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AN EXPERIMENTAL STUDY TO EVALUATE THE ROLE OF BUPRENORPHINE, MORPHINE AND NALTREXONE IN ANIMAL MODELS OF DEPRESSION

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ABSTRACT

Background and Purposes: Opioids were used for centuries to treat mood disorders, among the opioids receptors, kappa opioid receptors recently (KOR) been focus for antidepressant drug development, this led to interest in selective KOR antagonist as potential therapy agents for depression. The current study examined the antidepressant like activity of buprenorphine, morphine and naltrexone alone and the combination of buprenorphine and morphine with naltrexone in animal models of depression. Experimental Approach: This is a twelve months Experimental randomized study in which all mice were subjected to Unpredictable Chronic Mild Stress Test (UCMS) for 4weeks followed by treatment periods of 2 weeks before they were subjected to the following behavioral test namely Sucrose preference test (SPT), Open Field Test (OFT), Forced Swim Test (FST) and Tail Suspension Test (TST). **Results:** Daily injection for 2 weeks of buprenorphine (1mg/kg,i.p), naltrexone (1mg/kg,i.p), buprenorphine and morphine in combination with naltrexone, produced significant reductions in FST and TST immobility time, these drugs also showed significant increase in sucrose consumption but only in 6th week of treatment periods suggesting of antidepressant like activity in chronic stressed mice. Although morphine (5mg/kg, i.p) increase sucrose consumption but the effect is insignificant, however its effect on FST and TST immobility time is significant. Buprenorphine and buprenorphine in combination with naltrexone showed significant increase in locomotor activity, more pronounce in the 6th week of the treatment period. **Conclusion:** This study supports the evidence that buprenorphine alone and its combination with naltrexone could represent a novel rapid-acting antidepressant medication.

INTRODUCTION

Major Depression Disorder (MDD) is the most common and debilitating psychiatric illness worldwide that exerts a large cost, emotionally and economically on society. Current treatment strategies for depressive disorders have limited efficacy, leaving many patients unimproved or with significant residual symptom. Complicating the treatment of MDD and contributing to its chronicity are its frequent comorbidity with anxiety disorders and other medical comorbidity [1]. Moreover, most antidepressant produces their clinical effect after a lag period of 4-6 weeks which can be dangerous in severely depressed patients with suicidal ideation. Hence, there is currently unmet need to develop newer novel treatment strategies for treating depression.

Substantial evidence supports the theory that opioid system may have a role in depression [2]. Previous studies demonstrated that levels of the endogenous opioids released by the central nervous system may be reduced in important brain areas of patients with major depression"[3]. Among the opioids receptors, kappa opioid receptors have recently been a focus for antidepressant drug development. The endogenous opioids system especially the KORs and its endogenous ligand dynorphin are involved in depressive and anxiety disorders. KOR activation produces pro-depressive behaviour and dysphoric effects in human and rodents, they also induced psychotomimetic responses and increase levels of dynorphins in the Limbic region responses for regulation of mood disorders mediating the aversive property of stress [4].

Buprenorphine a partial mu (μ) receptor agonist and a kappa (κ) receptor antagonist shown to have antidepressants and anxiolytic like activity in mice [5].In recent clinical trials, an ultra-low dose of Buprenorphine significantly reduced suicidal ideation after 4 weeks of treatment in 62 patients [6]. However, a treatment with μ agonist carries a risk of abuse liability and dependence and there are no prior published reports of placebo-controlled studies of opioid agonists in the treatment of depression. In observational studies, treatment with μ opioid agonists has been associated with significant and rapid mood elevation in depression, including subjects with treatment-resistant MDD [7, 8]. If a co-administered μ antagonist was able to counteract the addictive properties of a μ agonist, without interfering with its antidepressant effects, then controlled opioid modulation via combined agonist–antagonist might provide a novel pharmacotherapeutic approach with broader applicability in the treatment of MDD. Naltrexone which is a relatively non selective opioid receptor antagonist with higher affinity for μ than k opioid receptors, when given in combination with Buprenorphine could reduce the potential abuse liability of Buprenorphine occurring via μ receptors. In some studies, Naltrexone alone also produced antidepressant like responses in mice[9].

