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Original Article

COMPARATIVE STUDY OF THE ANTINOCICEPTIVE ACTION OF *AMITRIPTYLINE* WITH *FLUOXETINE* AND EVALUATION OF THEIR PROBABLE MECHANISM OF THIS ACTION IN ALBINO MICE

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ABSTRACT

Objective: The aim of our study was to compare the anti-nociceptive action of *amitriptyline* with *fluoxetine* and evaluation of their probable mechanism of anti-nociceptive action by observing their individual interactions with *morphine, naloxone, yohimbine,* and *ondansetron*.

Methods: Albino mice weighing 25-35 grams were taken and divided into 12 groups. Group A-Control(distilled water), Group B-*amitriptyline* 20 mg/kg, Group C-*fluoxetine* 20 mg/kg, Group D-*morphine* 5 mg/kg, Group E-*amitriptyline* 20 mg/kg+ *morphine* 5 mg/kg, Group F-*amitriptyline* 20 mg/kg+ *naloxone* 3 mg/kg, Group G-*amitriptyline* 20 mg/kg+ *yohimbine* 2 mg/kg, Group H-*amitriptyline* 20 mg/kg+ *ondansetron* 0.1 mg/kg, Group I-*fluoxetine* 20 mg/kg+*morphine* 5 mg/kg, Group J-*fluoxetine* 20 mg/kg+ *naloxone* 3 mg/kg, Group K-*fluoxetine* 20 mg/kg+ *yohimbine* 2 mg/kg and Group L-*fluoxetine* 20 mg/kg+ *ondansetron* 0.1 mg/kg. Hot plate method and acetic acid writhing test were used to assess central and peripheral analgesic activity respectively.

Results: Both the *amitriptyline* and *fluoxetine*-treated animals showed significantly increased reaction time in a hot plate ($p<0.05$) and a significant decrease in the number of wriths in acetic acid writhing test ($p<0.05$), when compared with control. Animals in *amitriptyline* group showed significantly higher reaction time and less number of wriths when compared to *fluoxetine* group. *Morphine* increased, while *naloxone*, *yohimbine* and *ondansetron* decreased the reaction time in a hot plate. In the acetic acid writhing test, a number of wriths significantly decreased when co-treated with *morphine* and increased when co-treated with *naloxone*, *yohimbine* and *ondansetron*.

Conclusion: It is concluded that *amitriptyline* is a better antinociceptive agent than *fluoxetine*. Their central and peripheral mechanism of antinociception both involve opioidergic, serotonergic and noradrenergic pathway.

Keywords: Antinociceptive, *Amitriptyline*, *Fluoxetine*, *Morphine*, *Naloxone*, *Yohimbine*

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INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [1]. In the pain pathway, Aδ and C fibres carrying pain terminate in the dorsal horn and excite second order neurons to reach thalamus. From these thalamic areas, the signals are transmitted to basal areas of the brain, as well as to the somatosensory cortex [2]. Endogenous ascending pain modulation occurs at the level of dorsal horn by gate control mechanism. Major components of descending pain control are periaqueductal gray (PAG) and periventricular areas, raphe magnus nucleus (NRM) and pain inhibitory complex located in the dorsal horns of the spinal cord [2].

Several transmitter substances are involved in the analgesia system; especially involved are endogenous opioid peptides (encephalin), serotonin [2] and norepinephrine [3, 4]. The role of opioidergic pathway is well known. Encephalin causes both presynaptic and postsynaptic inhibition of incoming type C and types A**δ** pain fibers at their synapse in dorsal horn [2]. Regarding the role of serotonin, analgesia produced by spinal injection of serotonin (5-HT) appears to be mediated primarily through 5-HT3 receptors [5]. Pretreatment with tropisetran (5HT3 antagonist) reversed the antinociceptive action of intrathecally administered 5-HT [6]. 5HT3 receptors are implicated in the antinociceptive action of paroxetine (antidepressant of selective serotonin reuptake inhibitor class) as *ondansetron* (5-HT3 antagonist) inhibited its antinociceptive activity [7]. Regarding the role of the noradrenergic pathway in pain modulation, the noradrenergic pathway from the locus coeruleus also has an inhibitory effect on transmission in the dorsal horn [3]. It was found that α-2 play an important role in antidepressant-induced antinociception as RX821002 (α-2 antagonist) could block their antinociceptive action but prazosin (α-1 adrenoceptor antagonist) could not [8]. In the spinal cord, norepinephrine released from descending pathways suppresses pain primarily by acting through α-2 receptors [4].

