the neuromuscular junction, but does not prevent muscle contraction elicited by electrical stimulation. Since this observation, the neuromuscular junction has been used as a reference for synaptic transmission in physiology and pharmacology. It was also recognized a long time ago that ACh is the neurotransmitter that acts on the parasympathetic ganglia, but little was known about the precise mechanisms underlying this neurotransmission.

Binding of an agonist stabilizes the receptor in the active open state and causes cations to rapidly diffuse across the minute ionic pore. Significant differences in physiological properties, in terms of sensitivity to the agonist and time course of response, can be observed between different subtypes of nAChRs. One extreme is the α_7 receptors, which have a low sensitivity to ACh, but a very fast response; the other extreme is receptors like the $\alpha_4\beta_2$ receptor, which is highly sensitive to ACh and nicotine, but has a slow response[4]. α_7 responses are phasic, while $\alpha_4\beta_2$ responses are tonic. An additional and characteristic feature of α_7 nAChRs is their high permeability to calcium ions. Since these divalent cations have been shown to play an important role as a second messenger, it can be expected that $\alpha_7$activation could modify neuronal activity or gene expression. CNS nAChRs are widely distributed throughout brain regions associated with depression, such as the ventral tegmental area, locus coeruleus, and dorsal raphe nucleus. Their primary action is thought to be in the regulation of other neurotransmitter systems particularly dopamine, via direct and indirect effects and the n-methyl-D-aspartate (NMDA) glutamatergic system. nAChRs are also involved in other neurobiological systems that are dysregulated in depression, such as the hypothalamic-pituitary-adrenal axis. nAChRs are found on presynaptic terminals of corticotropin-releasing factor (CRF) neurons, and nAChR antagonists can block CRF release. Similarly, nAChRs appear to play a role in inflammation, which is the subject of increasing interest with regard to depression. nAChRs regulate the so called cholinergic ascending anti-inflammatory pathway, in which activation of the vagus nerve diminishes inflammation through decreased peripheral macrophage activity mediated via α7 nAChRs [5].

Antidepressants that block the reuptake of serotonin (5-HT) and noradrenaline (NA) are called 5-HT and NA reuptake inhibitors (SNRIs). SNRIs are agents that show “dual action” on 5-HT and NA[20]. These drugs bind the 5-HT transporters (SERT) and NA transporters (NAT) similar to tricyclic antidepressants (TCAs). However, SNRIs differ from TCAs in that SNRIs do not exert much affinity for other receptors. Although SNRIs are called “dual action” 5-HT - NA agents they increase dopamine levels in the prefrontal cortex via NAT inhibition. In this way, they have a third action on neurotransmitters in the prefrontal cortex. The main use of SNRIs is in the treatment of major depression. Other applications include treatment of pain disorders (including neuropathies and fibromyalgia), generalized anxiety, vasomotor symptoms of menopause and stress urinary incontinence. Venlafaxine is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class. This means it increases the concentrations of the neurotransmitters serotonin and norepinephrine in the body and the brain, first introduced by Wyeth is used primarily for the treatment of depression, general anxiety disorder, social phobia, panic disorder and vasomotor symptoms. At low doses (<150 mg/day), it acts only on serotonergic transmission. At moderate doses (>150 mg/day), it acts on serotonergic and noradrenergic systems, whereas at high doses (>300 mg/day), it also affects dopaminergic neurotransmission. Many doctors were starting to prescribe venlafaxine "off label" for the treatment of diabetic neuropathy (in a similar manner to duloxetine) and migraine. Duloxetine is a balanced dual serotonin and norepinephrine reuptake inhibitor recently approved for the treatment of stress urinary incontinence in women. It may be of benefit in women who are unable to perform pelvic floor muscle training, who are poor candidates for surgery or who wish to delay or avoid surgery[6]. Duloxetine is thought to exert its action by blocking the reuptake of these neurotransmitters in an area of the sacral spinal cord that contains a high density of serotonin and nor epinephrine receptors, thus stimulating specific motor neurons that regulate the urethral striated muscle sphincter. Duloxetine was created by Lilly researchers. In 2001 Lilly filed a New Drug Application (NDA) for duloxetine with the US Food and Drug Administration (FDA). Duloxetine was approved by the FDA for depression and diabetic neuropathy in 2004. In 2007 Health Canada approved duloxetine for the treatment of depression and diabetic peripheral neuropathic pain. Duloxetine was approved for use of stress urinary incontinence (SUI) in the Europe in 2004. Duloxetine (Duloxetine Hydrochloride: LY 248686, N-methyl-g- 1-napthalenyloxy-2-thiophene propanamine hydrochloride) is a new orally administered, balanced dual serotonin and norepinephrine (noradrenaline) reuptake inhibitor that has been developed for the treatment of stress urinary incontinence.