Therefore, the present study was planned to evaluate the antidepressant activity of buprenorphine, morphine and naltrexone alone and combination of buprenorphine and morphine with naltrexone in Unpredictable Chronic Mild Stress Test (UCMS) model of depression and in which the mice were further subjected to four behavioral test namely Sucrose preference test (SPT), Open Field Test (OFT), Forced Swim Test (FST) and Tail Suspension Test (TST) and whether these drugs can overcome the shortcoming of the existing drugs in treatment of depression

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi. Study was done according to the guidelines of CPCSEA, after approval of Institutional Animal Ethics Committee (IAEC) under Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying (MoFAH&D) constituted under the Prevention of Cruelty to Animals (PCA) Act, 1960.

ANIMALS

Male Swiss Albino mice weighing between 22-25g were utilized for this study. All mice were purchased from National Institute of Biologicals (NIB), UP-Noida. The animals were housed in standard laboratory conditions (12hrs light/dark cycle, 21± 1 °C and relative humidity of 55 ± 5%) with ad libitum access to food and water. After 7 days of acclimatization to laboratory conditions, the animals were randomly assigned to different experimental groups, each consisting of 6 mice [table 1]. Each animal was used only once in the experimental procedures. All experiments were carried out between 9:00 a.m. and 4:00 p.m. The Non-Stressed (without exposure to UCMS),Stressed group (with exposure to UCMS) and Standard group were studied concurrently with the Experimental groups.

STUDY DESIGN: Experimental Randomized Study

DURATION OF STUDY: Number of month = 12 month, Date of initiation April 2019**,** Date of completion April 2020

SAMPLE SIZE: 6 male Swiss Albino mice per group for 8 groups which is considered as appropriate sample size as per "Resource equation method"for animal studies [10]

ETHICAL CLEARANCE

All experiments procedures were carried out after being approved by the Institutional Animal Ethic Committee at the VMMC & SJ Hospital, New Delhi. The approval number was IAEC/VMMC/2018/05 dated 10/08/2018 (under CPCSEA guidelines)

DRUGS AND CHEMICALS

Buprenorphine was purchase from Neon laboratories limited, New Delhi Morphine, Naltrexone, Fluoxetine and normal saline (NS) (0.9% NaCl solution in distilled water) was issued from Drug store department, VMMC & SJH, New Delhi All the drugs were freshly prepared with distilled water and were administered intraperitoneally unless indicated otherwise

EXPERIMENTAL DESIGN AND PROCEDURE

Animal were randomly allocated into different groups as shown in the table 1, each group comprising of 6 animals. The antidepressants like activity were assessed using four behavioral test of depression – Sucrose preference test (SPT) [12], Open Field Test (OFT)[13], Forced Swim Test (FST) [14]and Tail Suspension Test (TST) [15]. In the UCMS model, drugs were administered for 14 days (5th and 6th week) intraperitoneally (i.p.) after completion of 4th week of Stressed induced.

Table 1. Animal were randomly divided into different treatment groups

Groups	Drugs/Placebo	Number of mice
A	Non-Stressed(without exposure to UCMS)	6
B	Stressed(with exposure to UCMS) (Normal saline 0.1-0.5ml i.p)	6
C	Fluoxetine (Flu) 10mg/kg i.p.	6
D	Buprenorphine (Bup) 1mg/kg i.p.	6
E	Morphine (Mor) 5mg/kg i.p.	6
F	Naltrexone (Nal) 1mg/kg i.p.	6
G	Morphine (Mor) 5mg/kg + Naltrexone (Nal) 1mg/kg i.p.	6
H	Buprenorphine (Bup) 1mg/kg + Naltrexone (Nal) 1mg/kg i.p.	6

Noted: The dose of each drug was selected on the basis of Pilot study

METHODOLOGY

Unpredictable Chronic Mild Stress Test (UCMS) [11]

The unpredictable chronic mild stress (UCMS) list protocol was applied to each mouse in an unpredictable manner which includes a variety of low-grade stressors administered over a long period of time. The presentation of different stressors was an essential feature of the model, as repeated presentation of a single stressor results in rapid behavioral habituation. The UCMS regime includes soiled cage, tilting of the cage, alterations of the light-dark cycle, periods of food or water deprivation, grouping etc. (table 2). Two stressors are applied simultaneously. UCMS induces anhedonic behaviour, one of the core symptoms of major depression that parallel symptoms observed in human depression and different antidepressants reverse these symptoms, the animals were exposed to each stressor for a short time (few hours to a day) over several (2-5) weeks. In this study the stressors each week were applied in the same manner throughout the experiment up to 4 weeks.

