IJPSR (2021), Volume 12, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 October 2021; received in revised form, 19 November 2021; accepted, 24 November 2021; published 01 December 2021

NEUROPHARMACOLOGICAL EVALUATION OF INTRANASAL GUAR GUM LOADED OLANZAPINE MICROSPHERES

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Keywords:

OLZ, Intranasal, Open field exploratory test, Forced swim test, Morris water maze test

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ABSTRACT: Background: The present work aimed to evaluate the sustained release antipsychotic activity of formulated biodegradable mucoadhesive guar gum microspheres of atypical antipsychotic drug Olanzapine (OLZ) for intranasal delivery and to reduce the dose-related side effects of the drug olanzapine. Guar gum microspheres of Olanzapine were fabricated and designed by O/W emulsion solvent evaporation technique using non-halogenated solvents and PVA and tween-80 as surfactant and stabilizer. The prepared guar gum microspheres were characterized for various physical and micrometric properties. Box Behnken design of experiment was applied for the formulation of various batches. An optimized batch was selected for *in-vivo* studies. **Objective:** This study solely emphasizes on sustained effect of formulated Olanzapine microsphere on different symptoms of schizophrenia. **Material and Methods:** Rats of Charles foster strain were subjected to a battery of various behavioral tests for representing positive, negative, and cognitive impairment symptoms found in schizophrenic patients. Different tests performed were open field exploratory, elevated plus maze, forced swim, passive avoidance and Morris water maze. **Results and Conclusion:** During *in-vivo* studies, prepared olanzapine formulation showed an antipsychotic effect that significantly prolonged over that of olanzapine solution for up to 48 h. The prolonged effect of Olanzapine was obtained from the optimized formulation of guar gum microspheres of Olanzapine administered intranasally, which may improve the treatment of psychotic disorders.

INTRODUCTION: Schizophrenia (SCZ) is major psychotic disorder having a devastating impact on various aspects of the patient's life, exposing them to a high risk of suicide and other life-threatening behavior. Non-adherence with antipsychotic medication remains main hindrance in providing better treatment outcomes in schizophrenia [1, 2].

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DOI: 10.13040/IJPSR.0975-8232.12(12).6725-35

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6725-35

More than 35% of patients demonstrate adherence problems during the first 4-6 weeks of treatment and within two years, up to 74% of patients are unable to adhere to their original prescribed treatment [3, 4, 5].

As a consequence of non-adherence to Pharmacological treatment, patients suffer from increased relapses, more frequent and longer hospitalizations, cumulative deterioration in functioning, and a diminished capacity to maintain employment and relationships [6]. Olanzapine (OLZ), {2 – methyl – 4 - (4-methyl – 1 - piperazinyl) - 10H-thieno (2, 3-b) benzodiazepine} belongs to the

second generation of atypical antipsychotic drugs used in the treatment of schizophrenia [7]. OLZ selectively binds to the central dopamine (D_2-R) and serotonin (5-HT_2C) receptors [8]. Following oral administration, OLZ undergoes extensive first-pass metabolism, and approximately 40% of the dose is metabolized before reaching systemic circulation [9]. Also, OLZ suffers from low brain permeability due to efflux by P-glycoprotein in the blood-brain barrier (BBB) [10]. In such case, dosage or dose frequency cannot be increased as it is commonly associated with dose related side effects; orthostatic hypotension, weight gain, dry mouth, *etc*.

Treatment of brain disorders is the greatest challenge because of various obstacles in effective drug delivery and maintaining therapeutic concentration in the brain. To overcome some of these problems, orally disintegrating tablets and IM injections of OLZ were presented [11]. The approved indications for OLZ IM injections are to rapidly control agitation and disturbed behaviors in patients with schizophrenia or manic episodes when oral therapy is inappropriate. A recent drug safety communication was issued to Eli Lilly (Australia) with the support of the Therapeutic Goods Administration. This communication reported adverse events associated with the administration of OLZ IM (Zyprexar) (WATAG Advisory Note). Besides the need for prompt therapeutic action, these adverse events make OLZ a possible candidate for the development of an alternative route of administration. However, intranasal drug delivery may be proved practical, the non-invasive alternative route of administration to circumvent BBB and achieve desired brain targeted drug concentration [12].

In the last few years, the intranasal route has been a point of attraction in the research area for brain-targeted drug delivery. Several advantages are associated with nasal route, such as rapid absorption, higher bioavailability allowing lower doses, fast onset of therapeutic action, bypass of liver and gastrointestinal metabolism, reduced risk of overdose, non-invasive administration, ease of convenience, and self-medication, and improved quality patient compliance. After intranasal administration, drugs travel via olfactory and trigeminal nerves to reach many regions in the central nervous system (CNS).

In experimental animals, intranasally administered drugs have been reported to reach the cerebrospinal fluid (CSF), olfactory bulb, and other parts of the brain [13, 14]. Kinetically and thermodynamically stable OLZ micelles were reported to transport a significant fraction of OLZ dose directly to the brain, circumventing the BBB, following intranasal administration. OLZ-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been reported to be capable of providing direct nose-to-brain delivery, thereby enhancing drug concentration in the brain [15].