Amitriptyline is a tricyclic antidepressant. *fluoxetine* is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class [9]. In the treatment of neuropathic and other pain disorders, antidepressants are being used, but among these which agents are preferable, there is a universal doubt. Studies reveal contradictory and insufficient results. Rani *et al.*, 1996[10] in their study on *amitriptyline* versus *fluoxetine* in pain conditions found that f*luoxetine* was superior. Nisha Michael *et al.*, 2012 [11] in their comparative study between *amitriptyline* and *fluoxetine* found *amitriptyline* to be superior. The mechanism of antinociceptive action of these antidepressant Compounds (*amitriptyline* and *fluoxetine*) are not yet known. But the onset of analgesic effect of Tricyclic antidepressants is earlier and it occurs at a lower dose than that is typically required for management of depression [12]. So, in our study, we have done a comparative analysis of the antinociceptive activity of *amitriptyline* and *fluoxetine* and evaluated the probable mechanism of this action.

MATERIALS AND METHODS

Drugs and chemicals

Amitriptyline was obtained from Himedia Labs, Bangalore. *fluoxetine* was obtained from Quality Pharma, Dibrugarh. *morphine* was obtained from Verve healthcare Ltd, Delhi. *naloxone* was obtained from Neon laboratories Ltd. *yohimbine* was obtained from himedia Laboratory Pvt. Ltd, Bangalore. *ondansetron* obtained from Alkem Laboratories Ltd, East Sikkim. *Diclofenac Sodium* was obtained from

NOVARTIS co. Pvt. Ltd. *Glacial acetic acid* was obtained from Qualigens (Fisher scientific).

Dose selection

Dose of *amitriptyline* used in this experiment was 20 mg/kg [13, 14], *fluoxetine* 20 mg/kg [15], *morphine* 5 mg/kg [16], *naloxone*3 mg/kg [17], *yohimbine* 2 mg/kg [18], *ondansetron* 0.1 mg/kg [7] and diclofenac 5 mg/kg [19].

Experimental animals

Healthy male swiss albino mice (25-35 grams) were taken from the Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages under standard conditions of light and dark cycle and maintained under normal room temperature. The animals were fed with a normal diet and water *ad libitum*. Before commencing the work permission from the Institutional Animal Ethics Committee was taken and conducted according to CPCSEA guidelines.

Experimental design

Albino mice (male) weighing 25-35 grams were taken and divided into 11 groups.

Group A-Control (Distilled water),

Group B-*Amitriptyline* 20 mg/kg,

Group C-*Fluoxetine* 20 mg/kg,

Group D-*Morphine* 5 mg/kg

Group E-*Amitriptyline* 20 mg/kg+*Morphine* 5 mg/kg,

Group F-*Amitriptyline* 20 mg/kg+*Naloxone* 3 mg/kg,

Group G-*Amitriptyline* 20 mg/kg+*Yohimbine* 2 mg/kg,

Group H-*Amitriptyline* 20 mg/kg+*Ondansetron* 0.1 mg/kg,

Group I-*Fluoxetine*20 mg/kg+*morphine* 5 mg/kg,

Group J-*Fluoxetine* 20 mg/kg+*Naloxone* 3 mg/kg,

Group K-*Fluoxetine* 20 mg/kg+*Yohimbine* 2 mg/kg

Group L-*Fluoxetine* 20 mg/kg+*Ondansetron* 0.1 mg/kg.

Central analgesic activity

The central analgesic activity was tested by Hot Plate method in albino mice. Mice were placed on an aluminum hot plate kept at 55 °C [20]. The reaction time is the time eclipsed between placing the animal on a hot plate to first paw licking (hind paw) or Jumping behavior shown by the animal (whichever appear first) [21]. The Pre-Drug reaction time for each animal was recorded. After that Distilled water and test, drugs were injected intraperitoneally and reaction time was noted at 20, 60 and 90 min after drug administration. Cut off time of 15 seconds was taken to avoid thermal injury to the paws of mice [22]. *Morphine* (5 mg/kg I. P.) was used as a reference standard [16].

Peripheral analgesic activity testing

The peripheral analgesic activity was tested by glacial acetic acid-induced Writhing test in albino mice. Writhing or stretching syndrome is characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs [23].

