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Evaluation of Anti-depressant and Analgesic- Like Activity of Ondansetron in Rodents Model of Co-morbid Pain and Depression

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Abstract

Although a clinical connection between pain and depression has long been recognized, how these two conditions interact remains unclear. Here we report the comorbidity of pain and depression-like behavior. The antidepressant and analgesic like activity of Ondansetron (OND, 0.25-2mg/kg) were investigated in animal models of acute depression (forced swim test) and pain (tail flick test) , existence of co-morbidity of pain and depression was simulated in surgical models in rats like olfactory bulbectomy (OBX) model of chronic depression and chronic constrictive injury (CCI) of chronic pain conditions. Acute administration of OND (0.5-2mg/kg i.p) significantly reduced the duration of immobility in mice in FST and increased the time latency in tail flick test. Behavioural anomalies shown by OBX rats in open field test was significantly reversed by OND (1-2 mg/kg p.o) but OBX rats failed to show the any kind of nociception. Further OND successfully reversed pain in CCI rats. In addition to reversal of pain, OND (1-2mg/kg p.o) significantly reduced the hyperactivity exhibited by CCI rats in open field test suggesting the effect of OND in co-morbid pain and depression. OND exhibited significant antidepressant and analgesic like effect as indicated by its ability to reduce swim stress induced immobility and reduction in writhing. The present study showed that OND has equal efficacy in comorbid pain and depression-like behavioural mediated through serotonergic system.

Keywords: *Comorbidity, Pain, Depression, 5-HT$_3$ Antagonist*

INTRODUCTION

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[1]. Pain, especially chronic pain, is an emotional condition as well as a physical sensation[2] that affects thought, mood and behavior and can lead to agitation, aggression, isolation and immobility. In these ways, it resembles depression and the relationship is intimate. Pain is depressing, and depression causes intensifies pain[3]. Pain slows recovery from depression, and depression makes pain more difficult to treat. Previously reported that, the occurrence of depression in patients with chronic pain is higher, ranging from 30% to 54%, than that in the general population[3]. Similarly, the presence of a depressive disorder significantly increases the risk of developing chronic pain[4]. The comorbidity of chronic pain and depression is so common and the conditions are so interwoven that it is difficult to pinpoint which usually comes first or whether one causes the other, still, the correlation is unclear. Both pain and depression feed on themselves, by changing both brain function and behavioural[4]. Depression leads to isolation and isolation leads to further depression; pain causes fear of movement, and immobility creates the conditions for further pain[5].

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[6]. 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,

Indian Journal of Pharmaceutical Education and Research
Received on 11/2/2009 ; Modified on 18/5/2009
Accepted on 18/12/2009

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 neurotransmitters (serotonin and norepinephrine) may be involved in the mechanisms of pain and depression[7-9]. In addition, 5-Hydroxytryptamine 3 (5-HT_3) receptor involved in the regulation of both mood and pain. 5-HT_3 receptors are present in many parts of the body including central nervous system, peripheral neurons, spinal cord etc. The antagonist's tropisetron and bemesetron inhibit peripheral 5-HT effects on nociception, indicating a role for 5HT_3 receptors for pain[10]. Ondansetron (OND) is a selective 5-HT_3 receptor antagonist [11] which showed potential anti-depressant-like effect [12] was evaluated for the efficacy in co-morbid pain and depression.

Preliminary screening of ondansetron for depression and pain included in the present investigation were forced swim test[13-14] (FST) and tail flick test [15,] where as olfactory bulbectomy[16-17] (OBX) and chronic constriction nerve injury (CCI) were used for the possible comorbid depression and pain evaluation. Based on the preceding information, the purpose of the present study is to evaluate 5-HT_3 antagonist (OND) for its antidepressant and analgesic like effect including its potential role in suppressing co-morbid pain and depression. The scope of the study also includes the discussion of the implications of these findings for future research in patients who suffer from both pain and depression.

MATERIALS AND METHODS

Drugs and Chemicals -Ondansetron (OND) and Amitriptyline (AMY) were procured from Ranbaxy Research laboratories, India. Ketamine and Xylazine were purchased from Indian Immunological India.

Animals- Male Swiss Albino mice (25–35 g) and male Wistar rats (220–250 g) were obtained from Hissar Agricultural University, Hissar, Haryana, India. All procedures were in adherence to Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No.IAEC/RES/4/1, dated 13.08.08). The animals were housed in laboratory cages and maintained under standard light condition (lights on from 7:00A.M. to 7.00P.M.), laboratory temperature (23±2ºC), and room humidity (50-60%) conditions for at least 1 week before the experimental sessions. The animals were given free access to food (Standard pellet 'chow' feed) and filtered water. The animals were used only once for each experiment.