Although the intranasal pathway has been proved to be useful for a variety of CNS active drugs, the total amount of drug reaching the brain is reported to be low, with low concentrations in cerebrospinal fluid (CSF) and olfactory bulb in the nanomolar range or a bioavailability from 0.01% to 0.1% [12]. Poor brain uptake of drugs may be due to their low membrane permeability, rapid mucociliary clearance from the nasal cavity, or during the passage across the epithelial barrier. Drugs with poor absorption must be either co-administered with absorption enhancers or encapsulated into an appropriate drug carrier system, such as liposomes, nanoparticles, microspheres. Microspheres on intranasal administration protect the encapsulated drug from biological and chemical degradation and offer improvement in extracellular transport by P-glycoproteins. Thereby increasing the CNS availability of the drug. The most commonly used degradable polymers in microsphere preparation are poly (lactic acid) (PLA), poly (glycolic acid) PGA, PLGA, chitosan, guar gum, beeswax, *etc*.

These polymers are biocompatible, biodegradable, and non-toxic. These have been widely investigated and used for biomedical applications. In the present study, an Optimized batch of guar gum microspheres of OLZ designed and fabricated using non-halogenated solvents and double emulsion solvent evaporation technique is evaluated for sustained release of drug OLZ in rat models representing negative, positive, and cognitive impairment symptoms of schizophrenia. This benefit would help to improve its clinical utility, decrease the dose and frequency of dosing, reducing side effects, and improve therapeutic efficacy.

MATERIALS AND METHODS:

1.1 Materials: Olanzapine and Guar gum (Polymer) were provided as gift samples from Enaltec Labs Pvt. Ltd. Navi Mumbai, India, and Dinesh Enterprises, Jodhpur, India, respectively. All other chemicals were purchased and of analytical grade.

1.2 Preparation of Microspheres: A new preparation method for OLZ microspheres based on an Oil/Water-type emulsion solvent evaporation method using non-halogenated solvents was investigated, the method is based on phase separation between acetone and aqueous glycerol. Tween-80 and polyvinyl alcohol (PVA) were used as surfactants. Box behnken design of experiment was employed to prepare different batches with drug, polymer, and surfactants in varying ratios. After characterization of all the formulated batches, in terms of production yield, particle size, drug entrapment efficiency, morphology, and various other micrometric properties; optimized batch with drug entrapment efficiency of 71.23% and particle size 20.57 µm and drug release of 56.03% at 48th hour was selected for neuropharmacological evaluation in an animal model.

1.3 Animals and Neuropharmacological Evaluation of OLZ Formulation: Adult Charles foster albino rats of either sex (248-300gms) were used. Animals were procured from central animal house IMS, B.H.U., Varanasi (U.P.), India. They were housed in groups of 6 in colony cages at an ambient temperature of 25±2ºC and 48-60% R.H. with 12 hours light/dark cycle. They had free access to pellet chow, and animals were exposed only once to every experiment. The experiment was performed after approval from the institutional ethics committee with IAEC number - 332, and principle of laboratory animal care (NIH) (publication No.86-23.received 1985) guidelines were followed throughout.

1.4 Experimental Design: The selected animals were grouped into four groups randomly irrespective of sexes, and each group comprised of six animals.

Group A: Control (Saline).
Group B: Ketamine Treated.
Group C: Olanzapine solution (OLZS).
Group D: Olanzapine formulation (OLZF).
Group E: Olanzapine Market preparation (OLZMP).

1.5 Routes of Drug Administration: Ketamine was administered intraperitoneally (i.p.). Olanzapine formulation and Olanzapine solution were administered intranasally. Olanzapine market preparation was administered subcutaneously. For intranasal administration, 10µl of the formulation (0.2mg/kg) was injected with the help of a silastic tube attached to a syringe inserted 2 cm into one of the nares towards the roof of the nasal cavity. The rats were held supine and received the dose, and were held for 10 minutes after administering the dose.

1.6 Doses & Schedule: Ketamine 30mg/kg, Olanzapine solution [OLZS] 0.2mg/kg, Olanzapine microsphere formulation [OLZF] 0.4mg/kg, Olanzapine market preparation [OLZMP] 0.4mg/kg. To all the groups except group A; Ketamine in a dose of 30mg/kg was administered intraperitoneally once a day for five days. On test day, OLZS, OLZF and OLZMP were administered intranasally 30 minutes to their respective groups of rats before subjecting them to behavioral tests.

1.7 Methods: A battery of behavioral tests was performed to evaluate the effects of an optimized batch of formulated olanzapine microspheres in Ketamine treated rat model of schizophrenia.

2. Anxiety & Restlessness, Hyperlocomotion (Positive Symptoms):

Open Field Exploratory Test: An open field apparatus similar to that of Bronstein (1972) [16] and modified by Jaiswal (2002) [17] was used to study the open field exploratory behavior in rats. It was made of plywood and consisted of a square (61x61cm) with high walls (61x61cm). The entire apparatus was painted black except for 6mm white lines that divided the floor into 16 squares. The entire room except the open field was kept dark during the experiment. The open field was lighted by a 60 W bulb focusing on to the field from a height of about 100cm from the floor. Each animal was centrally placed in the apparatus for 5min and the following behaviors were noted ambulation. This was measured in terms of the number of squares crossed by the animal.