Nicotinic antagonists inhibit the effects of acetylcholine on nicotinic receptors. According to their dominant effects, we distinguish the antagonists acting on the autonomic nervous system which are called ganglionic blocking agents, and those acting on neuromuscular junction which are called neuromuscular blocking agents. A nicotinic antagonist is a type of anticholinergic drug that inhibits the action of acetylcholine (ACh) at nicotinic acetylcholine receptors. These compounds are mainly used for peripheral muscle paralysis in surgery, the classical agent of this type being tubocurarine, but some centrally acting compounds such as bupropion, mecamylamine, and 18-methoxycoronaridine block nicotinic acetylcholine receptors in the brain and have been proposed for treating drug addiction.

Dextromethorphan (DM) is structurally closely related to levorphanol, codeine, and morphine, but unlike these opiates it has low affinity for opiate receptors and is not considered to be addictive. Nevertheless, it shares with

most opiates the ability to suppress cough and has been used as an effective antitussive drug for more than 40 years. DM and/or its demethylated major metabolite dextrorphan (DP) to block N-methyl-D- aspartate (NMDA) receptor channels, DM might also block nicotinic receptors in PC12 cells. Dextromethorphan is a synthetic compound. Dextromethorphan is 3 methoxy-17- methylmorphinan monohydrate, which is the d isomer of levophenol, a codeine analogue and opioid analgesic. Dextromethorphan is considered a synthetic opiate. It has been synthesized from a benzylisoquinoline (with a planar structure) by a process known as Grewe's cyclization (from the 1950's) to give the corresponding morphinan (with a three dimensional structure). The isoquinoline is 1,2,3,4,5,6,7,8-octahydro-1-(4- methoxybenzyl)isoquinoline (there is just one residual double bond at the fusion position of the two rings of the isoquinoline) is converted into the N-formyl derivative, cyclized to the N- formyl normorphinan, and the formyl group reduced to an N-methyl group, to give 3-methoxy-17-methylmorphinan, or Racemethorphan . Dextromethorphan is freely soluble in ethanol 96% and essentially insoluble in water. Dextromethorphan is commonly available as the monohydrated hydrobromide salt. However, some newer extended-release formulations contain dextromethorphan bound to an ion exchange resin based on polystyrene sulfonic acid. Dextrometorphan's specific rotation in water is + 27.6° (20°C, Sodium D-line)[7].

MATERIALS AND METHODS

Normal saline

0.9 % w/v sodium chloride solution was used for the study. Normal saline is the commonly used phrase for a solution of 0.90% w/v of NaCl, 308 mOsm/L or 9.0 g per liter. At first 9gm of corium chloride (Nacl) are weighted with balance. 600ml of distilled water is taken in a beaker/volumetric flask and added 9gm Nacl. Mixed it properly then distilled water is added up to 1000 ml again mixed it with the help of stirrer.

