

# Evaluation of Antidepressant and Antinociceptive Activity of Escitalopram

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## ABSTRACT

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Escitalopram, the S- (+) -enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities.

In this present study, antidepressant activity and antinociceptive effects of escitalopram (ESC, 40 mg/kg) have been studied in forced swim test, tail suspension test, hot plate test, acetic acid writhing and formalin tests in rodents. A significant ( $P < 0.05$ ) antidepressant-like effect was shown at dose of 40 mg/kg, which was due to reduction in the immobility period. ESC 40 mg/kg also presented an analgesic effect, in our studies. ESC 40mg/kg produced a significant ( $P < 0.05$ ) % increase in latency at time intervals 60, 90 and 120 min in thermal pain studies in mice. It also significantly inhibited acetic acid induced abdominal constrictions, with the maximal effect (55.95 % protection) at 40 mg/kg. In formalin induced paw licking pain model, ESC exhibited more pronounced effect in the inflammatory phase than the neurogenic phase (maximal effect was  $54.17 \pm 3.89$  s and  $87.83 \pm 1.64$  s, respectively, at 40 mg/kg, i.p.). The present study showed that ESC has equal efficacy in comorbid pain and depression-like behaviour mediated through serotonergic system.

**Keywords:** depression, forced swim test, tail suspension test, antinociceptive, pain models

## INTRODUCTION

The selective serotonin reuptake inhibitors (SSRIs) have gained extensive clinical use during the past two decades, and are drugs of choice for the treatment of depression and anxiety disorders.<sup>1</sup> The introduction of SSRI's such as paroxetine, fluoxetine, sertraline and fluvoxamine have offered patients new alternatives in the psychopharmacological management of depressive disorders. They are also used in treating fibromyalgia, diabetic neuropathy, migraine headache, tension-type headache, and mixed chronic pain.<sup>2</sup>

The primary mode of SSRI action is binding to the serotonin transporter (5-HTT), inhibiting its capacity to transport serotonin and, thus, raising the synaptic serotonin level, with consequently increased stimulation of one or more types of serotonergic (5-HT) receptor. The down-regulation of 5-HT receptors, particularly the 1A and 2 subtypes, may be linked to the antidepressant action of SSRIs and related pharmaceutical agents.<sup>3</sup>

Escitalopram, an allosteric serotonin reuptake inhibitor (ASRI)<sup>1</sup>, is the therapeutically active S-stereoisomer of racemic citalopram and is significantly more effective than citalopram, which also includes the R-stereoisomer.<sup>1</sup> In radioligand binding studies of cells expressing human

serotonin transporters, escitalopram proved to be approximately 30 times more potent than its enantiomer, R-citalopram in its capacity to bind to the serotonin transporter receptor site.<sup>3</sup> This finding confirms preclinical studies demonstrating that the antidepressant effect of citalopram primarily resides with S-enantiomer.<sup>4</sup> It is also extremely selective for serotonergic transport proteins relative to noradrenergic or dopaminergic binding sites, when compared directly with fluoxetine, paroxetine, fluvoxamine or sertraline.<sup>2</sup> Selectivity for serotonergic rather than muscarinic, histaminergic or adrenergic receptors suggests a lower potential for causing dry mouth, sedation or cardiovascular side effects.

The association of pain and depression represents an important health problem that is correlated with high rates of disability, morbidity, greater consumption of health care. Both are inexorably linked in a complex way.<sup>5</sup> Recognition of the overlap between persistent pain and depression has led to increased interest in the biological mechanisms linking pain and depression. Persistent pain conditions and depression, however, are heterogeneous.<sup>6</sup> The convergence of depression and pain is reflected in the circuitry of the nervous system. In the experience of pain, communication between body and brain goes both ways. Brain pathways that handle the reception of pain signals, including the seat of emotions in the limbic region, use some of the same neurotransmitters involved in the regulation of mood, especially serotonin (5-HT) and norepinephrine (NE). Previous studies have suggested the common biological pathways and

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neurotransmitters (serotonin and norepinephrine) may be involved in the mechanisms of pain and depression.<sup>7-9</sup>

The principal objective of the treatment of pain is to remove or abolish the cause of pain. But it is not always possible to do so; hence, analgesics are used for the symptomatic treatment of pain. Opioids are the most potent and commonly used group of analgesic drugs e.g. morphine and pethidine. But their analgesic action is associated with a greater degree of adverse drug reactions, most of which are dose-dependent. Recently, numerous open and controlled studies have shown that antidepressant drugs also have analgesic activity and particularly, selective serotonin reuptake inhibitors (SSRI) are effective in mixed, chronic pain.<sup>10</sup> It has also been shown that some antidepressants are superior to placebo in about 75% of studies.<sup>11</sup> Chronic administration of escitalopram desensitizes 5-HT<sub>1A</sub> receptors, controlling the release of 5-HT in the prefrontal cortex and enhances the effect of the drug on extracellular 5-HT thereby inhibiting reuptake.<sup>12</sup> With this background the present study revolves around establishing a proof of concept for the antidepressant activity and investigating the antinociceptive potential of escitalopram, on various experimental animal models of depression and pain.