Methodology

The animals were randomized into control and experimental groups (n = 6). All drugs were dissolved in distilled water and administered intraperitoneally and per-orally depend on acute and chronic treatment.

Dose–response studies

Dose–response studies were carried out using the mouse spontaneous locomotor activity (SLA) test, FST and tail flick test, to determine the appropriate doses of OND that significantly influenced the depressive state and analgesia without affecting the baseline locomotor status. The OND (0.25–2 mg kg-1, i.p.) was administered to mice 30 min before subjecting them to assessment of spontaneous locomotor activity (SLA), forced swim test and tail flick test in mice.

Comorbid test

Selected doses were used for the evaluation of comorbidity of pain and depression. *Depression and pain* assessment included behavioural study of OBX rats in modified open-field and nociceptive assays. In the OBX rat model of depression, after a post-surgical rehabilitation period of 14 days, the OBX rats received OND (1 and 2 mg $kg^{-1)}$, AMY (10 mg kg^{-1}) or vehicle, once a day for 14 days (15^{th} to 28^{th} day). On the 29^{th} day, the OBX rats were then subjected to open field (20 h after the last dose) and followed by nociceptive assessment on the 30^{th} day. Similarly for *pain and depression,* nociceptive assays and modified open field exploration was carried out in CCI rats. In CCI rat model of neuropathic pain after post surgical healing period of 7 days, the CCI rats received OND (1-2mg/kg p.o) and AMY (10mg/kg) or vehicle on 8^{th} day, one hour before nociceptive assays followed by modified open-field test on 9^{th} day.

Behavioural assays

Spontaneous locomotor activity

To evaluate spontaneous locomotor activity as described in boisser et al [18], each animal was individually placed in an actophotometer. The photocells of the actophotometer were checked before use and the animals were individually placed in a square arena (30 cm × 30 cm), with walls painted black. After an initial familiarization

period (2 min), the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with ethyl alcohol and dried between trials.

Forced swim test

The FST was carried out according to Porsolt et al. [13] with substantial modification. Mice were dropped individually into a glass cylinder (height: 30 cm, diameter: 22.5 cm) containing a depth of 15 cm of water maintained at 23–25 °C and were left in the water for 6 min. A mouse was judged immobile if it floated in the water in an upright position and exhibited only small movements to keep its head above the water or made other passive movements. First day 15 min training was give to each mouse. After 24 h of training duration of immobility was recorded during the last 4 min of the 6 min test.

Tail flick test

Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. The animals, which showed flicking response within 3-5 secs, were selected for the study. A cut off period of 15 seconds is observed to avoid damage to the tail. The measurement of withdrawal time using the tail flick apparatus was conducted at 30 min after administration of drugs[15].

Olfactory bulbectomy (OBX)

Surgery- The rats were bulbectomized at the age of 7– 8 weeks (bodyweight 220 - 250 g). Bilateral olfactory bulbectomy was performed with modification as described[12, 19]. Briefly, rats were anaesthetized with combination of xylazine (5mg/kg i.p) and ketamine (75 mg/kg, i.p). The animals were fixed in a stereotactic frame (Inco, India) and the skull was exposed by a midline incision and burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction, the holes were then filled with haemostatic sponge in order to control excessive bleeding and the scalp was sutured. Sham-operated rats were treated in the same way, including piercing of the dura mater but their bulbs were left intact. To prevent post surgical infection, the animals were given Sulprim injection (each ml containing 200 and 40 mg of sulphadiazine and trimethoprim respectively), intramuscularly (0.2 ml/300 g) once a day for 3 days of post-surgery. The OBX/ Sham animals were housed singly in cages. There were no differences in body weights between bulbectomized and control animals. Post 14 days of treatment, animals were subjected to both depression followed by nociceptive assessment on 15th day as mentioned in Fig 1.A.

Modified open field test - The open field exploration was conducted as described[12, 19] with substantial modification. The apparatus consisted of a circular (90-cm diameter) arena with 75-cm high aluminum walls and white floor equally divided into 10 cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. During the test each animal was individually placed in the center of the open field apparatus and the following parameters were recorded for 5 min by trained observer blind to the treatments. Ambulation scores (number of squares crossed), number of rearing episodes and fecal pellet were recorded. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly to eliminate the residual odor. Testing was performed in a temperature, noise and light controlled room. Each rat was transported, one hour before to the testing room using the home cage.