Distilled water and test drugs were injected intraperitoneally (I. P) 15 min before subjecting the animals to 0.6% acetic acid I. P. (1 ml/100 gram) [22]. The number of writhing responses was counted for 20 min. Index of analgesia is referred to as the percentage of protection against abdominal constriction [24]. It is calculated as:

$$\text{Index of analgesia} = \frac{\text{No.of writhing in control group} - \text{No.of writhing in treated group}}{\text{No of writhing in control group.}}$$

Diclofenac Sodium (5 mg/kg I. P.) was used as a reference standard [19].

Statistical analysis

The results were statistically analyzed using one-way ANOVA followed by dunnett's multiple comparison tests and bonferroni's test. Student's t-test (paired) was applied to compare pre-drug reaction time with post-drug reaction time at 20, 60 and 90 min individually. The statistical analysis was done using Graph pad prism software version 5.00. p values<0.05 were considered significant.

RESULTS

Analgesic activity of *amitriptyline* and *fluoxetine* on a hot plate and acetic acid-induced writhing test

The results are depicted in table 1. In hot plate method, both *amitriptyline* and *fluoxetine*-treated mice showed a significant increase in mean reaction time compared to the control group at 20, 60 and 90 min.

In acetic acid-induced writhing test, with regard to a number of writhing movement, both *amitriptyline* and *fluoxetine*-treated animals showed significant protection when compared to control group. Regarding percentage protection, *amitriptyline* group showed 79% protection and *fluoxetine* group showed 63% protection.

It is seen that *amitriptyline* is superior to *fluoxetine* with regard to analgesic activity in both the models both as a central analgesic and as a peripherally acting agent.

Table 1: Analgesic activity of *amitriptyline* and *fluoxetine* in hot plate test and acetic acid-induced writhing test

Group	Treatment	Reaction time in hot plate test (sec)				Acetic acid induced writhing test	
		Pre-drug	20 min	60 min	90 min	No of wriths	% protection
A	Control	4.6±0.24	4.36±0.18	3.888±0.35	4.080±0.25	59.2±1.9	
B	*Amitriptyline*	4.582±0.37	7.528±0.41a,b	8.266±0.37a,b	9.502±0.48a,b	12.4±0.5a	79%
C	*Fluoxetine*	4.226±0.36	6.524±0.33a,b,c	6.496±0.05a,b,c	6.432±0.53a,b,c	21.6±1.1a,c	63%
D	*Morphine*	4.236±0.11	7.794±0.11a,b	9.540±0.21a,b	10.11±0.24a,b	10±0.6a	82%

Values are expressed as mean±SEM; (n=5). Oneway ANOVA followed by dunnett's multiple comparison test. ANOVA followed by bonferroni's test was done between group B and C. Paired t test was done between pre-drug reaction time versus post drug reaction time at 20, 60 and 90 min. a p<0.05 when compared to control group. b p<0.05 when compared to pre-drug reaction time. c p<0.05 when compared to group B.

Analgesic activity of *amitriptyline* and its combination with *morphine*, *naloxone, yohimbine,* and *ondansetron* in hot plate and acetic acid writhing test

The results are depicted in table 2. In central analgesic model, *amitriptyline+morphine* group showed a statistically significant increase in mean reaction time at 60 and 90 min when compared to *amitriptyline* group. *Amitriptyline+naloxone*, *amitriptyline+ yohimbine,* and *amitriptyline+ondansetron* group recorded a significant decrease in mean reaction time was seen at 20, 60 and 90 min when compared to *amitriptyline* group.

In the peripheral analgesic model, *amitriptyline+morphine* group showed a significant decrease in the number of writhing and an increase in percentage protection while *amitriptyline+naloxone*, *amitriptyline+ yohimbine* and *amitriptyline+ondansetron* group recorded a significant increase in the number of writhing and a decrease in percentage protection when compared to *amitriptyline* treated group.