Rearing- Number of times the animal stood on the hind limbs. Grooming- The number of times the animal made these responses viz. grooming the face, licking, scratching the various parts of the body. Immobility Time- Time during which animals remained freezed. Fecal pellets-Number of fecal pellets excreted during the period.

Elevated Plus Maze Test [18]: The plus-maze consists of two opposite open arms(48x10cm) crossed with two opposite enclosed arms of the same dimension with walls 40cm high. The arms were connected with a central square (10x10cm) to give the apparatus a plus sign. The maze was kept elevated 48cm above the floor in a dimly lighted room. The rats were placed individually on the central square of the plus-maze facing an enclosed arm. The time spent and the number of entries made by the rats during the next five minutes on the open and enclosed arm were recorded. An arm entry was defined when all the four limbs of the rat were on the arm.

3. Depression (Negative Symptoms):

Behavioral Despair Test [19]: The rat was placed in the cylinder (45x20cm) containing 38cm water (25±2ºC), so that the rat could not touch the bottom of the cylinder with its hind limb or tail. Rats also could not climb over the edge of the cylinder. Two sessions of the swim were conducted, an initial 15 minutes pre-test. The 5 min test 24 h later. Drugs were administered after the pre-test. The period of immobility (remained floating in the water without struggling and making only those movements that are necessary to keep its head above water) during 5 min test period was noted.

4. Learning and Memory (Cognitive Impairment)

Morris Water Maze Test [20]: The maze consists of a black circular pool (diameter 2.15m, Height-80cm) filled to a depth of 44cm of water (25±2ºC). Water was made opaque by adding Indian ink. Spatial learning and memory were tested in the Morris water maze test. On the previous day, rats received a habituation trial (Exposure in water maze for 1 minute) in which there was no platform present. On the first day, a circular platform (Diameter 9cm) was kept hidden 2cm below the water level in the center of one of the quadrants. The platform remained in the same position during the training.

The random sequence of four starting poles along the perimeter of the pool was generated. All the animals follow the same sequence for the sessions. Each rat was placed in the water facing the pool's walls at the start location and was allowed for 90 seconds to find out the hidden platform. The latency to reach the platform was recorded. If the rat was unable to locate the hidden platform, it was lifted out and placed on the platform for 20 seconds. The procedure was repeated for all four start locations. Two sessions of four trials were conducted on the first day of testing, separated by different time intervals, and one session of four trials was conducted the next day. After that, the platform was removed, and a probe trial (without platform) was conducted at proper time intervals. Each rat was placed in the pool at the same randomly selected starting pole, and a swimming path was observed, and the time spent in the quadrant of the pool, which contained the platform, was measured. On the completion of the probe trial, a black platform that extended 1cm above the water's surface was placed in a quadrant other than that chosen for the submerged platform. Each rat was given four trials of 90 seconds to locate it. The latency to reach the platform was recorded (working memory procedure).

Passive Avoidance Test [21]: This test uses the normal behavior of rats developed by Kings and Glasser (1970). The step-through latency of passive avoidance behavior was evaluated by using the light-dark apparatus, which has two walls of wood and the remaining two walls of transparent plexiglass. It was divided into two equal compartments (30x25x30cm) by plexiglass with a 10x10cm opening in the center. A gullitone door between the two compartments controlled the opening. The light compartment was painted white, and a 15W lamp was used to illuminate it. The interior of the dark chamber was painted black and had a ceiling.

Each compartment had a copper grid floor. There was a gap of 1.5cm between the two floors in the light-dark box, at the opening between the two chambers to ensure electrical separation. On day 1, a rat was placed in the white box, and the time

taken to enter into the dark chamber was noted. As soon as the rat entered the dark box, the gullitone door was closed, and foot electric shock (0.5mA, for 3 seconds) was delivered. The rat was then replaced to its home cage. On the following day (24 h retention interval), each rat was again placed in the white box and was given a 5 min inhibition period. Latency to step through to the dark chamber was recorded. Electric shock was not delivered on day 2. If the animal remained in the white box for a test period of 5 min, the maximum score of 300 sec was assigned**.** At different time intervals, latency to step through was again recorded to test the retention of the passive avoidance learning.

Statistical Analysis: Results are given as mean ± Standard Error (SE). *In-vivo* data were analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett's test (control and test compounds) or Tukey's test (between the test compounds). Differences are considered significant at a level of P<0 .05 and highly significant at a level of P<0.001. Differences are considered insignificant at a level of P>0 .05.