Table 2. UCMS protocol for inducing depression each week for 4 weeks

Day	Stressor 1	Stressor 2
Monday	Cage tilting 45° (24hrs: 9AM-9AM)	Water deprivation (24hrs: 9AM-9AM)
Tuesday	Food deprivation (24hrs: 9AM-9AM)	Dark room (24hrs: 9AM-9AM)
Wednesday	Predator smell (Rat urine and feces (24hrs: 9AM-9AM)	Overnight illumination (24hrs: 9AM-9AM)
Thursday	Bed wetting – pouring 10-20 oz. of clean water into each standard cage (24hrs: 9AM-9AM)	Overcrowding – 15 mice in one cage (24hrs: 9AM-9AM)
Friday	Water deprivation (24hrs: 9AM-9AM)	Food deprivation (24hrs: 9AM-9AM)
Saturday	Cage tilting (24hrs: 9AM-9AM)	Soiled cage 100 ml water in 100 g sawdust bedding (24hrs: 9AM-9AM)
Sunday	Food deprivation (24hrs: 9AM-9AM)	Overcrowding (24hrs: 9AM-9AM)

(A) Sucrose preference test (SPT) [12]

The sucrose consumption test was conducted every week to assess anhedonia induced by the UCMS protocol, one of the core symptoms of major depression in humans. For checking anhedonic behaviors, animals were giving free access to 2 bottles one containing with 1% sucrose solution and the other water in their home cage, following 14 h of food and water deprivation, with anhedonia defined as a decrease in the voluntary preference for sucrose over water. Sucrose and water consumption will be measured by weighing pre-weighted bottles containing the sucrose solution and water, at the end of the test. Subsequently, sucrose and water consumption will be monitored, under similar conditions, at weekly intervals throughout the experiment.

Sucrose consumption test will be carried out once in a week during treatment period (30 minutes after drug administration) after stress induction. Increase in sucrose consumption will be compared with the Stressed group. P value < 0.05 will be taken as significant

(B) Open Field Test [13]

Decreased locomotor activity has been used as an index of low emotionality in mice and to evaluate the degree of depression.In this study, OFT was used to detect locomotor activity in each mouse.No stressor was performed to the animals for at least 24 h before OFT. The open field was made of white wood (50 X 50 cm), which was divided into 25 (5 cm x 5 cm) identical sectors by white stripes. The field was further divided into central and peripheral sector, where the central sector contained the 9 central squares (3 cm x 3 cm) and the peripheral sector were the remaining squares.Locomotor activity was measured at the end of 4th, 5th, 6th weeks in all the groups (30 minutes after drug administration). Horizontal Locomotion (number of line crossing), rearing

frequencies (the number of times an animal stood on its hind legs) and time spend in the center within 5min were measured to evaluate the locomotor activity.

(C) Forced Swim Test (FST) [14]

The cylindrical tank (30 cm height x 20 cm diameter) required for the mice were constructed of transparent Plexiglas. The water level was 15 cm from the bottom and was marked on tank to ensure that the volume of water was consistent across mice. Principle behind Forced Swim Test is when mouse is placed in a container filled with water, it will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behaviour despair.

The animals were transported in their home cages to the room at least 30 minutes prior to behavior testing in order to get acclimatize to the testing environment. There was one session of 6 minutes long for each mouse, divided into pretest (the first 2min) and test (the last 4 min). The cylinder was filled with tap water at 25 °C and the water depth is adjusted, usually up to 15cm from the bottom. The mouse was placed in the water for 6 minutes and the whole session was video recorded. Analysis made after the test session was for duration of time spent as immobility. Immobility was defined as the absence of movement of forelimb and hindlimb except that necessary to maintain the head above the water. The FST was performed on the 6th weeks, after 14days of drug administration

(D) Tail Suspension Test [15]