Chronic Constriction Injury (CCI)

Surgery was performed according to the method described [20]. Rats were anesthetized with combination of ketamine and xylazine. The left common sciatic nerve was exposed at the level of the mid-thigh. Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue and 4 ligatures (4-0 silk) were tied loosely around at about 1 mm spacing. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted. The incision was closed in layers. The same procedure was performed without sciatic nerve ligation for the sham group. Post seven days of healing nociceptive test was done followed by behavioural assessment in open field arena as given (Fig. 1.B).

Sensory testing using nociceptive assay in CCI rats- Four nociceptive assays aimed to determine the severity of neuropathic responses namely allodynia and hyperalgesia were performed.

Spontaneous pain

Spontaneous pain was assessed for total period of 5 min as described previously by Choi et al [21]. The operated rat was placed inside observation cage that was kept 5 cm from the working floor. An initial acclimatization period of 10 min was given to each of rats. A total number of six rats were assigned to each group. The test consisted of recording the cumulative duration that the rats hold its ipsilateral paw off the floor. The paw lifts associated with locomotion or body repositioning was not counted. It has been suggested that the paw lifts in the absence of any overt external stimuli are associated with spontaneous pain, and are correlated of ongoing pain.

Dynamic allodynia- All of the operated rats were assessed for dynamic allodynic response according to the procedure described [22]. The operated rats were placed inside observation cage 5 cm above the working floor. A positive dynamic allodynia consisted of lifting the affected paw for finite period of time in response to mild stroking on the plantar region using cotton-bud. This stimulus is non-noxious to normal-behaviouring rats. The latency to paw withdrawal was then noted down. If no paw withdrawal was shown within 15s, the test was terminated and animal were assigned withdrawal time. Hence 15s effectively represented no withdrawal.

Mechanical hyperalgesia- CCI rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described[23]. The Initial set up was same as previous test. Hind paw withdrawal duration was measured after mild pinprick stimulus to the mid plantar surface of the ipsilateral (left) hind paw. A withdrawal was defined as being abnormally prolonged if lasted at least 2s. The mean withdrawal duration was taken from a set of three responses.

Statistical analysis

All the data were expressed as mean ± s.e.m. The single treatment studies were analysed using one-way analysis of variance followed by post test tukeys.

RESULTS

Spontaneous locomotor activity - The locomotor activity of mice did not significantly influenced by OND treatment [F (4, 25) =0.18, NS Fig.2].

Forced swim test – Fig.3. Showed the characteristic immobility in FST resembles the behavioral despair. The acute treatment with OND significantly ($p<0.05$) decreased the duration of immobility in mice compared with vehicle treatment. This effect was dose dependent and was not observed at the lowest dose (0.25 mg kg-1).

Tail flick test -Fig. 4 displays effect of drug on time latency in tail flick test. Acute treatment with OND significantly ($p<0.05$) increased the tail flick latency when compared to vehicle group.

Olfactory bulbectomy

Modified open field test for OBX rats - The open ? eld test was done to evaluate the characteristic hyperactivity in OBX rats in this study (Table 1). OBX rats exhibited significant increase in ambulation ($p<0.05$), rearing ($p<0.05$), and fecal pellet ($p<0.05$), behaviour for 5 min after being put into the open ? eld arena. Both increased frequencies of ambulation and rearing were dose-dependently ameliorated by chronic OND (1 and 2mg/kg p.o) and AMY treatment (10 mg/kg). However, the ambulation and rearing in the sham group were not signi? cantly affected by the treatment with OND and AMY.

Nociceptive Assays

Nociceptive assessment in OBX rats showed no positive response. Thought the results were not significant at any level, data was not included.

Chronic constrictive injury in Rats

Nociceptive Assays- Behavioural studies have demonstrated that rats with the CCI experiences, spontaneous pain, dynamic allodynia and mechanical hyperalgesia. In this model, CCI rats showed significant intensity of spontaneous pain ($p<0.01$), dynamic allodynia response ($p<0.01$) and mechanical hyperalgesia ($p<0.01$). Pretreatment of AMY (10mg/kg) and OND (1-2 mg/kg) significantly ($p<0.01$) reversed the spontaneous pain. Dynamic allodynia response and mechanical hyperalgesia was suppressed significantly ($p<0.05$) by AMY and OND as shown Table 2.