Forced Swim Test: *In-vivo* pharmacological evaluation of guar gum-loaded Olanzapine microspheres were carried out with animal model-Chalers foster rats. The *in-vivo* effects of OLZS, OLZF, and OLZMP are presented in **Table 1**. OLZS significantly (p<0.01) decreased the immobility time up to 8 h as compared to Ketamine only treated Charles foster rat models representing a negative symptom of schizophrenia. OLZF significantly decreased (p<0.01) immobility time up to 48 h as compared to Ketamine only treated group. OLZMP significantly (p<0.05) decreased the immobility time up to 48 h as compared to Ketamine only treated group. Compared OLZS, OLZF and OLZMP showed a significantly prolonged decrease in immobility time from 16th to 48th h. The control of initial rapid release from the Olanzapine microspheres changed the therapeutic activity.

The highly significant (p<0.01) decrease in immobility time at 16th h and 32 h (using Tukey's test) were observed between OLZS and OLZF, OLZMP. On the other hand, the decrease in immobility time by OLZF and OLZMP was comparable, and no significant (p>0.05) difference was noted.

TABLE 1: EFFECT OF DIFFERENT DOSAGE FORMS ON AMBULATION IN OPEN FIELD EXPLORATORY TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	70.17±1.44	70.33± 1.33	70.5± 1.09	69.0±1.79	69.66± 1.36	69.33± 1.76
Ketamine	94.17 ±1.30	93.5± 1.29	93.66±1.71	93.0± 1.29	93.17± 1.38	93.83± 0.85
OLZS	71.0± 1.37 ^{c}	71.83±0.83^{c}	71.33±1.56 ^{c}	92.17± 2.02	92.5± 1.22	92.5± 1.49
OLZF	68.83± 1.74 ^{c}	69.83± 1.32 ^{c}	69.17±1.54 ^{c}	70.5± 1.14^{c,A}	69.83± 1.45 ^{c,B}	69.5 ±1.23 ^{c,B}
OLZMP	70.33 ±1.69 ^{c}	70.5± 1.69 ^{c}	70.75±1.78 ^{c}	70.9 ±1.48 ^{c,A}	71.33 ±1.23 ^{c,C}	71.5±1.58 ^{c,B}

*Value represents the Mean ± SEM ambulation score animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. ^{A}P<0.05, ^{B}P<0.01, ^{C}P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

TABLE 2: EFFECT OF DIFFERENT DOSAGE FORMS ON REARING IN OPEN FIELD EXPLORATORY TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	16.66±1.28	15.83± 1.60	16.17± 1.11	16.33±0.95	16.17± 0.95	16.66± 1.17
Ketamine	23.17 ±1.33	25.5± 1.48	23.0± 1.03	25.17± 1.60	24.5± 1.12	25.33± 1.36
OLZS	15.5± 1.20^{c}	16.5±1.08 ^{c}	15.17± 1.08 ^{c}	20.0± 0.86	22.17± 1.11	25.66 ±1.12
OLZF	15.0 ±1.34 ^{c}	14.83± 1.07 ^{c}	16.0± 0.86 ^{c}	16.33± 1.20^{c,A}	16.33± 1.12^{c,C}	16.66± 1.09 ^{c,,C}
OLZMP	16.16± 1.40 ^{c}	15.9± 1.40 ^{c}	15.83± 1.376^{c}	16.83± 1.01 ^{c,A}	15.33± 1.31 ^{c,C}	15.66 ±1.43^{c,C}

*Value represents the Mean ± SEM Rearing score animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. ^{A}P<0.05, ^{B}P<0.01, ^{C}P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

Morris Water Maze Test: In this behavioral evaluation of learning and memory, OLZF showed a sustained effect. Up to 8th h there was no significant difference (p>0.05) in escape latency of OLZS and OLZF, OLZMP. During 16th h there was no significant difference (p>0.05) between Ketamine only treated group and OLZS. While during 16th h, the escape latency of OLZF and

OLZMP was decreased. During probe trial on 32nd h no significant difference ($p<0.05$) was noted between Ketamine only treated group and OLZS while on comparing there was a highly significant difference ($p<0.001$) between OLZS and OLZF, OLZMP (using Tukey's test). In case of new platform trials, the escape latency of OLZS treated group and Ketamine only treated group was not significant ($p>0.05$), while the difference in escape latency of OLZF and OLZMP on comparing with Ketamine only treated group was highly significant ($p<0.01$).

Passive Avoidance Test: *In-vivo* Pharma-codynamic evaluation of guar gum-loaded Olanzapine microspheres was carried out with Ketamine treated Charles foster rats. The *in-vivo* effect of Olanzapine administered intranasally as OLZS, OLZF and OLZMP subcutaneously is presented in **Table 3**. In the case of OLZS, step-through latency was increased from 1st to 8th h that was significantly different ($p<0.05$) from Ketamine only treated group but from 16th to 48th h there was no difference ($p>0.05$) between both groups. Up to 8th hour there was no significant difference ($p>0.05$) in escape through latency of OLZS and OLZF, OLZMP (using Tukey's test). From 16th h there was a highly significant difference ($p<0.001$) in step-through latency of OLZS and OLZF, OLZMP. From 16th h there was no significant difference ($p>0.05$) between Ketamine treated group and OLZS.

On the other hand, there was a highly significant difference ($p<0.001$) in step-through latency of Ketamine only treated group and OLZF, OLZMP up to 48th h. Step through latency of OLZF and OLZMP was comparable.

