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Comparative Assessment of Opioid Reversal Nasal Spray Efficacy in Countering Fentanyl-Induced Opioid Toxicity in Wistar Albino Rats

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KEYWORDS
Anti-opioid, Open field test, Opioid reversal activity, Fentanyl induced anti-opioid activity

ABSTRACT:

Introduction: Anti-opioid nasal spray is already well established in the US market. The opioid reversal activity is characterized by displacement of opioid by anti opioid drug. The formulation in another dosage form studied for its behavioral changes in eperimental animals.

Objectives: The present study investigates a comparative analysis between a commercially available opioid reversal nasal spray and an in-house developed nasal spray, examining their impact on the locomotor activity of Wistar albino rats experiencing Fentanyl overdose.

Methods: The study comprises of 4 groups, first group was labeled as normal control and rest of 3 groups were overdose with fentanyl citrate injection, Group II was kept as an opioid control or negative control group, and rests of two groups Group III and Group IV were administered with marketed and in-house opioid reversal nasal spray respectively. After administration of drug the locomotor activity was measured by using the open field test apparatus. Activity calculated in terms of No of grid cross and freezing.

Results: Statistical data were analysed with a parametric two-way ANOVA followed by Dunnett's multiple comparison tests. The obtained results of test were equivalent as that of marketed formulation.

Conclusions: The comparative evaluation of anti-opioid nasal spray was carried out to treat fentanyl induced opioid overdose, the results of test were equivalent as that of the marketed formulation.

1. Introduction

The Western countries continue are deadly epidemic opioid overdose. As per United nation office of drug and crime in 2019, Opiod misused was around 1.2% of total world population, and in 70% of deaths opioid overdose was seen.(1) As per Center for Disease Control reported a 31% increased during the COVID-19 pandemic, around 75% of share was covered by opioid overdose in the age-adjusted rate of overdose deaths(2). As per report published in 2018, the opioid overdose death rate in male was 2-3 times more than Females(3) this shows higher usage of opioid by men than women(4). However, the occurrence of an opioid-involved deaths has increased doubled in women(5). The primary cause of death from opioid overdose is respiratory depression and ypoxia is major reason behind the deaths. Breathing characterized by an exchange of gases oxygen inhaled and carbon dioxide expelled in alveoli of lungs and is subject to voluntary as well as involuntary control(6). Brainstem mainly controls Involuntary ventilation, which connects central and peripheral sensory inputs. In rats, the medullary pre-Bötzinger complex in rat, coupled with the retrotrapezoid/parafacial respiratory group, gets an input from the pontine Kölliker-Fuse/parabrachial complex. In conjugation, with this network of pattern generators along with nuclei in surrounding regions respiratory drive and rhythm used to produce and modulate(7). This rhythm-generation network drives along with coordinates respiratory muscle activity by spinal and cranial motor neurons, with an input from forebrain to the respiratory network via the nuclei tractus solitaries.(8) Stimulation to the respiratory network alters not only breathing rhythms/patterns but also the rate and depth of breathing.

An opioid induce respiratory depression cause by direct actions on breathing circuits present in the brainstem, with ancillary blunting of central and peripheral chemo

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sensitivity like hypoxia (lack of O2) and hypercapnia (abundance of CO2) with an actions on respiratory motor neurons muscle.[9]

In the brainstem, opioids bind to μ-opioid receptors (MORs), activates Gi-protein-mediated inhibitory intracellular pathways which reduces neuronal excitability and disrupt respiratory rhythms.[10] MOR activation causes decrease in breathing frequency by affecting inhalation and exhalation speed and by increasing the "apneic pause" between breathing cycle. Opioids overdose can reduce the amount of air exchanged per breath (tidal volume) and CO2 sensitivity.[11] Sedation may cause on higher dosage, which decreases the activity of cortical regions, some of which regulate voluntary breathing. [12]

The present study characterized the effects of in-house opioids formulation against marketed one, by sung fentanyl induce opioid overdosing on wister albino rats.[13] The investigation carried out to study bioequivlence study of marketed and in-house nasal spray. Overdosing of fentanyl was made that decreases locomotor activity of the rat due to the depression characterized by breathing rhythm and flow, decrease in heart rate results in decrease in the locomotor activity.[14] The marketed opioid reversal nasal spray formulation considered as standard and the test of was analyzed against standard.