Modified open field test for CCI rats - Exploratory hyperactivity in CCI rats was summarized in (Table 3). CCI rats exhibited significant increase in ambulation ($p<0.01$), rearing ($p<0.01$) and fecal pellet ($p<0.01$), behaviour for 5 min after being put into the open ? eld arena. Both increased frequencies of ambulation, rearing and defecation were dose-dependently ameliorated by chronic OND and AMY treatment. However, the ambulation and rearing in the sham group were not signi? cantly affected by the treatment with OND and AMY.

DISCUSSION

Even though chronic pain is comorbid with a broad array of mental disorders, it is its co-occurrence with depression that has been most studied[1]. The presence of depression increases the disablement of chronic pain and complicates the rehabilitation of patients with the condition[5]. It is there-fore a pressing research question to ?nd the intervention package that will alleviate not only the symptoms of depression but also relieve the comorbid pain condition. Preliminary investigation in FST and tail flick test gives an idea for anti-depressant and analgesics like activity of OND and peak dose effect in this acute model was selected for further evaluation in comorbid pain and depression in OBX and CCI model respectively.

FST is the most extensively used mice behavioural models for antidepressant screening [13-14].The antidepressant-like effects of OND in the FST are not due to hyper-locomotive effects as indicated by the spontaneous locomotor activity test in actophotometer. Pretreatment with OND significantly reduced the duration of immobility in mice FST indicating anti-depressant like effect. Antidepressant-like activity of OND might be attributed to selective 5-HT_3 receptor blockade modulating neuronal release of neurotransmitters[24].

In the tail flick test, a thermal stimulus is focused on the skin of the animal's tail, activating nociceptors in the surface layers of the skin[25]. The tail flick latency was significantly increased by OND. From the results it could be concluded that OND exhibits anti-nociceptive activity consistent with previous reporting, that activation of spinal 5-HT_3 receptors produce a nociceptive effect that is reversed by speci?c 5-HT_3 receptor blockade[10].

The OND dose which showed peak effect in FST and tail flick test was tested for the chronic model of depression and pain. The rat olfactory bulbectomy model of chronic depression (with adequate face and predictive validity) is known to respond a novel agents that are unmarked by more conventional models of depression[16-17]. The OBX rats exhibited a specific abnormal behavioural pattern in the brightly lit, circular, open field arena[17, 26, 27] resembling the extreme of psychological state similar to person attempting suicide [19]. The various behavioural changes included increased ambulation, rearing and defecation compared to sham controls [28]. AMY (10mg/kg) and OND at both doses (1 and 2 mg/kg being more effective) reduced the hyperactivity in bulbectomised rats. Nociceptive assays mentioned above were performed in OBX rats following the open field test. Though the data were not presented as induction of spontaneous pain, dynamic allodynia and mechanical hyperalgesia in OBX rats compared to the sham rats were statistically not significant.

Further to assess possible comorbid pain and depression, chronic constriction injury was done. Neuropathic pain in the rat CCI is characterized by intense persistent pain and hypersensitivity to mechanical and/or thermal stimuli, re?ecting presumably abnormal spontaneous afferent activity, alterations in central processing, and/or increased afferent sensitivity[29]. 5-HT_3 receptor contributes to peripheral sensitization and hyperalgesia in in?ammation and nerve injury. The present study clearly demonstrated that oral administration of OND could induce signi?cant, dose-dependent attenuation of spontaneous, dynamic and mechanical allodynia in a rat CCI neuropathic pain model which is consistent with report of McCleane [30] showing the possible use of 5-HT_3 receptor antagonists for the treatment of neuropathic pain. Activation of 5-HT_3 receptors on peripheral afferent neurons in animal models of tissue injury leads to acute and persistent nociceptive effects that can be blocked by 5-HT_3 receptors antagonists [31]. Based on the result we suggest that, the net reversal of spontaneous pain, dynamic and mechanical allodynia by OND observed in CCI rats is mediated through 5-HT_3 receptor.

Individuals with chronic pain have a higher prevalence of depression, anxiety, alcohol and drug abuse or dependence than those without pain [32-34]. Chronic pain is associated with increased risk of suicide [35-36] assessed in modified open field test following nociceptive assays. MOFT resembles the psychological state of depressed person committing suicide. CCI rats showed significant increase in ambulation, rearing and defecation in open field test suggesting the associated depressive condition linked to chronic pain [37]. This test also indicated the worsening of depression-like behavior in CCI rats compared to sham rats and an improvement after administration of OND, which is consistent with the results from open field test.