TABLE 3: EFFECT OF DIFFERENT DOSAGE FORMS ON GROOMING IN OPEN FIELD EXPLORATORY TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	9.17±0.79	9.5± 0.62	9.0± 1.03	9.0±1.09	8.83± 0.60	9.0± 2.89
Ketamine	15.0 ±1.24	15.33± 1.28	15.66± 1.05	16.0± 1.29	16.33 ±1.19	16.66 ±1.52
OLZS	9.0± 1.19 [c]	9.66±1.20 [c]	8.5± 0.85 [c]	15.0± 1.09	15.83± 1.12	16.5± 3.07
OLZF	9.17± 0.98 [c]	9.16± 1.16 [c]	9.5 ±0.92 [c]	8.66± 1.26 [c,B]	9.33± 0.95 [c,B]	8.66± 2.88 [c,B]
OLZMP	9.33± 1.17 [c]	9.33± 1.12 [c]	9.0± 1.00 [c]	9.0± 0.93 [c,B]	9.0 ±0.89 [c,B]	8.83± 1.47 [c,B]

*Value represents the Mean ± SEM Grooming score animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. [a]P<0.05, [b]P<0.01, [c]P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

Elevated Plus Maze: In the case of OLZS counts of entries and time spent in closed and open arms were comparable to the control group up to 8th h. There was a significant difference ($p<0.05$) when compared with the ketamine-only treated group. In OLZF treated group counts of entries in and time spent in open and closed arms was significantly different ($p>0.05$) from Ketamine only treated group and was maintained up to 48th h. A group that was treated with s.c. OLZMP showed activity comparable to the control group up to 48th hour. From 16th h; when compared with OLZS, OLZF and OLZMP exhibited a highly significant ($p<0.001$) prolonged effect.

TABLE 4: EFFECT OF DIFFERENT DOSAGE FORMS ON IMMOBILITY TIME IN OPEN FIELD EXPLORATORY TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	69.33±1.45	68.17± 2.55	68.66± 1.86	68.33±1.64	68.33± 1.65	68.66± 2.46
Ketamine	21.17± 1.56	19.66± 1.20	20.66± 1.86	19.22± 1.67	19.5± 1.31	21.66± 1.17
OLZS	68.66± 1.82 [c]	70.5±1.38 [c]	69.5± 1.20 [c]	48.83± 1.51	36.5± 1.57	20.0 ±1.39
OLZF	69.5± 2.16 [c]	70.33± 1.56 [c]	70.33± 1.54 [c]	70.66± 1.15 [c,A]	69.17± 1.62 [c,A]	69.83 ±1.66 [c,C]
OLZMP	70.00± 1.87 [c]	69.5± 1.88 [c]	69.66 ±1.15 [c]	69.83± 1.85 [c,A]	70.0 ±1.92 [c,A]	70.0 ±2.11 [c,C]

*Value represents the Mean ± SEM immobility time animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. [a]P<0.05, [b]P<0.01, [c]P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tucky's test following significant one-way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tucky's test following significant one-way ANOVA.

Open Field Exploratory Test: Table 5 shows that activities of OLZS treated group compared to control group and significantly different ($p<0.05$) from Ketamine only treated group upto 8th h. OLZF treated group exhibited behavioral activities comparable to control group up to 48th h. Group treated with OLZMP showed behavioral activities comparable to the control group. There is a highly significant difference ($p<0.01$) between Ketamine only treated group and OLZF, OLZMP. Compared with OLZS, OLZF and OLZMP displayed significantly ($p<0.05$) prolonged effect after 8th h.

TABLE 5: EFFECT OF DIFFERENT DOSAGE FORMS ON FECAL PELLET IN OPEN FIELD EXPLORATORY TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	1.66±0.33	1.66± 0.33	1.66± 0.33	1.66±0.21	1.33 ±0.42	1.16± 0.31
Ketamine	3.5± 0.43	3.66± 0.33	3.17± 0.60	3.33 ±0.61	3.0± 0.36	3.8± 0.54
OLZS	1.5± 0.22[b]	1.36±0.33[b]	1.66± 0.33[b]	1.83± 0.31[b]	2.17± 0.60[a]	2.0± 0.37[a]
OLZF	1.66± 0.33[b]	1.66± 0.33[b]	1.83± 0.31[b]	1.3± 0.37[b]	1.66± 0.33[b,A]	1.5 ±0.43[b,A]
OLZMP	1.83± 0.31[b]	1.83± 0.31[b]	1.5± 0.43[b]	1.66 ±0.33[b]	1.16 ±0.30[b,A]	1.83± 0.48[b,A]

*Value represents the Mean ± SEM fecal pellet animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. [a]P<0.05, [b]P<0.01, [c]P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