This test was performed as described by Stenu et al. In our laboratory, we use specially manufactured tail suspension boxes, made of plastic with the dimensions (55 height X 60 width X 11.5 cm depths). In order to prevent animals from observing or interacting each other, each mouse is suspended within its own three-walled rectangular compartment (55 height X 15 width X 11.5 cm depths). The mice is suspended by tail shows alternate periods of agitation and immobility, for this metallic gallows were connected to a nylon catheter with hook attached to its extremity with distance of 55 cm to floor. Mice are hanged on the hook by an adhesive tape placed 1 cm from the extremity of its tail. Observe for movements like running movement (forward and backward), body torsion with attempts to catch the suspended bond and body jerks recorded by camera for 6 minutes duration. Mice were considered immobility when they hang passively and completely motionless.The TST was performed on the 6th weeks, after 14days of drug administration

STATISTICAL ANALYSIS

The results were expressed as mean ± SD data of n (number of animals studied). Observation parameters were examined separately by analysis of variance across all groups. One way ANOVA was used in evaluating differences between groups in Sucrose Preference Test (SPT), the data was further evaluated using independent t test. ANOVA was used in evaluating differences between groups in Forced Swim Test (FST) and Tail Suspension Test (TST), the data was further evaluated using Bonferroni Post Hoc Test. Kruskal Wallis Test was used in evaluating differences between groups in open Filed Test (OFT), the data was further evaluated using Dunn Pairwise Test. A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.

RESULTS

(A) Sucrose Preference Test (figure 1 & 2)

In the present study, the drug treatment was started after the end of the 4th week and there was no significant finding in first four weeks (fig 1). Therefore, we have described the results for 5th and 6th week only (fig 2).

Chronic stress model caused a gradual decrease in the consumption of 1% sucrose solution as compared to non-stressed animals, with maximum and significant decrease seen in 4th week. This decrease in sucrose consumption was reversed by chronic treatment with buprenorphine and the combination of buprenorphine with naltrexone for two weeks after the end of 4th week i.e. 5th and 6th week. Buprenorphine (1mg/kg, i.p.) showed significant increase in sucrose consumption in 6th week as compared to saline treated animals with UCMS (Stressed) group ($p < 0.004$) (F value = 3.93). However, there is increase consumption of sucrose on the 5th week but the effect was not significant as compared to stressed group. Whereas Fluoxetine showed significant effect on both 5th ($p<0.001$) (F value = 5.467) and 6th ($p<0.002$) (F value = 3.93) weeks. The effect of buprenorphine was comparable to fluoxetine which is used as reference antidepressant on the 6th week only.

Similarly, the combination of buprenorphine (1mg/kg, i.p.) with naltrexone (1mg/kg, i.p.) also caused significant increase in sucrose consumption on 6th week only ($p < 0.04$) (F value = 3.93)but the effect was less compared to buprenorphine alone.

On the other hand, Morphine (5mg/kg, i.p.), showed increase consumption of sucrose especially on the 6th week but the effect was not significant on both weeks as compared to stressed group. But Morphine (5mg/kg, i.p.) in combination with Naltrexone (1mg/kg, i.p.)showed increase in sucrose consumption as compared to stressed group (p value <0.03), and Naltrexone (1mg/kg, i.p.) also showed significant increase in sucrose consumption(p value <0.01)on the 6th week respectively.

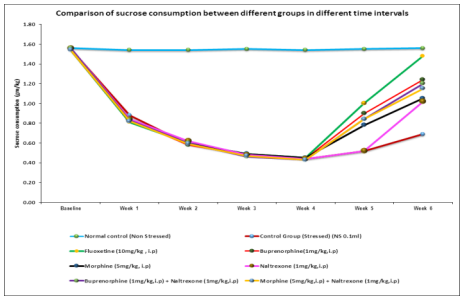

Figure 1: Effect of Fluoxetine, Buprenorphine, Morphine, Naltrexone, and the combination of Buprenorphine and morphine with naltrexone on UCMS induced depression measured as reduced intake of sucrose solution. The baseline indicate sucrose consumption before starting of UCMS, the first four week indicate sucrose consumption during induction of UCMS, the maximum decrease in sucrose consumption was seen at the end of four week of UCMS, and the next two week (week 5 and 6) indicate sucrose consumption in treatment period. Mean intake in 1hour sucrose test is shown for weekly test session compared to control saline treated at the same time point.