2. Objectives

To compare opioid reversal activity of In–house anti-opioid nasal spray Vs Marketed anti-opioid nasal spray on fentanyl induced opioid overdosed rats. Understand the impact on locomotor activity of fentanyl induced opioid overdose in wistar albino rats using open field test apparatus. To study the comparative opioid reversal activity to treat the opioid overdose of fentanyl of rats. The record the behavioural changes of animals in terms of the locomotor actity. To apply statistical evaluation parameters like 2 way ANOVA to the results obtained interms of no. Of greeds crossed and freezing understand the tools

3. Methods

Animals: Twenty-four male Wistar rats, weighing, 180-200 gm were randomly grouped into 4 different sets of 6 animals each.[15]

Drug: Fentanyl citrate injection, Marketed opioid reversal nasal spray, test opioid reversal nasal spray.[15,16]

Open-Field Test

In order to assess the gross locomotor activity, the animals were tested in an open-field arena. The open-field apparatus consisted of an open-top box of polypropylene (72 cm × 72 cm) with 36cm high walls. Blue visible lines were drawn on the floor using a marker into sixteen 18 × 18 cm squares.[17]

Procedure: The normal control group (Group I) comprised of normal, no treatment was given to this group of animals. Group II (Disease Control Group) comprised of rats that were injected by subcutaneous route (SC) with 50μg/kg of Fentanyl and no treatment was given. Group III (Marketed formulation treated Group) comprised of rats that were injected (S.C.) with 50μg/kg of Fentanyl and 5 minutes later treated with nasal spray. Group IV (Test formulation treated Group) comprised of rats that were injected (S.C.) with 50μg/kg and 5 minutes later treated with nasal spray containing test formulation.[18, 19]

The fentanyl dose was initially optimized on three animals at 2.5μg/kg, 5.0μg/kg and 50μg/kg. Animal injected with 50μg/kg, showed most significant opioid overdose reaction, so selected for study.[20, 21, 22]

Table No 1 Groups of Animals

Sr. No.	Group	Treatment
1	I	No Fentanyl + No treatment
2	II	50μg/kg Fentanyl + No treatment
3	III	50μg/kg Fentanyl + Marketed Nasal Spray Treatment
4	IV	50μg/kg Fentanyl + Test formulation Nasal Spray Treatment

The animals from group II, III and group IV went into the unconscious state. After 5mins the animals from group III and group IV were given opioid reversal formulation standard and test respectively.

The animals were transferred to the testing room in their home cages and allowed to acclimatize to this room prior to testing. Each rat was gently placed in the center of the open-field arena after treatment and left freely to explore the arena for 15 minutes while recording scores of its behaviors.[23] At the end of the 15-minute test period, animals were returned to their respective home cages.

To determine the reversal effect of test formulation on locomotor activity of the animals were assessed:

No. of line/grid crossing: frequency with which the rats crossed one of the grid lines with all four paws.

Freezing: Duration time in which the animal was completely stationary.

Locomotor activity for each animal was assessed by the sum of line/grid crosses with all four paws. The animal behavioral domains used to assess unconscious behavior were freezing.

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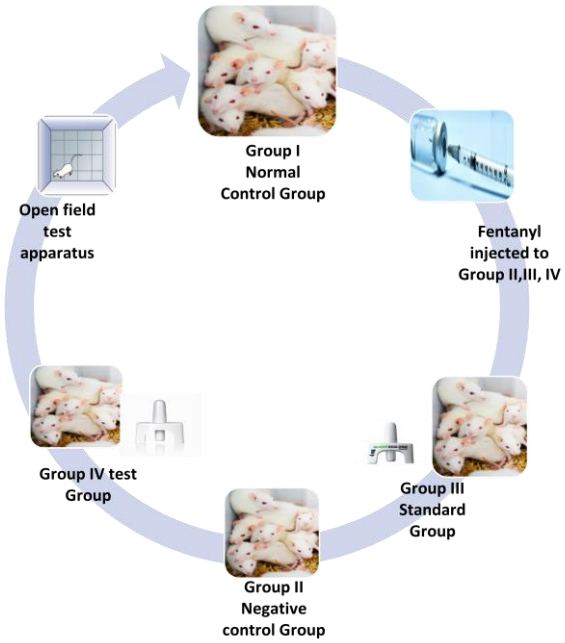
The locomotor activity increases as the opioid reversal formulation displace the opioid available in the brain of the wister albino rat.

Statistical analysis: Data obtained were analysed with a parametric two-way ANOVA followed by Dunnett's t-tests.[24, 25]



4. Results

The animals from group III and group IV went into an unconscious state on overdosing of fentanyl injection in unconscious state the breathing decreases heart rate decreases and mainly function of brain altered this leads to decrease in movement of the animals, this locomotor activity of the animals on administration of opioid reversal nasal spray increased. The animals in group II were having opioid in their brain, the locomotor activity in terms of grid crossed decreased and freezing increases as that of group I. the locomotor activity in terms of grid crossing and freezing were recorded and data obtained were analysed with a parametric two-way ANOVA followed by Dunnett's t-tests. This helped to understand the comparative study in better manner. This gave direct indication that efficacy of test formulation is comparable with the marketed formulation.