## MATERIALS AND METHODS

**Animals-** In-bred male Swiss Albino mice (20–25 g) and male Wistar rats (220–250 g) were used. The animals were maintained under standard light condition with food and water ad libitum, under 12 hour light/ 12 hour dark cycle. The experimental protocols were approved by IAEC (Institutional Animal Ethics Committee) of Bombay College of Pharmacy.

**Drugs and Chemicals-** Amitriptyline (Glenmark Pharmaceuticals, Mumbai), Fluoxetine hydrochloride (Sandoz, Mumbai), Nortriptyline hydrochloride, Tramadol, Aspirin (MKR Laboratories, Mumbai) and Escitalopram (Shodhana Laboratories, Hyderabad) were obtained as gift samples. The drugs were freshly prepared in saline and injected intraperitoneally in a constant volume of 10 ml/kg.

### Screening Antidepressant activity

Male Swiss Albino mice were divided into five groups, six animals in each for forced swim and tail suspension tests. Group I (control; vehicle treated), group II [amitriptyline (AMI) 10 mg/kg]<sup>13</sup>, group III [fluoxetine (FLU) 20 mg/kg]<sup>14</sup>, group IV [nortriptyline (NOR) 20 mg/kg]<sup>15</sup> and group V (Escitalopram (ESC) 40 mg/kg). All the animals were subjected to the respective treatments intraperitoneally for a period of 5 days for forced swim test and 10 days for tail suspension test.

### Forced Swim test

The forced swim test was carried out as described elsewhere<sup>16</sup>

with slight modifications. Mice were dropped individually into a plexi-glass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water to a depth of 15 cm and maintained at 23–25 °C. In this test, after an initial vigorous activity (2 min), the mice acquire an immobile posture which was characterized by motionless floating in the water, making only those movements necessary to keep the head above the water. The duration of immobility which reflects the state of depression was recorded during the last 4min of the 6 min test. On day 5, the mice were subjected to 15 min-training session under similar conditions and dosed thereafter. On day 6, the period of immobility was noted, one hour post dosing.

### Tail suspension test

On 10<sup>th</sup> day, the mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm).<sup>17</sup> Typically, mice exhibited several escape-oriented behaviour interspersed with temporally increasing bouts of immobility.<sup>9</sup> The duration of immobility (in seconds) during the 6-min test session was recorded.

### Screening Antinociceptive activity

Male Swiss Albino mice were divided into ten groups, six animals in each for hot plate test. Group I (control; distilled water p.o), group II (control; 0.9% saline i.p), group III (AMI 10 mg/kg i.p),<sup>18</sup> group IV (FLU 10 mg/kg i.p),<sup>19</sup> group V (FLU 20 mg/kg i.p),<sup>14</sup> group VI (NOR 10 mg/kg i.p),<sup>20</sup> group VII (NOR 20 mg/kg i.p),<sup>15</sup> group VIII [tramadol 25 (TRA) mg/kg i.p],<sup>21</sup> group IX [aspirin (ASA) 100 mg/kg p.o]<sup>21</sup> and group X (ESC 40 mg/kg i.p).

In acetic acid induced writhing test, mice were divided into nine groups, six animals each. Tramadol was not included in this test, since it is a centrally acting analgesic.

In formalin test, male Wistar rats were divided into seven groups of six animals each. Group I (control; 0.9% saline i.p), group II (control; distilled water p.o), group III (amitriptyline 10 mg/kg i.p), group IV (fluoxetine 20 mg/kg i.p), group V (nortriptyline 20 mg/kg i.p), group VI (aspirin 100 mg/kg p.o) and group VII (escitalopram 40 mg/kg i.p).