Drugs

The drugs used in studies such as duloxetine, venlafaxine and dextromethorphan were purchased from Yarrow chem products, mumbai in the pure form. ED50 doses of two antidepressants were fixed according to previous research work done on its. ED50 doses were then used in combination with dextromethorphan to study for synergistic potential. The dose of dextrometorphan 15 mg/kg i.p was selected based on previous research work done on dextrometorphan. Animals were randomized on the basis of their body weight into different groups such as vehicle p.o. (Group 1), Venlafaxine 8mg/kg (Group 2), Duloxetine 30mg/kg (Group 3) Dextromethorphan (DXM) 30mg/kg (Group 4), Venlafaxine 4mg/kg+ DXM15mg/kg (Group 5), Duloxetine 15mg/kg+DXM15mg/kg (Group 6).

Animals

Swiss albino mice of either sex weighing 25-30g were used for this study. The animals were purchased from Sree chithra Tirunal Institute of Medical Science and Technology, poojapura, Trivandrum. Those animals were housed in the groups of 6 mice/cage in standard cages in a room temperature of 22 ± 2°C, under natural light/dark cycle and had free access to water and food (standard laboratory pellets) before the experiments. The mice were acclimatized at lab conditions for 5 days before the start of the experiment. All the experimental work had been carried out from 9:00 to 16:00. All experimental pharmacologic studies were done after getting permission from the Institutional Animal Ethics Committee, Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom (2/IAEC/Pharmacology/ECP S/2015) and care of animals was taken as per CPCSEA guidelines; Department of Animal Welfare, Government of India.

Prediction of Biological activity

The biological activities were predicted by using Pass online software.

(http://www.pharmaexpert.ru/passonline/)

The concept of biological activity spectrum was introduced to describe the properties of biologically active substances. The PASS (Prediction of Activity Spectra for Substances) software product, which predicts more than 30000 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, may be effectively used to find new targets (mechanisms) for some ligands and conversely, to reveal new ligands for some biological targets. 51 Prediction of activity spectra for substances (PASS) is hosted by the V. N. Orechovich Institute of Biomedical Chemistry under the aegis of the Russian Foundation of Basic Research. The web based application predicts the biological activity spectrum of a compound based on its structure. It works on the principle that the biological activity of a compound equates to its structure. PASS prediction tools are constructed using 20000 principal compounds from MDDR (MDL (Molecular Design Laboratory) Drug Data Report) database (produced by Accelrys and Prous Science). The database contains over 180000 biologically relevant compounds and is constantly updated.

Analgesic Activities

Evaluation of Analgesic Activities - Eddy's Hot plate method

Algesia (pain) is an illness-defined as unpleasant sensation, usually evoked by an external or internal noxious stimulus[8]. Analgesic is a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Excessive pain may produce other effects-sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP, tachypnoea. Analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as adjuvants to more etiological approach to pain. Hot plate method of assessing analgesic activity was first

described by wolf and Donald. In this method, heat is used as a means of evoking pain experimentally. The paws of mouse and rats are very sensitive to heat and temperature which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

Each mouse was placed on the top of the hot-plate maintained at a temperature of 55 °C and the latency of the reaction to this nociceptive stimulus (number of seconds for the animal to start licking the paw of the caudal limb or jumping) was calculated[9].

Evaluation of Analgesic Activities - Tail Immersion Test in Hot Water

Pain is a warning signal, primarily protective in nature, but causes discomfort and suffering; may even be unbearable and incapacitating[10]. It is the most important symptom that brings the patient to the physician. Excessive pain may produce other effects-sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP, tachypnoea. Analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as adjuvants to more etiological approach to pain. The analgesic effect of drug brings about reduced awareness to pain. This activity can be tested using tail-dip method (thermal method). The method has been developed to be selective for morphine- like compounds[11]. The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. In this method, the tail of the rat is immersed in an organ bath where temperature maintained at 55^0c and the reaction of the rat to the painful heat stimuli is noted before and after the administration of analgesics. The ability of rat to withstand the painful heat stimulus for longer duration is an indication of analgesic effect of the drug [12].

Statistical Analysis

The data were evaluated by one-way analysis of variance followed by individual comparisons using Tukey's post-hoc test. All results are shown as mean ± standard error of the mean. ED50 was calculated using Graph Pad Prism 7 Software developed by Graph Pad Software, Inc., USA.