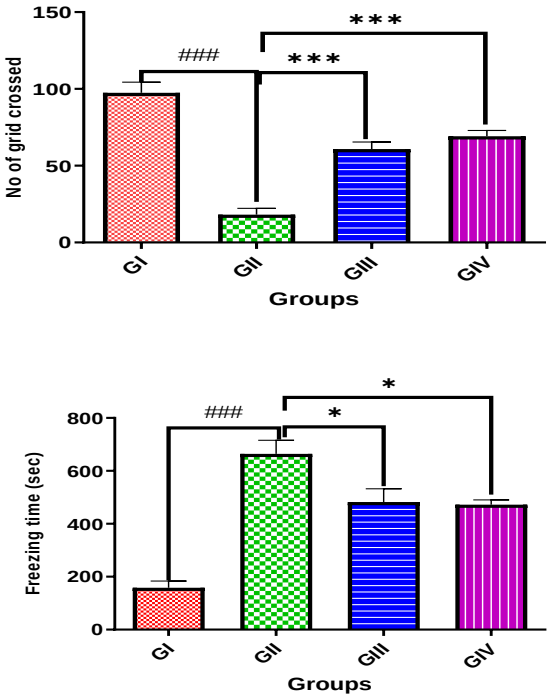


Figure 1 Comparative locomotor activity of anti-opioid nasal spray

Table: Activity of Test Formulation on Locomotion and Freezing Activity.

Sr. No.	Group	Treatment	Group Mean±SD	
			Grid Crossing (no.)	Freezing (Sec)
1	Group I	No Fentanyl + No treatment	97.5±16.909	158.50±61.020
2	Group II	50µg/kg Fentanyl + No treatment	18.17±9.928$^{###}$	664.00±126.649$^{###}$
3	Group III	50µg/kg Fentanyl + Marketed Nasal Spray Treatment	60.83±11.268***	482.50±121.291*
4	Group IV	50µg/kg Fentanyl + Test formulation Nasal Spray Treatment	69.17±9.218***	472.33±49.624*

Results are expressed as mean ± SD. Values are expressed as mean ± SD (n=6), Data was analysed using one-way ANOVA followed by Dunnette's multiple comparison test. Normal Control vs. Disease Control:

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(###p<0.001); Disease Control vs. Treated Groups (*p<0.1, **p<0.01, ***p<0.001)

Effect of test formulation on Locomotor Activities, Anxiety- Group II resulted in significant decrease in the grid cross activity (###p<0.001) as compared to Group I. Whereas group III and Group IV resulted in significant increase in the grid crossing activity as compared to Group II (***p<0.001).

Immobility time (freezing duration) was significantly (###p<0.001) Group II as compared to Group I, an indicator of increased impaired brain functioning leads to impaired locomotor activity. Whereas, both the standard and treated groups ie. Group III and Group IV showed significant decrease (*p<0.1, *p<0.1) in freezing activity as compared to Group II.

5. Discussion

The fentanyl induced opioid overdosed animals treated with the marketed anti-opioid nasal spray, the results obtained using open field test apparatus was analysed in terms of locomotor activity. As the locomotor activity of the animals with fentanyl treatment were showing the decrease in locomotor activity. This can be reverse by administrating anti-opioid nasal spray. The marketed formulation used as a standard to check the efficacy of the test formulation. The locomotor activity in group II is decreased and the group II and group IV activity was compared with the stand and test as the the anti-opioid nasal spray enhance the locomotor activity which was better understand using the open field test apparatus where animal on fentanyl administration can show increase in freezing time and decrease in the no. of grid crossing Where as the animals with anti-opioid nasal spray administration shows enhancement in locomotor activity of the animals and the decrease in freezing time. The locomotor activity of test and standard were compared with the negative control group to which opioid overdose administered. The animals from group III and group IV were administered with opioid reversal nasal spray which displaces opioid from the brain by the anti-opioid drug ultimately increase Locomotor activity.

The locomotor activity was analysed and to it the parametric two-way ANOVA followed by Dunnett's t-tests. This helped to understand the comparative study in better manner. This gave direct indication that efficacy of test formulation is comparable with the marketed formulation. Group III and Group IV resulted in significant increase in the grid crossing activity as compared to Group II (***p<0.001). Group III and Group IV showed significant decrease (*p<0.1, *p<0.1) in freezing activity as compared to Group II.

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