### Hot-plate test

The hot-plate test was used to measure the response latencies according to the method described by Viana *et al.*<sup>22</sup> In these experiments, the hot plate apparatus (Ugo Basile, Model-DS 37) was maintained at 53 ± 1°C. Animals were placed on the heated surface and the time between placement and licking of the paws or jumping was recorded as latency.<sup>23</sup> The animal was immediately removed from the hot plate by the investigator to prevent any tissue damage to the hind paws. Each mouse was tested twice before administration of

drugs and the reaction times were averaged to obtain a baseline.<sup>23</sup> In order to avoid burns, the maximal time of the hot plate experiment did not exceed 60 seconds. The standard and test compounds were administered after animal selection on time of 30 minutes. The selection was made on the basis of the reactivity on the test.<sup>23</sup> Pre-treatment times 0 and 30 minutes were used for assay adaptation and selection of the animals, respectively. Only mice showing a reaction time within the range of 4-10 sec. were used in this test. The latency of the reaction to nociception was measured at time 0 and then at 30, 60, 90, 120 and 180 minutes.<sup>23</sup>

### Writhing test

In brief, the selected groups of animals, consisting of six mice per group were pretreated (60 minutes prior to acetic injection) with standard and test drugs.<sup>23</sup> Five minutes after the i.p. injection of 0.6 % acetic acid (0.25 ml), the number of writhing exhibited by each mouse was counted for 20 min. The antinociceptive activity was expressed as the reduction on the number of abdominal writhing. For scoring purpose, a writh is indicated as stretching of the abdomen with simultaneous stretching of at least one hind limb. Effect of drugs on acetic-acid induced writhing was expressed as % Protection (i.e. compound producing 100% protection prevents acetic acid-induced abdominal constriction).<sup>24</sup>

### Formula for calculating % Protection<sup>25</sup>

$$\% \text{ Protection} = 1 - \frac{\text{Number of writhes in drug treated}}{\text{number of writhes in control}} \times 100$$

### Formalin-induced nociception in rats

The formalin test was performed according to the method of Hunskaar and Hole.<sup>26</sup> Briefly, 20  $\mu$ l of a 2.5% (v/v) solution of formalin in saline was injected into the sub plantar region of the right hind paw and the quantification of the time that the animal spent licking the right hind paw during the first 5 min (first phase) and from 15 to 30 min (second phase) of post-injection time was recorded. The test was performed at ambient temperature of 22-26°C and care was taken to exclude environmental disturbances (high temperature, noise and excessive movement) that might interfere with the animal's response.<sup>27</sup>

### Statistical analysis:

Data obtained from animal experiments were expressed as the mean standard error (Mean  $\pm$  S.E.M.). Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests.  $P < 0.05$  was considered to be significant.

## RESULTS

**Forced swim test and Tail suspension test-** These tests are most widely used tool for assessing antidepressant activity pre-clinically. Table.1. showed the mean duration of immobility in the FST and TST in the vehicle, amitriptyline-, nortriptyline-, fluoxetine- and escitalopram- treated groups. As can be seen, escitalopram, fluoxetine, amitriptyline and nortriptyline significantly ( $P < 0.05$ ) decreased the duration of immobility in mice compared with vehicle treatment. The duration of immobility period obtained with escitalopram ( $37.17 \pm 2.32$  seconds) treatment in FST was comparable with the values obtained by amitriptyline ( $33.67 \pm 1.59$  seconds) and nortriptyline ( $35.67 \pm 1.09$  seconds). But, the duration of immobility period obtained with escitalopram ( $40.17 \pm 1.60$  seconds) treatment in TST was more compared to the values obtained by amitriptyline ( $38.17 \pm 2.11$  seconds), nortriptyline ( $37.17 \pm 2.18$  seconds) and fluoxetine ( $35.67 \pm 1.43$  seconds).

**Hot-plate test** – Fig.1. showed that the onset of antinociceptive activity of escitalopram (40 mg/kg) in the hot-plate paradigm was at 60 min after its intraperitoneal administration, an effect that lasted up to 120 min post-treatment. The change in response latency for escitalopram, 60 min after injection ( $5.99 \pm 0.15$  s), was significant ( $p < 0.05$ ) when compared with corresponding vehicle levels ( $1.37 \pm 0.06$  s). It was also significant ( $p < 0.05$ ) with respect to the change in response latency for fluoxetine 20 mg/kg ( $4.23 \pm 0.4$  s) and nortriptyline 10 mg/kg ( $3.34 \pm 0.4$  s) 60 min after injection.