Table 2: Analgesic activity of *amitriptyline* and its combination with *morphine, naloxone, yohimbine* and *ondansetron* in the hot plate and acetic acid writhing test

Group	Treatment	Reaction time in hot plate (sec)				Acetic acid induced writhing test	
		Pre-drug	20 min	60 min	90 min	No of wriths	% protection
B	*Amitriptyline*	4.58±0.37	7.53±0.41b	8.27±0.37b	9.5±0.48b	12.4±0.5	79%
E	*Amitriptyline +Morphine*	4.4±0.39	7.99±0.43b	13.11± 0.62a,b	13.83±0.65a,b	0a	100%
F	*Amitriptyline +Naloxone*	4.8±0.59	1.77±0.55a,b	1.044±0.17a,b	1.3±0.17a,b	54.2±1.3a	8%
G	*Amitriptyline +Yohimbine*	4.3±0.53	1.68±0.44a,b	1.238±0.22a,b	1.67±0.40a,b	58±0.9a	2%
H	*Amitriptyline+ Ondansetron*	4.68±0.41	2.85±0.44a,b	2.998±0.76a,b	1.09±0.15a,b	50.4±0.9a	14%

Values are expressed as mean±SEM; (n=5). One-way ANOVA followed by dunnett's test. Paired t-test was done between pre-drug reaction time versus post drug reaction time at 20, 60 and 90 min. a=p<0.05 when compared to group B. b=p<0.05 when compared to pre-drug reaction time.

Analgesic activity of fluoxetine and its combinations with morphine, naloxone yohimbine and ondansetron in hot plate and acetic acid writhing test

The results are depicted in table 3. In central analgesic model, *fluoxetine+morphine* group showed a statistically significant increase in mean reaction time at 60 and 90 min when compared to *fluoxetine* treated group. *Fluoxetine+naloxone*, *fluoxetine+yohimbine* and *fluoxetine+ondansetron* group showed a significant decrease in mean reaction time at 20, 60 and 90 min, when compared to fluoxetine, treated group.

In the peripheral analgesic model, the *fluoxetine+morphine* group showed a significant decrease in the number of writhing and an increase in percentage protection while *fluoxetine+naloxone*, *fluoxetine+yohimbine* and *fluoxetine+ondansetron* group recorded a significant increase in the number of writhing and a decrease in percentage protection when compared to *Fluoxetine* treated group.

Table 3: Analgesic activity of fluoxetine and its combinations with morphine, naloxone yohimbine and ondansetron in hot plate and acetic acid writhing test

Group	Treatment	Reaction time in Hotplate (sec)				Acetic acid induced writhing test	
		Pre-drug	20 min	60 min	90 min	No of wriths	% protection
C	*Fluoxetine*	4.22±0.35	6.52±0.33b	6.49±0.51b	6.4±0.53b	21.6±1	63%
I	*Fluoxetine +Morphine*	4.11±0.35	7.48±0.29b	10.02± 0.97a,b	11.92±0.39a,b	0a	100%
J	*Fluoxetine +Naloxone*	4.032±0.26	1.49± 0.31a,b	0.88± 0.18a,b	1.65± 0.41a,b	54.8± 0.86a	7%
K	*Fluoxetine +Yohimbine*	4.124±0.41	2.67± 0.76a,b	2.62±0.12a,b	2.92± 1.56a,b	50± 0.70a	15%
L	*Fluoxetine+ Ondansetron*	3.838±0.27	1.17± 0.11a,b	0.97± 0.09a,b	0.99± 0.22a,b	57.4± 1.36a	3%

Values are expressed as mean±SEM; (n=5). One-way ANOVA followed by Dunnett's test. Paired t-test was done between pre-drug reaction time versus post-drug reaction time at 20, 60 and 90 min. a p<0.05 when compared to group C. b p<0.05 when compared to pre-drug reaction time.

DISCUSSION

In our present study, we found that both *amitriptyline* and *fluoxetine* are effective as an analgesic agent in both the central model and peripheral model of analgesic activity.