RESULTS

Data Computed from PASS software

Different biological activities of selected drugs were predicted using PASS software and the result obtained are summarized in Table 1.

From the PASS value obtained for dextromethorphan it is understood that it have antagonistic action on various nicotinic subreceptors like Nicotinic alpha4beta4 receptor, Nicotinic alpha6beta3beta4alpha5 receptor, Nicotinic alpha2beta2 receptor with Pa values 0.748, 0.712, 0.618 respectively , If Pa> 0.7: chance to find the activity in experiments is very high, which shows that it is noncompetitive nicotinic receptor antagonist. From the PASS values obtained for venlafaxine it is clear that it has antidepressent activity with a Pa value of 0.618. Also, it possess various other activities like Mood disorders treatment, 5 Hydroxytryptamine uptake inhibitor, Phobic disorders treatment, analgesic with Pa values 0.666, 0.660, 0.607, 0.525 respectively. If 0.5< Pa< 0.7: chance to find out the activity in experiment is less, but it had the activity.

The Pa value for the antidepressent activity of duloxetine was 0.634 other than this activity it also has Posttraumatic stress disorder treatment with Pa value 0.609, Mood disorders treatment with Pa value 0.634, 5 Hydroxytryptamine uptake inhibitor with Pa value 0.575, analgesic with Pa value 0.621. If 0.5< Pa< 0.7: chance to find out the activity in experiment is less, but it had the activity. From the PASS value obtained for each drug it is clear that each drug alone has the desired activity. So, by combining these drugs it may produces the synergistic activity.

Evaluation of Analgesic Activity

Eddy's Hot plate method

The hot plate test is commonly used for evaluating thermal pain sensitivity. The hot-plate test evaluates thermal pain reflexes due to footpad contact with a heated surface. The analgesic activity by Eddy's hot plate method was done by the method discussed previously. The number of seconds for the animal to start licking the paw of the caudal limb or jumping was calculated as reaction time. The mice were treated with different drugs such as venlafaxine, duloxetine, dextromethorphan, Venlafaxine with dextromethorphan, duloxetine with dextromethorphan. The control group animals show the absence of analgesia. The Venlafaxine treated groups shows significant increase in reaction time compared with control group (P≤ 0.01). Simillarly the duloxetine treated group of shows significant increase in reaction time as compared to control group (P≤ 0.01). In dextromethorphan treated group there is significant increase in reaction time compared to control group but when comparing it with Venlafaxine and duloxetine the effect is less. Group of animals receiving the combination of dextromethorphan and Venlafaxine shows subadditive effect compared with group receiving Venlafaxine alone (P≤ 0.001). Similarly, the group receiving combination of dextromethorphan and duloxetine shows subadditive effect compared to group receiving duloxetine alone (P≤ 0.001). The change in analgesic activity was observed are summarized in table 2 and figure 1.

Evaluation of Analgesic activity - Tail immersion method

The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. The time required for the mice to withdraw its tail from the hot water was recorded (tail flick latency). The analgesic activity of duloxetine by tail immersion method was done by the method discussed previously. The mice were treated with different drugs such as venlafaxine, duloxetine, dextromethorphan, Venlafaxine with dextromethorphan, duloxetine with dextromethorphan. The control group animals show less tail flick latency which shows the absence of analgesia. The Venlafaxine treated groups shows significant increase in tail flick latency compared with control group (P≤ 0.01). Similarly the duloxetine treated group of shows significant increase in tail flick latency as compared to control group (P≤ 0.01). In dextromethorphan treated group there is significant increase in latency compared to control group but when comparing it with Venlafaxine and duloxetine the effect is less. Group of animals receiving the combination of dextromethorphan and Venlafaxine shows subadditive effect compared with group receiving Venlafaxine alone (P≤ 0.001). Similarly, the group receiving combination of dextromethorphan and duloxetine shows subadditive effect compared to group receiving duloxetine alone (P≤ 0.001). The change in analgesic activity was observed are summarized in Table 3 and figure 2.