The relationship between chronic pain and depression is undeniable, yet these two neuropsychiatric phenomena's share a relationship analogous to siamese twins. The diverse results suggested that this preclinical model of combined nociceptive and depression-like behaviours may be useful to investigate the mechanisms underlying the interaction between pain and depression and peripheral and central effect of 5-HT_3 receptors antagonist, potential agent for treating combined symptoms of pain and depression. Preliminary investigations like FST and tail flick test revealed the anti-depressant like and analgesic like activity of ondansetron without affecting the base line locomotion. Peak dose of OND were tested in chronic depression and pain model. OBX rats exhibited the characteristic hyperactivity in MOFT [12, 19], but failed to show pain in nociceptive assays. It could be due to the fact that removal of olfactory bulb lead to aggression only [17], sense of pain was not observed. Peripheral tissue damage due to CCI resulted significant increase in intensity of pain consisted with previous finding [28]. In addition to the nociceptive pain, CCI rats showed exploratory hyperactivity in MOFT suggesting the comorbidity of depressive like behaviour in chronic pain. It could be due the central and peripheral effect induced by CCI[38]. The development of depression and other psychiatric symptom is more in chronic pain compared to without pain[39] as observed in CCI rats when placed in open field arena.

The relationship between pain and clinical depression is an important and under-investigated issue. Although antidepressants have been used in clinical pain management, their mechanisms of action remain unclear. The current study revealed that CCI can be the model for comorbidity of depressive like behaviour in pain and the possible role of OND successfully reversing the comorbid condition. Our results provide evidence that this preclinical model of combined nociceptive and depression-like behaviors may be useful to investigate the mechanisms underlying the interaction between pain and depression and to explore new treatment options for this significant clinical issue. Our data also suggest that OND may be a useful agent for treating combined symptoms of pain and depression.

Day 0	**0^{th} -1^{st} day**	**1^{st} -14^{th} day**	**15^{th} - 28^{th} day**	**29^{th}-30^{th} day**	
				Behavioural Assessments	
Surgery	Recovery from surgery (continuous care)	Rehabilitation period (Daily handling and observation)	Drug/vehicle treatment (Once a day i.p. administration for 14 days)	Modified open field test	Pain Assessments

Fig.1A displays the Surgery and treatment schedule to assess the effect of OND and AMY on behavioural anomalies resulted in olfactory bulbectomized rats.

Day 0	**0^{th} -1^{st} day**	**1^{st} -7^{th} day**	**8^{th} - 9^{th} day**	
			Behavioural Assessments	
Surgery	Recovery from surgery (continuous care)	Rehabilitation period	Treatment and Pain assessment	Modified open field test

Fig.1B showed the Surgery and treatment schedule to examine the effect of OND and AMY on Chronic constrictive injury in rats

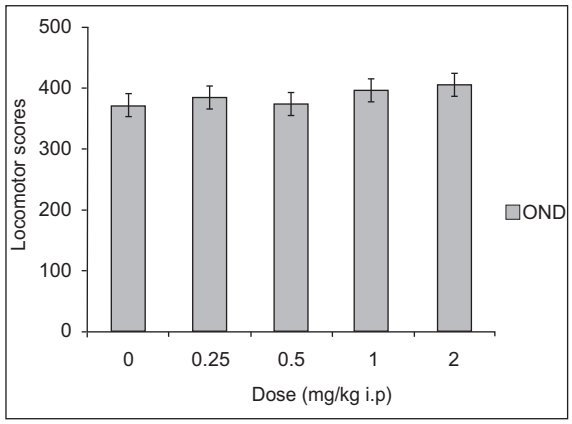

Fig.2 Effect of OND on spontaneous locomotor activity in mice. The columns represent mean of digital locomotor scores, recorded during a 10 min observation period. Error bars represent ± S.E.M. n= 6 per group

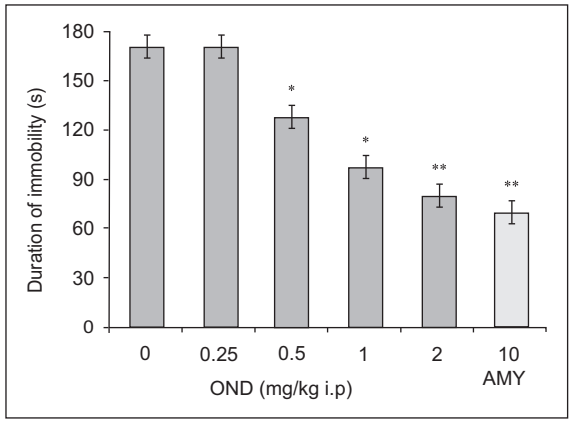

Fig.3 Effect of AMY and OND on duration of immobility (s) in mice FST. The columns represent mean of values. Error bars indicate ± S.E.M. * p <0.05, **p<0.01 vs. vehicle control group. AMY and OND significantly [F(4,25=43.16 , p<0.05] decrease the duration of immobility in mice FST. (n = 6 per group)