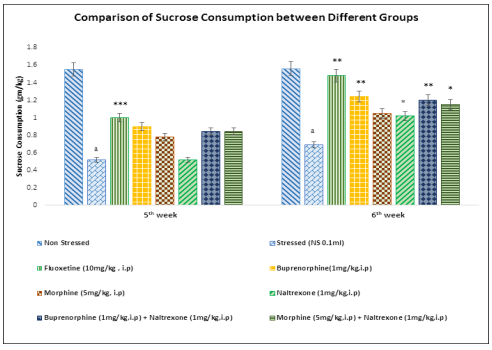

Figure 2: Effect of Fluoxetine, Buprenorphine, Morphine, Naltrexone and the combination of Buprenorphine and morphine with naltrexone on Sucrose consumption (Mean ± SD) in gm/kg at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non stressed group
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compare to Normal Saline treated stressed group.

(B) Open Field Test: (Figure 3, 4 & 5)

Marked decrease in locomotors activity (horizontal locomotion, rearing frequency and time spend in center) was seen in stressed animals at the end 4th week of UCMS, as compared to non-stressed. Chronic administration of Buprenorphine (1mg/kg,i.p.) caused significant increase in horizontal locomotion($p<0.001$, $p<0.0003$), rearing frequency($p<0.007$, $p<0.001$), and time spend in the center ($p<0.001$, $p<0.0003$), as compared to normal saline treated Stressed group at the end of both the weeks respectively (5th and 6th weeks), with slightly greater effect seen in 6th week. Similar significant increase in locomotor activity was also seen with the combination of buprenorphine and naltrexone; however the effect was slightly less compare to buprenorphine group.

Also, Fluoxetine (10mg/kg, i.p.) which is the reference standard antidepressant showed highly significant effect on total locomotion as compared to normal saline injected Stressed group (horizontal locomotion($p<0.0002$, $p<0.0001$), rearing frequency($p<0.001$, $p<0.0001$), and time spend in the center ($p<0.0003$, $p<0.0001$)). Morphine (5mg/kg, i.p.), Naltrexone (1mg/kg, i.p.), and Morphine (5mg/kg, i.p.) in combination with Naltrexone (1mg/kg, i.p increases the total locomotion; however, the effect is insignificant as compared to normal saline treated Stressed group at the end of both the weeks respectively (5^{th} and 6^{th} weeks)

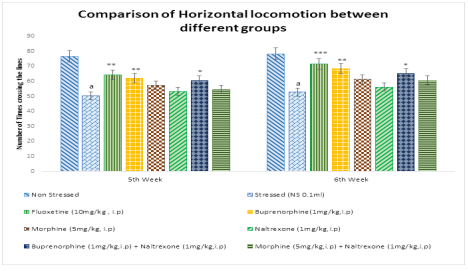

Figure 3: Effect of different drugs on Horizontal locomotion (Mean ± SD) in 5 minutes at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non stressed group
* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$ compared to Normal Saline treated stressed group

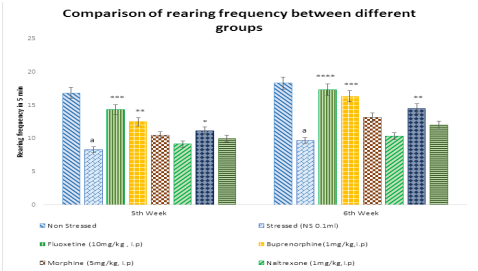

Figure 4: Effect of different drugs on rearing frequency (Mean ± SD) in 5 minutes at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non stressed group
* $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$, **** $P < 0.0001$ compare to Normal Saline treated stressed group

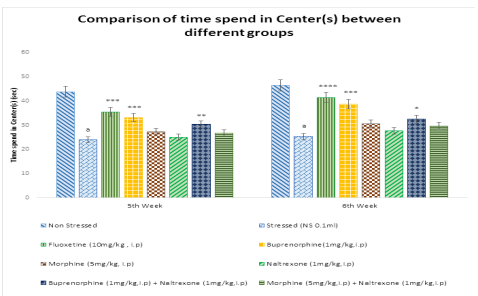

Figure 5: Effect of different drugs on time spend in Center(s) (Mean ± SD) in seconds at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non stressed group
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compare to Normal Saline treated stressed group

(C) Forced Swim Test (FST)