Regarding central anti-nociceptive action, in our experiment, antinociceptive actions of *amitriptyline* and *fluoxetine* were synergized by *morphine,* and it was antagonized by *naloxone* (opioid receptor antagonist), *yohimbine* (α-2 antagonist) and *ondansetron* (5-HT3receptor antagonist) in hot plate method. Thus, it seems that their central analgesic action involves opioidergic, serotonergic and noradrenergic pathways. *Fluoxetine* and *amitriptyline* are reported to increase the density of opioid receptors [25-27]. They also cause the release of endogenous opioid peptides [27]. Acetorphan (encephalinase inhibitor) potentiated the antinociceptive activity of antidepressants indicating a clear role of opioidergic system in antidepra essant-induced nociception [28]. These drugs are also reported to act directly on opioid receptors [29]. Antidepressants can displace opioids from binding sites in radioligand binding assays [30]. For their opioidergic action, fluoxetine may be acting through μ [31, 32] opioid receptor. Amitriptyline seems to be acting through μ [28], κ [29th] and δ [28, 29] opioid receptors. Regarding the involvement of serotonergic and noradrenergic pathway, NE and 5-HT may activate pain inhibitory interneurons in the superficial layers of dorsal horn [33]. 5-HT facilitates the release of beta-endorphin [34]. Antidepressants which increase the availability of NE and 5HT leads to enhancement of opioid pathways [35]. The opioid peptides also modulate Locus Coeruleus (LC). Activation of presynaptic delta-opioid receptors inhibits GABA release and thus excites spinally projecting noradrenergic LC neurons [36]. Alpha-2 adrenoceptors modulate pain in many ways. They may cause presynaptic inhibition (at the level of primary afferent nociceptors), inhibition of pain-relaying spinothalamic neurons (postsynaptic inhibition). Alpha-2 Cadrenoceptors present on excitatory interneurons in the dorsal horn of spinal cord contributes to spinal control of pain [4].

Coming to peripheral antinociceptive action, anti-nociceptive actions of *amitriptyline* and *fluoxetine* were synergized by *morphine* and antagonized by *naloxone*, *yohimbine,* and *ondansetron* in the peripheral analgesic model (acetic acid-induced writhing test). Thus, it can be said that their peripheral analgesic action also involves opioidergic, noradrenergic and serotonergic mechanisms. A prostaglandin synthesis inhibitory action may also be present. Regarding the involvement of opioidergic system in the peripheral antinociceptive action, it is a known fact that immune cells contain numerous opioid peptides but the predominant opioid peptides involved in immune-cell mediated antinociception are thought to be endorphin and encephalin [37]. Endogenous opioid peptides are released by immune cells to reduce inflammatory pain. noradrenaline, corticotropin-releasing factor (CRF) and interleukin-1α stimulate immune cells to secrete their opioid peptides [37]. These opioid peptides cause inhibition of pro-inflammatory neuropeptide release and sensory neuron excitability [38]. Opioid mimicking or enhancing a property of the antidepressants may also play a role in their peripheral analgesic action [30]. Regarding the involvement of serotonergic pathway in the peripheral antinociceptive action, in our study, in the peripheral analgesic model, 5-HT3 antagonist *ondansetron* antagonized the antinociceptive action of both *amitriptyline* and *fluoxetine* in the acetic acid writhing model. Similar observation was also made by Kesim M *et al.*, 2005 [7] with paroxetine. But the exact mechanism of how peripheral 5-HT3 modulation is involved in the peripheral antinociceptive action of both the drugs is still not clear. Pain sensation in acetic acid induced writhing method is due to prostaglandin biosynthesis (Via cyclooxygenase pathway) triggered by localized inflammatory reaction [39, 40]. So, agents which reduce the number of writhing will render their effect preferably by inhibition of prostaglandin biosynthesis [39, 40]. In our study, both the antidepressants were effective in preventing acetic acid-induced writhing movements in mice. So, a prostaglandin synthesis inhibitory action of the drugs (*amitriptyline* and *fluoxetine*) also may be present and which may be responsible in part for their peripheral antinociceptive action. This needs further investigation.

CONCLUSION

In this study, it was found that both *amitriptyline* and *fluoxetine* has antinociceptive property and *amitriptyline* is a better antinociceptive agent than *fluoxetine*. Their mechanism of central antinociceptive action may involve opioidergic actions, serotonin reuptake inhibitory action, and norepinephrine reuptake inhibitor property of both the drugs. A complex interaction of all the three systems may be playing the main role.

Regarding peripheral antinociceptive action, it has been observed that their interaction with opioidergic system and noradrenaline mediated enhancement of peripheral opioidergic system seems to play the main role. Additional 5-HT3 receptor-mediated or prostaglandin synthesis inhibitory action may also be involved in their peripheral analgesic action. Again, antidepressants are known to interact with many other receptor systems. So, some of these systems may also take part in their analgesic action. This needs further investigation.

AUTHORS CONTRIBUTIONS

Both the authors contributed equally in protocol preparation, ethical approval, the conduct of the experiment, data collection, analysis, and manuscript preparation.

CONFLICT OF INTERESTS

Nil

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