TABLE 6: EFFECT OF DIFFERENT DOSAGE FORMS ON NUMBER OF ENTRIES IN OPEN ARM IN ELEVATED PLUS MAZE TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	20.33±0.76	21.0±0.97	21.3±0.88	21.83±1.01	21.66±0.95	22.0±0.77
Ketamine	6.66±0.33	6.83±0.48	6.83±0.60	6.5±0.56	6.17±0.87	6.0±0.97
OLZS	20.5±0.96[c]	21.83±1.11[c]	20.33±1.12[c]	15.17±0.65[b]	10.66±0.76[a]	8.66±0.81
OLZF	19.33±0.95[c]	18±0.80[c]	17.33±0.95[c]	20.17±1.42[c,B]	22±1.52[c,C]	21.66±1.41[c,C]
OLZMP	21.33±0.89[c]	17.66±0.93[c]	18.17±1.40[c]	19.33±1.01[c,B]	22±1.09[c.C]	22.83±0.70[c,C]

*Value represents the Mean ± SEM number of entries of animals (n=6) in open arm during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. [a]P<0.05, [b]P<0.01, [c]P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

TABLE 7: EFFECT OF DIFFERENT DOSAGE FORMS ON NUMBER OF ENTRIES IN CLOSED ARM IN ELEVATED PLUS MAZE TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	5.17±0.65	4.66±0.67	5.5±0.62	3.0±0.52	6.0±1.09	4.17±0.79
Ketamine	14.83±0.87	13.83±0.95	15.0±0.73	15.83±0.79	14.33±0.95	15.16±1.12
OLZS	3.5±0.43[c]	3.67±0.61[c]	4.33±0.76[c]	10.33±1.05[a,A]	12.66±1.42	14.83±0.70[c]
OLZF	2.33±0.42[c]	3.33±0.80[c]	5.33±0.56[c]	3.33±0.92[c,A]	4.83±0.83[c,A]	5.33±0.61[c,C]
OLZMP	3.17±1.01[c]	5.5±0.62[c]	3.17±0.79[c]	3.0±0.52[c,A,]	5.17±0.95[c,A]	4.0±0.93[c,C]

*Value represents the Mean ± SEM number of entries of animals (n=6) in the closed arm during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control .[a]P<0.05, [b] P<0.01, [c]P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA.

TABLE 8: EFFECT OF DIFFERENT DOSAGE FORMS ON TIME SPENT IN OPEN ARM IN ELEVATED PLUS MAZE TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	244.66±1.69	245.66±1.59	244.48±1.77	244.33±1.56	245.0±1.41	25.17±0.75
Ketamine	154.0±1.65	148.33±2.99	152.83±1.99	153.83±2.26	155.0±1.51	124.33±0.76
OLZS	245.83±1.60[b]	244.17±1.42[b]	245.0±1.55[b]	160.8±1.46	154.17±1.38	125.0±1.13
OLZF	245.0±2.11[b]	244.66±1.28[b]	244.17±1.99[b]	245.17±1.25[b,A]	244.33±0.95[b,A]	24.33±0.95[b,A]
OLZMP	244.83±2.49[b]	245.0±1.98[b]	244.66±1.52[b]	245.5±1.56[b,A]	246.0±1.48[b,A]	24.5±0.92[b,A]

*Value represents the Mean ± SEM of time spent by animals (n=6) in open arm during observation period of 1,4,8,16,32,48 hours after different test compounds, saline control . aP<0.05, b P<0.01, cP<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA. [A]P<0.05, [B]P<0.01, [C]P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA.

TABLE 9: EFFECT OF DIFFERENT DOSAGE FORMS ON TIME SPENT IN CLOSED ARM IN ELEVATED PLUS MAZE TEST

Group	1st Hour	4th Hour	8th Hour	16th Hour	32nd Hour	48th Hour
Control	26.0±0.77	26.66±0.67	26.5±0.76	25.66±0.80	24.66±1.15	25.17±0.75
Ketamine	126.5±2.43	127.33±1.31	127.17±0.95	125.17±2.37	122.33±0.92	124.33±0.76
OLZS	24.66±1.28 c	25.66±0.80 c	27.0±1.41 c	80.17±1.72 b	100.66±1.20	118.0±1.13
OLZF	25.66±1.30 c	26.33±0.61 c	26.83±1.30 c	25.0±0.97 c,A	24.17±1.17 c,C	24.33±0.95 c,C
OLZMP	26.17±1.08 c	26.48±0.76 c	26.66±1.38 c	25.33±0.80 c,A	24.5±0.96 c,C	24.5±0.92 c,C

*Value represents the Mean ± SEM time spent by animals (n=6) in the closed arm during the observation period of 1,4,8,16,32,48 hours after different test compounds, saline control. aP<0.05, b P<0.01, cP<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA. A P<0.05, B P<0.01, C P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

TABLE 10: EFFECT OF DIFFERENT DOSAGE FORMS ON FORCED SWIM TEST

Time (Hours)	Control	Ketamine	OLZS	OLZF	OLZMP
1st	39.17±1.22	235.17±2.10	38.5±1.87	40.83±1.87	41.33±2.31
4th	40.17±2.02	230.17±2.08	41.66±1.41	43.33±1.67	38.0±1.75
8th	39.33±1.67	243.17±2.12	40.83±1.60 c	35.5±2.03 c,	42.12±1.34 c
16th	39.0±1.85	248.83±2.39	100.17±2.18 a	42.66±2.12 c,A	43.5±2.05 c,A
32nd	43.0±1.51	242.5±2.62	148.66±2.23 b	41.5±1.79 c,B	45.83±1.48 c,B
48th	40.17±1.28	251.0±1.93	200.33±1.43 b	39.17±2.41 c,C	41.48±2.30 cC

*Value represents the Mean ± SEM of behavioral despair of animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control .aP<0.05, b P<0.01, cP<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA. ^{A}P<0.05, ^{B}P<0.01, ^{C}P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA.