Table 1: PASS value of drugs

Drug	Biological Activity	Pa Value	Pi value
Dextromethorphan	Nicotinic alpha4beta4 receptor antagonist	0.748	0.013
	Nicotinic alpha6beta3beta4alpha5 receptor antagonist	0.712	0.031
	Nicotinic alpha2beta2 receptor antagonist	0.618	0.044
	Nicotinic receptor alpha7 subunit antagonist	0.213	0.005
	Acetylcholine neuromuscular blocking agent	0.621	0.016
	Antidepressant	0.517	0.019
Venlafaxine	Antidepressant	0.681	0.008
	Mood disorders treatment	0.666	0.009
	5 Hydroxytryptamine uptake inhibitor	0.660	0.004
	Phobic disorders treatment	0.607	0.120
	Analgesic	0.525	0.032
Duloxetine	Antidepressant	0.634	0.011
	Mood disorders treatment	0.634	0.010
	Posttraumatic stress disorder treatment	0.609	0.001
	5 Hydroxytryptamine uptake inhibitor	0.575	0.004
	Analgesic	0.621	0.017

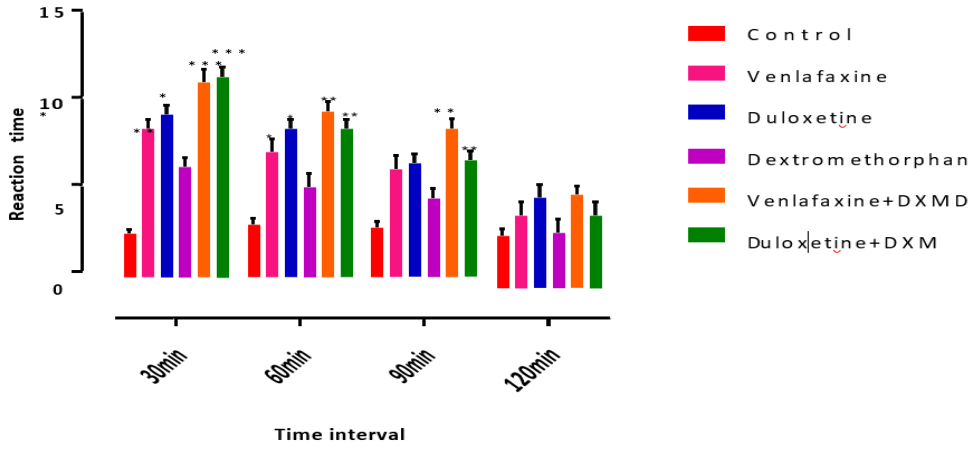

Figure 1: Evaluation of Analgesic Activity - Eddy's Hot plate method

Mice were treated with venlafaxine, duloxetine, dextromethorphan, venlafaxine with dextromethorphan and duloxetine with dextromethorphan. All values are expressed as Mean ± SEM (n = 6). The statistical analysis was carried out by one way ANOVA followed by individual comparisons using posthoc Tukey's multiple comparison tests. Where each group compared with that of control group. Where ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

Table 2: Evaluation of Analgesic Activity - Eddy's Hot plate method

	Reaction time in sec (Mean ±SEM)			
	30 min	**60min**	**90 min**	**120 min**
Control	2.500±0.244	3.000±0.365	2.833±307	3.000±0.365
Venlafaxine (8mg/kg)	8.500±0.224**	7.164±0.307*	6.167±0.307	4.167±0.307
Duloxetine (30mg/kg)	9.33±0.211**	8.500±0.226*	6.500±0.224	5.176±0.307
Dextromethorphan (30mg/kg)	6.330±0.211	5.167±0.307	4.500±0.224	3.167±0.307
Venlafaxine+DXM (4+15mg/kg)	11.167±0.307***	9.50±0.224**	8.500±0.224**	5.370±0.211
Duloxetine+DXM (15+15m g/kg)	11.500±0.224***	8.50±0.244**	6.667±0.211**	4.160±0.307