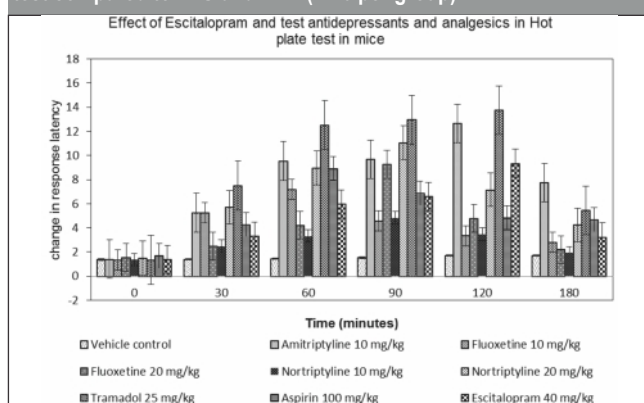
**Writhing test-** The results depicted in Table. 2 show that escitalopram, given 60 minutes beforehand, produced an inhibition of acetic acid induced abdominal constrictions in mice. The treatment of mice with amitriptyline, fluoxetine, nortriptyline, aspirin and escitalopram produced marked inhibition of acetic acid induced writhing response. The inhibitions were 77.08%, 75.70%, 61.47%, 53.04%, 55.95% respectively.

### Formalin-induced nociception in rats-

In assessing the antinociceptive activity in this test of nociception induced by formalin depicted in Table.3., escitalopram exhibited more pronounced antinociceptive activity in the inflammatory phase than the neurogenic phase ( $54.17 \pm 3.89$  seconds and  $87.83 \pm 1.64$  seconds, respectively), which was significant ( $P < 0.05$ ) compared to the vehicle control ( $129.17 \pm 3.43$  seconds and  $85.83 \pm 2.17$  seconds, respectively). The treatment with amitriptyline (10 mg/kg, i.p), fluoxetine (20 mg/kg, i.p.), nortriptyline (20 mg/kg, i.p.) and aspirin (100 mg/kg, p.o.) were also able to reduce the licking time by  $45.83 \pm 0.87$ s,  $34.50 \pm 2.79$ s,  $33.33 \pm 1.63$ s and  $35.33 \pm 0.88$ s, respectively, in the inflammatory phase as compared to the neurogenic phase.

**Table 1: Effect of AMI, FLU, NOR and ESC on duration of immobility (s) in mice FST and TST. Values are mean  $\pm$  S.E.M. for 6 mice ( $p < 0.05$ )**

Groups	Immobility Period (seconds) in FST	Immobility Period (seconds) in TST
Vehicle Control (i.p Saline)	134.67 $\pm$ 3.09	83.33 $\pm$ 4.1
Amitriptyline 10 mg/kg (i.p)	33.67 $\pm$ 1.59*	38.17 $\pm$ 2.11*
Fluoxetine 20mg/kg (i.p)	67.83 $\pm$ 4.4*	35.67 $\pm$ 1.43*
Nortriptyline 20 mg/kg (i.p)	35.67 $\pm$ 1.09*	37.17 $\pm$ 2.18*
Escitalopram 40 mg/kg (i.p)	37.17 $\pm$ 2.32*	40.17 $\pm$ 1.60*

**Fig.1. Effect of AMI, FLU, NOR, TRA, ASA and ESC on latency time of mice exposed to hot plate test. The columns represent mean of values. Error bars indicate  $\pm$  S.E.M. \*  $p < 0.05$  vs. vehicle control group. ESC significantly ( $P < 0.05$ ) increased the latency of hot plate test compared to FLU and TRA (n= 6 per group).**

**Table.2. Effect of AMI, FLU, NOR, ASA and ESC in acetic acid induced abdominal writhing in mice. Values are mean  $\pm$  S.E.M. for 6 mice ( $p < 0.05$ )**

Treatment (mg/kg)	No. of Writhes in 20 min	% Protection
Vehicle Control (i.p)	36.33 $\pm$ 0.76	--
Vehicle Control (p.o)	35.50 $\pm$ 0.62	--
Amitriptyline (10 mg/kg i.p)	8.33 $\pm$ 1.38*	77.08
Fluoxetine (10 mg/kg i.p)	15.83 $\pm$ 1.43*	56.43
Fluoxetine (20 mg/kg i.p)	8.83 $\pm$ 0.65*	75.70
Nortriptyline(10 mg/kg i.p)	16.67 $\pm$ 0.88*	54.12
Nortriptyline(20 mg/kg i.p)	14.00 $\pm$ 0.86*	61.47
Aspirin (100 mg/kg p.o)	16.67 $\pm$ 1.11*	53.04
Escitalopram (40 mg/kg i.p)	16.00 $\pm$ 1.61*	55.95

**Table.3. Effect of AMI, FLU, NOR, ASA and ESC in formalin induced nociception in rats. Values are mean  $\pm$  S.E.M. for 6 mice ( $p < 0.05$ )**