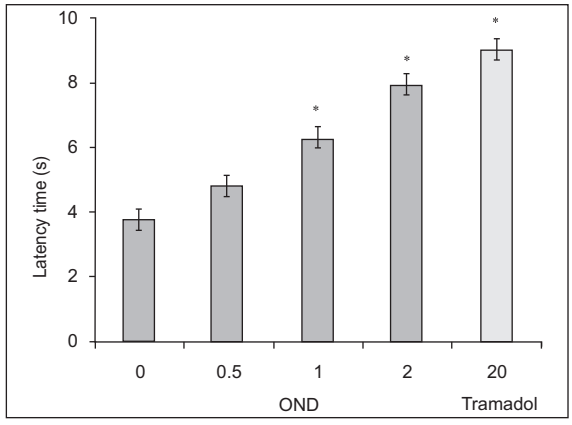

Fig.4 Effect of Tramadol and OND on latency time of mice exposed to tail flick test. The columns represent mean of values. Error bars indicate ± S.E.M. * p <0.05 vs. vehicle control group. Tramadol and OND significantly [F(4,25=65.96, p<0.05)] increased the latency of tail flick compared to vehicle control group. (n = 6 per group)

Effect of AMY and OND in the behavioural assessment of OBX /sham rats in the modified open field test

S.No.	Group	Dose	Ambulation	Rearing	Fecal pellet
1	Sham	0	127.83±6.27	12.33±1.21	3.16±0.50
2	Sham+ AMY	10	122.0±5.9	13.5±1.21	2.66±0.34
3	Sham+ OND	1	124.33±6.53	11.5±1.50	3.33±0.30
4	Sham+ OND	2	123.00±8.16	10.50±0.99	3.00±0.33
5	OBX	2	212.50±9.40 *	24.66±1.55 *	6.16±0.89 *
6	OBX + AMY	10	102.00±8.39 #	8.33±0.80 #	2.50±0.39 #
7	OBX + OND	1	130.16±5.53 #	13.16±1.14 #	3.00±0.33 #
8	OBX + OND	2	123.16±5.31 #	11.83±1.21 #	2.66±0.65 #

Table 1. Effect of AMY and OND on ambulation, rearing and fecal pellet in OBX /sham rats. The columns represent mean number of ambulation, rearing and fecal pellet and error bars indicate s.e.m. * $p<0.05$ vs sham group, $^{\#}$ $p<0.05$ vs vehicle treated OBX group. OBX significantly increased the behavioural anomalies (ambulation, rearing and fecal pellets) compared to sham groups. Statistical analysis (ANOVA) showed that AMY and OND significantly reversed the behavioural anomalies like Ambulation [$F(7, 40) = 18.39$], rearing ([$F(7 , 40)= 12.17$], and fecal pellet [$F(7, 40) = , 4.38$] compared to the vehicle treated OBX rats . n =6 per group

Effect of AMY and OND on the Nociceptive assessment like spontaneous pain , dynamic allodynia response and mechanical hyperalgesia in CCI /sham rats

S.No.	Group	Dose (mg/kg p.o)	Spontaneous Pain (s)	Dynamic Allodynia (s)	Mechanical Hyperalgesia(s)
1	Sham	0	22.63±1.50	4.08±0.67	4.66±0.69
2	Sham+ AMY	10	21.00±1.60	3.80±0.36	4.10±0.52
3	Sham+ OND	1	20.5±1.36	4.11±0.54	4.66±0.50
4	Sham+ OND	2	18.33±0.96	3.66±0.45	4.00±0.33
5	CCI	0	248.50±6.9 **	67.83±4.4 **	102.83±5.03 **
6	CCI + AMY	10	121.60±6.5 ##	48.5±3.6 #	57.10±4.7 #
7	CCI + OND	1	188.50±12.2 #	47.8±4.5 #	85.00±5.03 #
8	CCI + OND	2	145.86±5.9 ##	44.38±3.9 #	67.7±4.2 #