Effect of Buprenorphine, Morphine, Naltrexone, Fluoxetine and the combination of Buprenorphine and morphine with naltrexone on Immobility time in forced swim test (figure 6)

In this experiment, mice treated with fluoxetine (10mg/kg,i.p.), buprenorphine (1mg/kg,i.p.) and buprenorphine (1mg/kg,i.p.) in combination with naltrexone (1mg/kg,i.p.) demonstrated significantly reduced immobility time of 75.17±3.76 ($p<0.0001$), 80.17±5.38 ($p<0.0001$) and 82.5±5.65 ($p<0.0001$) (F value = 42.579, $p < 0.0001$) respectively and increase duration of movement when compared to normal saline treated stressed group suggesting an antidepressant like activity. This decrease in immobility time was more pronounced and more significant with Buprenorphine alone than Buprenorphine in combination with naltrexone On the other hand, Naltrexone (1mg/kg, i.p.), Morphine (5mg/kg,i.p.) and the combination of naltrexone with morphine also reduced the immobility time of 96.83±8.45 ($p<0.0001$), 89.83±4.54 ($p<0.0001$), 90.±6.07 ($p<0.0001$) respectively and increase duration of movement as compared to normal saline injected control group , however the decrease in immobility time is less pronounced as compared to buprenorphine and the combination of buprenorphine with naltrexone though the comparative effect among them is insignificant.

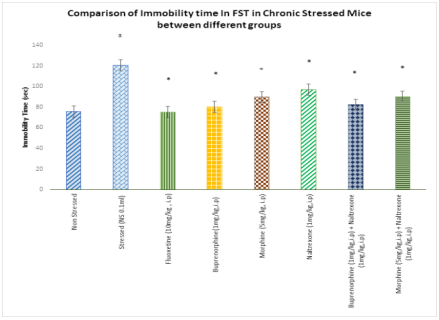

Figure 6: Effect of Fluoxetine, Buprenorphine, Morphine, Naltrexone and the combination of Buprenorphine and morphine with naltrexone on Immobility time in FST in unpredictable chronic stressed mice (UCMS) at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non stressed group
* $P < 0.001$, * * $P < 0.0001$ compare to Normal Saline treated stressed group

(D) Tail Suspension Test (TST)

Effect of Buprenorphine, Morphine, Naltrexone, Fluoxetine and the combination of Buprenorphine and morphine with naltrexone on Immobility time in tail suspension test (figure 7)

In this experiment, mice treated with fluoxetine (10mg/kg,i.p.), buprenorphine (1mg/kg,i.p.) and buprenorphine (1mg/kg,i.p.) in combination with naltrexone (1mg/kg,i.p.) demonstrated significantly reduced immobility time of 75.17±6.31 ($p<0.0001$), 78.33±3.01 ($p<0.0001$) and 80.5±3.78 ($p<0.0001$) (F value = 20.944, $p < 0.0001$) respectively and increase duration of struggle when compared to normal saline stressed group suggesting an antidepressant like activity. This decrease in immobility time was more pronounced and more significant with Buprenorphine alone than Buprenorphine in combination with naltrexone

On the other hand, Naltrexone (1mg/kg, i.p.), Morphine

(5mg/kg,i.p.) and the combination of naltrexone with morphine also reduced the immobility time of 93.5±3.45 ($p<0.01$), 88.33±4.32 ($p<0.0001$), 88.33±4.32 ($p<0.0001$) respectively and increase duration of struggle as compared to normal saline injected control group , however the decrease in immobility time is less pronounced as compared to buprenorphine and the combination of buprenorphine with naltrexone though the comparative effect among them is insignificant

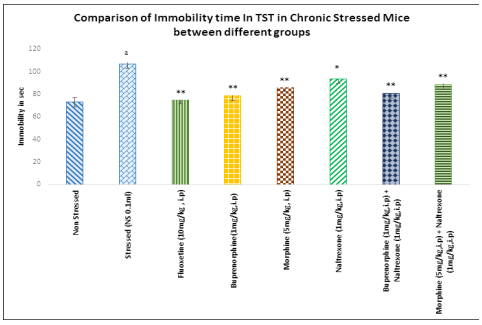

Figure 7: Effect of Fluoxetine, Buprenorphine, Morphine, Naltrexone and the combination of Buprenorphine and morphine with naltrexone on Immobility time In TST in Chronic Stressed Mice at the end of 5th and 6th weeks of drug treatment.