Mice were treated with venlafaxine, duloxetine, dextromethorphan, venlafaxine with dextromethorphan and duloxetine with dextromethorphan. The study was carried out as discussed in 4.2.2.2.1. The statistical analysis was carried out by one way ANOVA followed by individual comparisons using posthoc Tukey's multiple comparison tests. Where each group compared with that of control group. Where ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

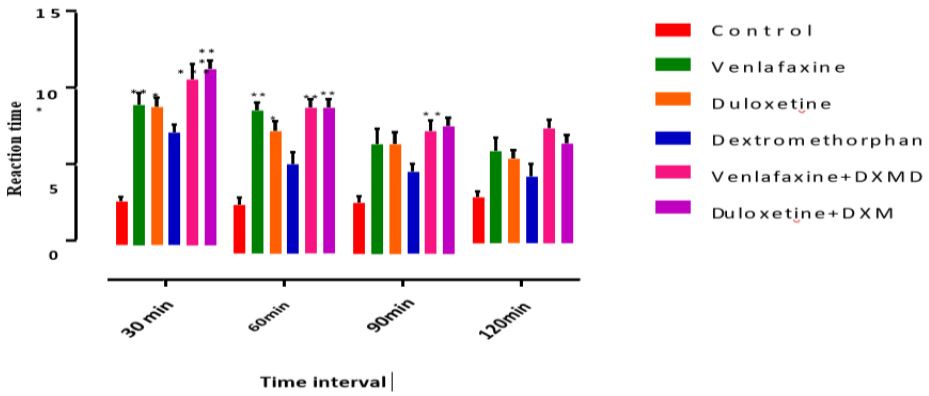

Figure 2: Evaluation of Analgesic Activity–Tail immersion method

Mice were treated with venlafaxine, duloxetine, dextromethorphan, venlafaxine with dextromethorphan and duloxetine with dextromethorphan. All values are expressed as Mean ± SEM (n = 6). The statistical analysis was carried out by one way ANOVA followed by individual comparisons using posthoc Tukey's multiple comparison tests. Where each group compared with that of control group. Where ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

Table 3: Evaluation of Analgesic Activity–Tail immersion method

Group	Reaction time in sec (Mean ±SEM)			
	30 min	**60min**	**90 min**	**120 min**
Control	2.883±0.307	3.167±0.477	3.333±0.422	3.000±0.365
Venlafaxine (8mg/kg)	9.167±0.307**	9.333±0.211**	7.167±0.401	6.000±0.365
Duloxetine (30mg/kg)	9.000±0.258**	8.000±0.258*	7.167±0.307	5.500±0.224
Dextromethorphan (30mg/kg)	7.333±0.211	5.833±0.307	5.333±0.211	5.313±0.121
Venlafaxine+DXM (4+15mg/kg)	12.167±0.307***	10.50±0.224**	8.500±0.224*	5.370±0.211
Duloxetine+DXM (15+15m g/kg)	12.500±0.224***	8.500±0.244**	6.667±0.211*	4.160±0.307

Mice were treated with venlafaxine, duloxetine, dextromethorphan, venlafaxine with dextromethorphan and duloxetine with dextromethorphan. The study was carried out as discussed in 4.2.2.2.1. The statistical analysis was carried out by one way ANOVA followed by individual comparisons using posthoc Tukey's multiple comparison tests. Where each group compared with that of control group. Where ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

DISCUSSION

The main mechanism of antidepressants that inhibit neuropathic pain is first, to increase noradrenaline in the spinal cord, and second, to act on the LC, thereby directly inhibiting pain and activating the impaired descending noradrenergic inhibitory system. Dopamine and 5-HT also increase in the central nervous system and may enhance the inhibitory effects of noradrenaline in an auxiliary manner [13]. The hot plate test is a test of the pain response in animals. It is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat. This method was used as a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with latency, and encompasses an emotional component of escaping. Venlafaxine, duloxetine and dextromethorphan

shows analgesic effect when used as single drugs. When these are combined the analgesic effect was increased [14].