Table 2. Effect of AMY and OND on spontaneous pain, dynamic and mechanical allodynia in CCI /sham rats. The columns represent mean number of spontaneous pain, dynamic and mechanical allodynia and error bars indicate s.e.m. * $p<0.05$, ** $p<0.01$ vs sham group, # $P<0.05$ $^{\#\#}P<0.01$ vs vehicle treated CCI group. Statistical analysis (ANOVA) showed that AMY and OND significantly decreased the chronic pain as indicated by spontaneous pain [$F(7 , 40)= 192.4$], dynamic allodynia response [$F(7 , 40)=67.19$] and mechanical hyperalgesia [$F(7 , 40)=122.4$] in CCI rats compared to the vehicle treated CCI rats . n =6 per group

Effect of AMY and OND on the Behavioural parameter of CCI / sham rats in the Modified open field test

S.No.	Group	Dose (mg/kg p.o)	Ambulation	Rearing	Fecal pellet
1	Sham	0	116.00±4.40	12.66±0.80	3.66±0.52
2	Sham+ AMY	10	104.16±6.0	12.16± 0.98	3.50± 0.35
3	Sham+ OND	1	113.83±6.27	13.30±1.06	3.80±0.36
4	Sham+ OND	2	115.33±7.07	12.5±1.21	3.33±0.56
5	CCI	0	192.16±11.75 **	22.83±1.57 **	8.80±0.43 **
6	CCI + AMY	10	134.86±7.63 #	9.33±0.96 #	4.50±0.39 #
7	CCI + OND	1	157.33±6.41 #	14.83±0.69 #	5.33±0.60 #
8	CCI + OND	2	131.16± 5.25 #	12.00±1.13 #	4.33±0.50 #

Table 3. Effect of AMY and OND on ambulation, rearing and fecal pellet in CCI /sham rats. The columns represent mean number of ambulation, rearing and fecal pellet and error bars indicate s.e.m., **p<0.01, * p<0.05 vs sham group, # p<0.05 vs vehicle treated CCI group. CCI rats showed significant pain compared to the Sham group. Statistical analysis (ANOVA) showed that chronic treatment with AMY and OND significantly decreases the spontaneous [F(7 , 40)= 13.67], rearing [F(7 , 40)= 9.76] and fecal pellet [F(7 , 40)= 11.48] compared to vehicle treated CCI rats. n =6per group

REFERENCES

1. Bair M J, Robinson RL, Katon W, Kroenke K. "Depression and Pain Comorbidity: A Literature Review," Archives of Internal Medicine 2003; 163: 2433–2445.
2. Bao Y. "A National Study of the Effect of Chronic Pain on the Use of Health Care by Depressed Persons," Psychiatric Services 2003; 54: 693–697.
3. Ohayon M M, Schatzberg A F. Using pain to predict depressive morbidity in the general population. Arch Gen Psychiatry 2003; 60:39–47.
4. Parker C. "Management of Depression and Rheumatoid Arthritis: A Combined Pharmacologic and Cognitive-Behavioral Approach," Arthritis and Rheumatism 2003; 49: 766–777.
5. Mosse J, Gallagher R M, Tirumalasetti F. Pain and depression reduce physical functioning in elderly residents of a continuing care retirement community residents: Implications for health management. Pain Medicine 2000; 4: 340-350.
6. Claw D J, Chrousos G P. Chronic pain and fatigue syndromes: Overlapping clinical and neuroendocrine features and potential pathogenic mechanisms. Neuroimmunomodulation 1997; 4:134–153.
7. Delgado P L. Depression: The case for a monoamine deficiency. J Clin Psychiatry 2000; 61: 7–11.
8. Nelson J C. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. Biological Psychiatry 1999; 46:1301–1308.
9. Von Knomng L. Affect and pain: neurochemical mediators and therapeutic approaches. In: Dubner R, Gebhart G F, editors. Proceedings of the Vth world congress on pain. Amsterdam: Elsevier Science Publishers, 1988:276-285.
10. Glaum S R, Proufit H K, Anderson E G. '5HT$_3$ receptors modulate spinal nociceptive reflexes.' Brain Research 1990; 26: 12-16.
11. Wilde M I, Markham A. Ondansetron: a review of its pharmacology and preliminary clinical findings in novel applications. Drugs 1996; 52:773–794.
12. Ramamoorthy R, Radhakrishnan M, Borah M. Antidepressant-like effects of serotonin type-3

antagonist, Ondansetron: an investigation in behaviour -based rodent models. Behavioural Pharmacology 2008; 19:29-40.