TABLE 11: EFFECT OF DIFFERENT DOSAGE FORMS ON ESCAPE LATENCY IN MORRIS WATER MAZE TEST

Time (Hours)	Control	Ketamine	OLZS.	OLZF	OLZMP
1st	59.5±2.23	86.83±1.64	61.5±1.73 a	61.66±1.67 a	58.0±5.33 a
4th	41.0±1.57	68.0±1.44	42.66±1.23 a	42.0±1.29 a	40.33±1.20 a
8th	33.33±0.98	62.0±0.77	38.17±1.08 a	33.0±1.32 a	33.0±1.24 a
16th	20.5±1.08	53.5±2.89	61.5±1.82	20.83±0.91 c,A	20.66±1.15 c,A
32nd PT	75.33±0.71	42.0±2.31	44.5±1.59	77.66±0.95 c,C	78.66±1.17 c,C
48th NPT	14.17±1.11	56.17±2.06	55.00±1.86	13.83±1.08 c,C	13.0±1.16 c,C

PT-Probe Trial, NPT-New Platform Trial. *Value represents the Mean ± SEM escape latency of animals (n=6) in during observation period of 1,4,8,16,32,48 hours after different test compounds, saline control .^{a}P<0.05, ^{b} P<0.01, ^{c}P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA. ^{A}P<0.05, ^{B} P<0.01, ^{C}P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one-way ANOVA.

TABLE 12: EFFECT OF DIFFERENT DOSAGE FORMS ON STEP THROUGH LATENCY PASSIVE AVOIDANCE TEST

Time (Hours)	Control	Ketamine	OLZS	OLZF	OLZMP
1st	11.48±1.48	22.66±0.42	11.83±1.08 b	11.66±1.05 b	11.0±1.18 b
4th	293.66±1.82	140.0±1.39	289.83±1.25 c	291.0±1.89 c	290.0±2.73 c
8th	290.66±2.12	128.17±0.91	286.66±0.99 c	291.66±2.69 c	291.83±1.96 c
16th	291.33±1.12	125.66±1.33	130.17±0.70	293.17±1.62 c,A	289.0±1.24 c,A
32nd	282.83±2.76	122.83±1.01	128.0±2.16	283.33±1.65 c,A	284.33±1.74 c,A
48th	266.66±2.29	113.66±2.09	118.17±2.02	270.0±1.71 c,B	269.66±2.01 c,B

*Value represents the Mean ± SEM step-through latency exhibited by animals (n=6) during an observation period of 1,4,8,16,32,48 hours after different test compounds, saline control .^{a}P<0.05, ^{b} P<0.01, ^{c}P<0.001 compared between Ketamine, OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA. ^{A}P<0.05, ^{B} P<0.01, ^{C}P<0.001 compared between OLZS, OLZF, OLZMP using Tukey's test following significant one way ANOVA

DISCUSSION: Schizophrenia (SCZ) is a heterogeneous chronic disease characterized by severe behavioral perturbation. Atypical antipsychotic agent OLZ is used because it is among the most appropriate choices for the treatment of SCZ in terms of a long-term delivery system based on efficacy, potency, stability, side effects and risk profile. The result suggests that guar gum-loaded OLZ microsphere significantly prevented positive, negative, and cognitive symptoms in a ketamine-induced animal model of SCZ and also the effect was sustained up to 48 h. Agents that block the N-Methyl-D-aspartate subtype of glutamate receptor, such as Ketamine, induce a SCZ like psychosis in adult humans.

Ketamine-induced model mimics the core behavioral model deficient in SCZ including positive, negative, and cognitive symptoms The effect persists even after the drug withdrawal period. The reversal of all symptoms by atypical antipsychotic drugs validates this model. Since Ketamine has these properties we elected this drug to induce SCZ conditions in this study. Pharmacodynamic activity of OLZ by this model is predictive of efficacy against positive, negative, and cognitive symptoms of psychosis and demonstrates prolonged in vivo antagonistic activity of D-2 receptor of OLZ loaded guar gum formulation. Decrease in immobility time was found to be less for OLZS than OLZF and OLZMP.