a $P < 0.01$ compared to non Stressed group
* $P < 0.05$, ** $P < 0.0001$ compare to Normal Saline treated Stressed group

DISCUSSION

In the present study we have employed a single and combination of two drugs as an alternative approach to get kappa antagonism. First one is buprenorphine which is a semi-synthetic opioid and acts as a partial μ-receptor agonist and a κ-receptor antagonist with additional nociception/orphanin FQ receptor (NOP-receptor, also known as ORL1) partial agonist activity [16]. Second one is naltrexone which is a relatively non-selective opioid receptor antagonist, with a higher affinity for μ- than κ-opioid receptors [17]. The antidepressant effects of all these drugs were compared mainly with normal saline treated stressed group and the reference drug fluoxetine.

The unpredictable chronic mild stress (UCMS) model is the validated and most widely used paradigm in rodents, which comprises systematic and repeated exposures to variable, unpredictable, and uncontrollable stressors lasting days or weeks [18,19]. The effects of the UCMS model are frequently monitored by measuring the reduction of sucrose preference or consumption in the sucrose preference test (SPT) [20], which was assumed as a measure of anhedonia. This concept refers to a markedly decreased ability to experience pleasure, which represents one of the core symptoms of depression [21,22].

In our study chronic sequential exposure of mice to variety of unpredictable stressors over a period of 6 weeks, produced a marked increase in depressive behavior, as evident by gradual decrease in sucrose consumption in SPT, with maximum decrease seen at the end of 4th week of UCMS (fig 1). The drug treatment to stressed animals was started after 4th week once a day (single dose) for 14 days. In the present study, both buprenorphine and naltrexone were administered in the dose of 1mg/kg, intraperitoneally. This dose was selected on the basis of previous study which reported that this combination was neither rewarding nor aversive in the conditioned place preference paradigm and was without significant locomotor effects [9]

Effect of different drugs on Sucrose consumption

The chronic administration of buprenorphine (1mg/kg, i.p.) alone and in combination with naltrexone (1mg/kg, i.p.) caused significant increase in sucrose consumption compared to saline treated stressed group, suggesting antidepressant like activity. The effect was comparable to fluoxetine which is reference antidepressant drug. In our study, the sucrose consumption was slightly more with buprenorphine alone than buprenorphine combination with naltrexone. This implies that even though buprenorphine could be producing its effect through its actions at KORs, it does not rule out a possible role for other receptors like the DOR and the nociceptin receptor (NOP), since buprenorphine and its active metabolites bind to multiple opioid receptors [5]. Buprenorphine action as a low efficacy partial agonist at NOP receptors, as well as norbuprenorphine's potent agonist activity at DOR, could also contribute to its antidepressant effects [23]. Also, of interest a literature detailing the effects of buprenorphine and other compounds following social defeat stressand a report looking at the ability of buprenorphine to restore hedonic function following stress exposure [24,25].

In our study, naltrexone (1mg/kg ,i.p.), and the combination of morphine (5mg/kg,i.p.) with naltrexone (1mg/kg ,i.p.) also showed significant increase in sucrose consumption as compared to saline-treated stressed group (figure 2).However, morphine (5mg/kg,i.p.) alone showed less effective in SPT behavioral test as compared to other drug treatment group, the reason for this could be that different opioids receptors mediate their effects through a complex system which is currently unknown and requires further studies to fill the knowledge gap.

Effect of different drugs on locomotor activity

In the present study buprenorphine alone and in combination with naltrexone showed significant increase in locomotor activity as compared to normal saline control group. Maximum locomotor activity was seen with buprenorphine (1mg/kg, i.p.) at the end of 6th week of treatment period. This study was consistent with previous study where buprenorphine dose produced a significantly higher locomotor response when compared to normal saline 30 minutes post administration [5]. Morphine (5mg/kg,i.p.), and the combination of morphine (5mg/kg, i.p.) with naltrexone (1mg/kg,i.p) showed insignificant effect on total locomotors activity as compared to normal saline stressed group. The results of this study were inconsistent with previous preclinical evidence which suggested that activation of μ opioid receptors has antidepressant-like effects [26,27]. Also naltrexone (1mg/kg,i.p.) showed no significant effect on total locomotors activity (figure 3,4,5). In Previous literature, rodent studies have assessed the influence of morphine on locomotor effects and have demonstrated contrasting results depending on dose and time of administration, with both stimulant and depressive effects being reported [28,29]. Kappa receptor activation has also been reported to affect rearing and locomotion activity, with lower doses increasing this activity and higher doses decreasing it. In our study, buprenorphine and its combination with naltrexone significantly reversed the stress-induced decrease in locomotor activity in UCMS model, probably through antagonism of Kappa receptors.