Treatment (mg/kg)	Licking time (s)	
	0-5 min	20-30 min
Vehicle Control (i.p)	129.17 $\pm$ 3.43	85.83 $\pm$ 2.17
Vehicle Control (p.o)	128.00 $\pm$ 1.99	86.33 $\pm$ 2.11
Amitriptyline (10 mg/kg i.p)	72.00 $\pm$ 2.96*	45.83 $\pm$ 0.87*
Fluoxetine (20 mg/kg i.p)	67.83 $\pm$ 3.55*	34.50 $\pm$ 2.79*
Nortriptyline(20 mg/kg i.p)	82.83 $\pm$ 1.58*	33.33 $\pm$ 1.63*
Aspirin (100 mg/kg p.o)	89.33 $\pm$ 2.97*	35.33 $\pm$ 0.88*
Escitalopram (40 mg/kg i.p)	87.83 $\pm$ 1.64*	54.17 $\pm$ 3.89*

## DISCUSSION

In the present study, the antidepressant like effects of escitalopram was evaluated in animal models of depression. The practice of using whole animal assays has become the rapid method to predict the psychopharmacological effect of compounds and drug interaction studies have made a pivotal contribution to unearth antidepressant mechanism.<sup>28, 29</sup> Thus, this investigation included the acute animal models of depression like forced swim and tail suspension tests in mice.

The anti-depressant-like activity of a drug is expressed by a decreased duration of immobility in FST and TST. Both these models of depression are widely used to screen new chemical entities for anti-depressant potential.<sup>30, 31</sup> These tests are quite sensitive and relatively specific to all class of antidepressants. Pimozide, a novel 5-HT<sub>7</sub> receptor antagonist (1 and 2 mg/kg) produced a significant reduction in the duration of immobility as compared to control group.<sup>32</sup> Pretreatment with escitalopram significantly reduced the duration of immobility in mice FST and TST indicating antidepressant like effect. Antidepressant-like activity of escitalopram might be attributed to selective 5-HT<sub>1A</sub> receptor desensitization and by modulating neuronal release of neurotransmitters.<sup>12</sup>

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage. Pain is always a subjective feeling.<sup>33</sup> One of the objectives of the treatment of pain is to remove the cause of pain. Opioids have been the mainstay of pain treatment for even today. Opioids exert their therapeutic effect by mimicking the action of endogenous opioid peptides at opioid receptors.<sup>34</sup> Several clinical and laboratory studies have reported antinociceptive activity of antidepressants. Antidepressants are reported to be more effective than opioid analgesics in treating neuropathic or differentiation pain.<sup>35</sup> Inhibition of the reuptake of monoamines is considered to be a major effect of antidepressants.<sup>35</sup>

Several acute and chronic pain models in rodents were employed in evaluating the analgesic effect of escitalopram. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of analgesic properties of a substance using behavioral nociceptive tests.<sup>36</sup> The results obtained indicate that escitalopram possesses an analgesic effect on the various pain models used.

Acetic acid causes inflammatory pain by increasing capillary permeability.<sup>37</sup> Writhes induced by noxious chemicals injected intraperitoneally is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild analgesic non-steroidal anti-inflammatory compounds.<sup>38</sup> Escitalopram caused a significant inhibition of

writes in mice. The effect was comparable to that of acetylsalicylic acid, a cyclooxygenase inhibitor. This suggests that escitalopram may have a peripheral analgesic action. Escitalopram showed significant effects in the hot plate. Centrally acting analgesic drugs alleviate pain threshold of animals to heat. The hot plate induced pain indicates narcotic involvement,<sup>39</sup> whereas the tail flick test is considered selective for opioid-like analgesic compounds. The results obtained indicate a significant, time related analgesic activity of escitalopram in both the hot plate assay. The effect of escitalopram on these pain models indicate that it might be centrally acting. Escitalopram inhibited both phases of the formalin-induced pain, with a more intense effect at the inflammatory phase than the neurogenic phase. Formalin exhibits neurogenic, inflammatory and tonic pain as in clinical pain situations. Drugs which act mainly centrally such as narcotic analgesics inhibit both phases of pain in this model while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase.<sup>40</sup> The inhibitory effect of the extract on both phases of the formalin pain model confirms its analgesic activity via central and peripheral mechanisms.

## CONCLUSION

With the results of the present study taken together, it is concluded that escitalopram possesses analgesic activity, which may be mediated via peripheral and central mechanisms. This provides evidence for its use in human medicine to relief pain in the treatment of ailments accompanied with pain and depression.

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