Inhibition of immobility time was observed up to 8 hrs. 48 h after intranasal and subcutaneous injection of OLZS, OLZF, and OLZMP, respectively. During the passive avoidance test, representing the cognitive symptoms of SCZ, the step-through latency of OLZ treated group is nearly the same as control, OLZF and OLZMP treated groups up to 8hrs but after 16 hr step-through latency increased, and at 32 hr is was nearly the same as Ketamine only treated group. On the other hand, in the case of OLZF and OLZMP formulation, step-through latency was nearly the same as of the control group from 1^{st} to 48^{th} hr. this indicates retention of memory was exhibited by OLZF and OLZMP up to 48^{th} h due to sustained release. In another test for cognitive symptoms that is Morris Water Maze test, similar results were found. During 1^{st} hr the escape latency of OLZS, OLZF, OLZMP, and control group was nearly the same. Up to 8 hr difference in escape latency in OLZS, OLZF and OLZMP treated group was not significant was nearly same as of control group. During probe trial time spent in quadrant where the platform was placed earlier by OLZF and OLZMP as nearly same as the control group while there was a highly significant difference between OLZS and OLZF, OLZMP treated groups. The difference in time spent in that quadrant by OLZS treated group and Ketamine only treated group was insignificant. This shows the tapering effect of OLZS because the drug solution was not a sustained release formulation. On 48^{th} h; during new platform trial OLZF and OLZMP treated group shows escape latency nearly as of control group.

This indicates that OLZF and OLZMP have sustained effect on 48^{th} hr. So guar gum-loaded OLZ microsphere has activity comparable to OLZMP. An elevated plus-maze behavioral test was employed to test the effect of OLZF on ketamine-induced negative symptoms in rats. It was seen that the OLZS treated group entered and spent more time in the open arm up to 8^{th} hour. But after 8^{th} ho counts of entries as well as time spent in closed arm was increased while counts of entries and time spent in the open arm was decreased and from 16^{th} to 48^{th} hour there was an insignificant difference in a number of entries and time spent in open as well as closed-arm between OLZS and Ketamine only treated group.

This indicates that OLZS has inhibited depressive behavior in Ketamine treated group up to 8^{th} hour but from 16^{th} h its effect starts tapering. Whereas OLZF and OLZMP inhibited the ketamine-induced depressive behavior up to 48^{th} h proved by increased counts of entries and time spent in open arms as compared to closed arms. Rats having depressive symptoms tend to enter frequently in closed and spend more time in closed arm while rats having antipsychotic effect of drug will show more entries in open arms and will also spend more time in open arms; exploring the arms of elevated plus maze suggesting active behavior. Open exploratory field test is a measure of positive symptoms of SCZ. In this test ketamine induced anxious and restless rat model will show more numbers of ambulation means crosses more number of squares, increased number of grooming, rearing and fecal pellets and decreased immobility

time. When the anxious and restless behavior is affected by drug administration, then the number of ambulation, grooming, rearing, and fecal pellets will decrease. Ketamine only treated group showed anxious and restless behavior mimicking positive symptoms of SCZ. This group was running from one side to the other crossing the squares. This group was also grooming itself again and again and stood many times on their hind limbs and excreted fecal pellets. And also, rats of this group were mobile for only a few seconds. It was noted that up to 8 hours OLZS decreased the number of ambulation grooming, rearing, fecal pellets, and increased immobility time for few more seconds. From 16th hour gave a significant difference between OLZS, OLZF and OLZMP. In OLZS treated rats number of various behavioural parameters was increased, indicating the tapering effect of drug solution. On the other hand there was no significant difference in behavioral parameters exhibited by OLZF and OLZMP treated rats and control groups up to 48th hour; suggesting sustained release effect of guar gum loaded OLZ microsphere and OLZMP.

CONCLUSION: So, from the above study, it has been concluded that Ketamine induces rat model of positive, negative and cognitive models of SCZ have predictive validity and can be used to evaluate antipsychotic effect of drugs. In our study when compared; up to 8th h OLZS, OLZF and OLZMP treated group exhibited insignificant difference ($p>0.5$) in their behavioral pattern when subjected to different behavioral evaluation conditions indicating different symptoms of SCZ. But after 8th hour that is from 16th h the difference in behavioral pattern between the ketamine-only treated groups OLZS treated group was insignificant, suggesting the diminished effect of the drug OLZ contained in OLZ drug solution. This might have occurred because the concentration of OLZ in rats was not sufficient to overcome the effect of Ketamine. Surprisingly, it was noted that a group of rats was displayed behavioral patterns treated with intranasal OLZF and s.c. OLZMP; were insignificantly ($p> 0.5$) different from the control group, while highly significant differences ($p<0.001$) were observed between Ketamine only treated group versus OLZF and OLZMP treated group from 1st to 48th h. This proves the sustained effect of the drug contained in our formulation of guar gum-loaded microsphere that is comparatively insignificant from sustained-release marketed preparation of drug Olanzapine.

ACKNOWLEDGEMENT: Author is thankful to Dr. B. L. pandey, Professor, Department of pharmacology, IMS, BHU, for his invaluble support and the management of Rama medical College Department of Pharmacology for support and encouragement.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

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How to cite this article:
Singh C: Neuropharmacological evaluation of intranasal guar gum loaded olanzapine microspheres. Int J Pharm Sci & Res 2021; 12(12): 6725-35. doi: 10.13040/IJPSR.0975-8232.12(12).6725-35.