Effect of different drugs on immobility time in FST and TST

Another main finding in this study was the clear demonstration of the antidepressant-like effect exerted by buprenorphine (1mg/kg, i.p.) and the combination of buprenorphine and morphine with naltrexone as witnessed by a decrease in immobility in FST and TST and increase duration of struggle in TST. These findings are consistent with previous studies using the TST or other behavioral tests to screen for depression or antidepressant effects of these opioids [30-33]. In previous study, remarkable increase in immobility time for the forced swimming test and tail suspension test was found in the groups that received a supra-therapeutic dose of buprenorphine, independent of gender [34]. Other literature showed that KORs mediated the antidepressant activity of buprenorphine in the FST, this is also relevant as this was the first report of the effect of buprenorphine alone following chronic mild stress exposure[35].

In our study, the most remarkable effective drug treatment group was of buprenorphine and in combination with naltrexone (figure 6, 7). In several animal studies, opioids like morphine, codeine and

tramadol have shown to decrease immobility in a tail suspension test [36]. In another study, both morphine and agmatine (an endogenous aminoguanidine) have decreased immobility time in FST model and these effects were blocked by pretreatment with naloxone (a μ-opioid receptor antagonist)suggesting the role of μ-opioid receptor in depression[23]. Moreover, μ-opioid receptors are known for their role in reward, analgesia and also intricately involved in mood regulation. Agonism, or activation, of these receptors is associated with improved mood, or what is considered antidepressant-like activity [37, 38]. Interestingly, some study has reported that naltrexone (an opioid receptor antagonist) produces the effects of antidepressants in both forced swim test and tail suspension test as well as a foot shock-induced behavioral despair paradigm [39]. These antidepressant effects of morphine and naltrexone were also seen in our study by showing reduction in immobility time.

It is evident from the result of present study that buprenorphine alone and in combination with naltrexone has significant antidepressant activity as demonstrated by increase sucrose consumption in UCMS model and increase in immobility time in FST and TST model. This potential antidepressant activity of Buprenorphine could be attributed to its kappa antagonistic action. Previous studies have also shown to increase immobility with κ-opioid receptor activation in the forced swim test and elevate brain reward thresholds, indicative of an anhedonic depressive- like effect[40]. Conversely, administration of a putative κ- opioid receptor antagonist reverses these effects indicative of an antidepressant-like effect [41]. Additional pre-clinical studies have also demonstrated the ability of κ- opioid receptor antagonists to have antidepressant-like effects as well as reduce repeated forced swim stress induced immobility and decrease anhedonia-like responses in a cocaine withdrawal paradigm [42,43]. In the present study we have combined naltrexone to buprenorphine, this will decrease abuse potential occurring due to mu agonism and their effect in all our studied models were significant. Therefore, together these data suggest that buprenorphine alone and in combination with naltrexone may have potential to be used as an antidepressant agent, however further studies are needed to exactly define its role in depression.

Limitation of study

1. In the present study we have used only single dose of buprenorphine, morphine, and naltrexone due to constraint of animals.
2. Further studies also needed to be done for biochemical estimation of the neurotransmitter and histopathological examination for tissues or cells damage.
3. Dose range study was not conducted due to restraint of animals

CONCLUSION

It can be concluded from the results that buprenorphine alone and in combination with naltrexone has significant antidepressant activity as observed in all these behavioral tests. This effect of buprenorphine could probably be mediated through antagonism of KOR, although possibility of other opioid receptors cannot be ruled out. Further, addition of naltrexone with buprenorphine reduces the risk of abuse potential. However, further studies both animal and clinical, need to be carried out to exactly define the role of Buprenorphine in depression particularly concerning its efficacy and safety in clinical settings.

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