13. Porsolt R D, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977; 229: 327–336.
14. Bourin M, Hascoe M, Colombel M C, Coutts R T, Baker G B. Clonidine potentiates the effects of tranylcypromine, phenelzine and two analogues in the forced swimming test in mice. J Psychiatry Neuroscience 2002; 27:178–185.
15. Grossman ML, Basbaum AI, Fields HL. Afferent and efferent connections of the rat tail flick reflex (a model used to analyze pain control mechanisms). J Comp Neurol 1982;206:9–16.
16. Cairncross K D, Cox B, Forster C, Wren A F. Olfactory projection systems, drugs and behavior: a review. Psychoneuroendocrinology 1979; 4: 253–272.
17. Song C, Leonard B E. The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Review 2005; 29: 627–647.
18. Boissier J R, Simon P. Action of caffeine on the spontaneous motility of the mouse. Arch Int Pharmacodyn Ther 1965; 158:212–222.
19. Kelly J P, Wyrnn AS, Leonard B E. Olfactory Bulbectomised rat as a model of depression: An update. Pharmacology and Therapeutics 1997; 74: 299–316.
20. Bennett G J, Xie Y K. A peripheral mono-neuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87- 107.
21. Choi Y, Yoon Y W, Na H S, Kim S H, Chung J M. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 1994; 59:369–376.
22. Field M J, Scott M , Hughes J , Singh L. Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozocin in the rat. Pain 1999; 80: 391–398.
23. Field M J, Bramwell S, Hughes J, and Singh L. Detection of static and dynamic components of mechanical allodynia in rat models of neuropathic pain: are they signalled by distinct primary sensory neurones? Pain 1999; 83:303–311.
24. Blandina P, Goldfarb J, Walcott J, Green J P. Serotonergic modulation of the release of endogenous norepinephrine from rat hypothalamic slices. Journal of Pharmacology and Experimental Therapeutics 1991; 256: 341-347.
25. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001; 53:597–52.
26. Kelly J P, Leonard B E. The effect of Tianeptine and sertraline in three animal models of depression. Neuro-psycho-pharmacology 1994; 33: 1106–1116.
27. Mahesh R, Rajkumar R, Minasri B, Venkatesha Perumal R. Potential antidepressants: pharmacology of 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile in rodent behavioral models. Pharmazie . 2007;62: 919–924.
28. Slotkin T A, Miller D B, Fumagali F, Mccook E C, Zhang J, Bissette G, Seidler F J. Modeling geriatric depression in animals: biochemical and behavioural effects of olfactory bulbectomy in young versus aged rats. Journal of Pharmacology and Experimental Therapeutics 1999; 289: 334–345.
29. Martin T J, Eisenach J C. Pharmacology of opioid and non-opioid analgesics in chronic pain states. Journal of Pharmacology and Experimental Therapeutics 2001; 299: 811–817.
30. Gary McCleane. Novel analgesics Do $5HT_3$ antagonists antinociceptive have an Effect?. Anesthesia and Analgesia 2003; 97 :1474-1478.
31. Sufka K, Schomburg F, Giordano J. Receptor mediation of 5-HT-induced inflammation and nociception in rats. Pharmacol Biochem Behav 1992; 41: 53-56.
32. Hestbæk L, Leboeuf-Yde C, Engberg M. The course of low back pain in a general population: results of a five-year prospective survey. J Manipulative Physiol Ther 2003; 26: 213-219.
33. Currie S R, Wang J. Chronic back pain and major depression in the general Canadian population. Pain 2005; 107:54–60.
34. Von KorffM, Crane P, Lane M. Chronic spinal pain and physical-mental comorbidity in the United States: results from the National Comorbidity Survey Replication. Pain 2005; 113:331–339.
35. Fishbain D A, Goldberg M, Rosomoff R S, Rosomoff H. Completed suicide in chronic pain.

Clin J Pain 1991; 7:29–36.

36. Penttinen J. Back pain and suicide among Finnish farmers. Am J Public Health 1995; 85:1452–1453.
37. Collins S L, Moore R A, McQuay H J, Wiffen P. Antidepressants and anticonvulsants for diabetic neuropathy and postherapeutic neuralgia: a quantitative systematic review. J Pain Symptom Manage 2000; 20: 449–458.
38. Song X, Song J, Cao H, Li J , Zheng X. Upregulation and redistribution of ephrinB and EphB receptor in dorsal root ganglion and spinal dorsal horn neurons after peripheral nerve injury and dorsal rhizotomy. European Journal of Pain 2002; 12: 1031-1039.
39. Mark A I, Kara Z, Ryan J, McCammon A B, Marcia V. Pain and suicidal thoughts, plans and attempts in the United States. General Hospital Psychiatry 2008; 30: 521-527.