The hot water tail Immersion test unit serves to assess the tail flick reaction of mice and rats when their tail is immersed in a constant temperature bath. the study was conducted by holding, wrapping or placing the animal in a restrainer with the tail exposed. The animal's tail is then immersed into the water bath. The temperature range is from room temperature to 65 degrees Celsius. When the animal reacts by flicking their tail you can stop the timer by either the front panel pushbuttons or footswitch that is supplied, this unit will store and display the time of reaction. Venlafaxine, duloxetine and dextromethorphan shows analgesic effect when used as single drugs[15]. When these are combined the analgesic effect was increased. Many antidepressants block 5-HT transporters, leading to increased 5-HT in the synaptic cleft. The role of 5-HT on the inhibitory effects of antidepressants against neuropathic pain, however, is unclear. Although SSRIs are popular drugs for the treatment of depression, they are not recommended for the treatment of neuropathic pain. Despite some reports of their effectiveness in randomized controlled trials in patients with chronic pain, the NNT for SSRIs is higher than that for TCA and SNRI. For this reason, 5-HT is thought to play a less important role than noradrenaline in the inhibition of neuropathic pain, but simultaneous administration of noradrenaline and 5-HT selective reuptake inhibitors in an animal experiment produce a synergistic analgesic effect, suggesting that 5-HT has auxiliary actions. T in the dorsal horn of the spinal cord greatly contributes to pain modulation. Inhibitory 5-HT_{1A} receptors, and excitatory 5-$HT_{2A/2C}$, 5-HT_3, and 5-HT_7 receptors that strongly contribute to nociceptive transmission are expressed in the dorsal horn of the spinal cord. These receptors are present in the pre-synaptic terminals of primary afferent nerve fibers [16], inhibitory interneurons, excitatory interneurons and projection neurons, and modify nociceptive transmission. When the pain sensation reaches the brain, the descending inhibitory system is mobilized from a variety of sites in the cerebral cortex and activates the periaqueductal gray (PAG). The PAG closely controls the rostroventromedial medulla (RVM) and modifies pain via projecting fibers from the RVM to the dorsal horn of the spinal cord[17]. The RVM includes the nucleus raphe magnus, which projects abundant serotonergic fibers to the spinal cord dorsal horn. Descending serotonergic projections from the RVM to the spinal cord dorsal horn exert both inhibitory and facilitatory effects on pain processing depending on the pain state, acute or chronic. In neuropathic pain models, many studies reported that ablation of descending 5-HT pathways inhibit pain hypersensitivity, and have also demonstrated that nerve injury induces descending facilitation by activating spinal 5-HT_3 receptors. Activation of descending serotonergic neurons form the RVM is required[18], however, for this descending facilitation to occur. In contrast, direct intrathecal injection of 5-HT or a 5-HT_3 agonist inhibits allodynia in a rat neuropathic pain model. Systemic administration of paroxetine[19], an SSRI, produces an anti-hyperalgesic effect in a rat model of neuropathic pain through spinal 5-HT_3 receptors, because the drug directly increases 5-HT in the spinal cord by inhibiting 5-HT transporters[20].

CONCLUSION

The main mechanism of antidepressants that inhibit neuropathic pain is first, to increase noradrenaline in the spinal cord, and second, to act on the LC, thereby directly inhibiting pain and activating the impaired descending noradrenergic inhibitory system. The higher concentration of 5-HT in the central nervous system and may enhance the inhibitory effects of noradrenaline in an auxiliary manner. This may cause the analgesic effect of the antidepressants coming under the category of selective serotonin and noradrenaline reuptake inhibitors. The co administration of dextromethorphan shows a sub additive increase in analgesic action this may due to the increased level of serotonin produced by the dextromethorphan.

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