International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.543.550

Research Article
Enhanced Brain Uptake and Behaviour Study of Buspirone Loaded *in situ* Nanoemulsion Gel

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Abstract

Background and Objective: Nose-to-brain delivery is the most fascinating application, bypassing the blood-brain barrier and directly targeting the brain receptor. Buspirone is indicated for the treatment of generalized anxiety disorder, relieve symptoms of anxiety and unipolar depression. This drug exhibit low bioavailability (approximately 5%), extensive first-pass metabolism and non-targeted delivery results in numerous side effects. The reported experimental study was conducted to explore the potential of buspirone-loaded *in situ* nanoemulsion gel (BNG) to the brain delivery via nasal route. **Materials and Methods:** Effect of single daily nasal administration was compared in Wistar rats for locomotor activity, marble burying and elevated plus maze model. Thereafter, buspirone concentration in brain tissue was determined by multiple time point pharmacokinetic study. The analysis of the drug was carried out on the HPLC system. **Results:** A statistically significant effect of Buspirone-loaded Nanoemulsion (BNE) and BNG as compared to the nasal solution was observed in the present investigation. Biodistribution of BNE, BNG, Buspirone Plain Solution (BHP) and Buspirone Solution (BHS) in the brain and blood of swiss albino rats following Intranasal (IN) and Intravenous (IV) administration was examined. The brain blood uptake ratio of 0.889, 2.398, 2.779 and 0.102 for BHP (intranasal), BNE (intranasal), BNG (intranasal) and BHS (IV), respectively at T_{max} are indicative of direct nose to brain transport bypassing the blood-brain barrier. Higher direct transport percentage (93.00%) and Drug Transport Efficiency (DTE>1) confirm the direct pathway from the nose to the brain. **Conclusion:** Reports imply that the pharmacokinetic and pharmacodynamics study used in this investigation is well suited for optimization of spatial and temporal targeting. The finding of reported results reveals, a rapid and larger extent of transport of BNG and BNE, which confirms that *in situ* nanoemulsion gel containing buspirone could be used as an intranasal formulation for targeted brain delivery.

Key words: Buspirone hydrochloride, brain uptake, nose to brain delivery, nanoemulsion, brain targeting, intranasal administration, olfactory pathway

Citation: Tripathi, K. and N.K. Manna, 2022. Enhanced Brain uptake and behaviour study of buspirone loaded *in situ* nanoemulsion gel. Int. J. Pharmacol., 18: 543-550.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anxiety is a widespread condition of stress, uncontrollable and persistent nervousness, worry in clinical practices and associated with psychiatric or psychological intervention[1]. We know that these constitute the 6th most common chronic condition in clinical practice. Approximately two-thirds of anxious patient respond to the currently available treatment but shows the disappointing magnitude of improvement till now[2]. Although many anti-anxiety drugs are available today the systemic delivery of therapeutic moieties to the brain is not effective due to the presence of the Blood Brain Barrier (BBB) and Blood Cerebrospinal Fluid Barrier (BCSFB)[3], which prevents the release of drugs from the circulating blood to the brain or tightly segregates the brain from the circulating blood[4]. These necessities the development of a controlled release formulation[5] (buspirone *in situ* nanoemulsion gel), whose psychotherapeutic potential has been assessed in animal models. Buspirone [8-(4-(4-(2-Pyrimidinyl)-1-piperizinyl)butyl)-8-azaspiro(4,5)decane-7,9-dione] is a psychotropic drug with anxiolytic properties that belongs chemically to the class of azaspiro decanediones and is used primarily as an anxiolytic, specifically for generalized anxiety disorder, relieve symptoms of anxiety and unipolar depression[6].

Orally administered buspirone is rapidly absorbed but it undergoes first-pass metabolism, resulting in a low bioavailability of less than 5%. So thereby a strategy of the nose to brain delivery of therapeutic moiety improves the bioavailability by preventing first-pass metabolism, by-passes the blood-brain barrier and provides spatial delivery to the receptor site[7]. Earlier studies have demonstrated that Intranasal administration of therapeutics avoids first-pass metabolism, there by protecting it from the nasal metabolizing enzymes[8], enhancing the uptake of the therapeutic moiety to the brain, avoiding systemic circulation[9] and hence enhancing the amount of drug in the brain and improving brain bioavailability[10]. Martins *et al.*[11] suggested that nasal delivery technologies are designed to overcome inherent anatomical and physiological challenges and facilitate more efficient and targeted drug delivery for Central Nervous System (CNS) disorders[11]. Bonferoni *et al.*[12] evaluated that nasal nanoemulsion seems to be an effective system to achieve temporal and spatial delivery for neurological treatment. Keller *et al.*[13] suggested that the olfactory pathway appears to be a promising route for the long-term treatment of Alzheimer's disease since it is less expensive, more convenient to use and less prone to systemic side effects than other methods currently in use[13].

In the present research, we strive to investigate the effect of buspirone-loaded *in situ* nanoemulsion gel (BNG) on the various animal models by examining the possibilities of effects for brain delivery via the nasal route in the rat brain.

MATERIAL AND METHODS

Study area: The study was carried out at the Deshpande Laboratories limited, MP (India) from January-March, 2020.

Materials: Buspirone was procured from Yarrow Chem Products, India. Pluronic F127 (PF127) was acquired from BASF, Germany. Methanol and acetonitrile of HPLC grade were procured from SD Fine-Chem Limited (Ahmedabad, India). All other reagents and solvents used were of analytical grade and procured from local vendors.

Animals: Adult male 120 Wistar rats (250-300 g) were obtained from the animal house, Deshpande Laboratories limited (regd. No. 1410/c/11/CPCSEA). The animals were accommodated under standard laboratory conditions, individually ventilated cage, maintained on a 12:12 hrs light: Dark cycle, 25°C temperature, controlled humidity and noise, animals had free access to food. Animals were fed with autoclaved standard pellet diet and RO water *ad libitum*. Animals were acclimatized to laboratory conditions before the tests. All experiments were carried out between 9 and 17 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and performed in agreement with the guidelines of the Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Experimental design: The animals were randomly divided into 3 experimental groups with 6 animals in each. Group I (Control) comprised of control animals receiving saline as a vehicle. Group II animals (Standard) were administrated buspirone solution (IV) while group III (Test) animals were administrated BNG (Nasal route) (Table1). The dose of buspirone (5 mg kg^{-1}) was selected based on previous studies stating buspirone effects[14]. For the pharmacokinetic study, the animals were randomly divided into 4 experimental groups with 6 animals in each. Group I (positive control) comprised of control animals receiving buspirone solution as a vehicle through the intranasal route. Group II animals (Standard) were administrated buspirone solution (intravenous route) while group III and IV (Test) animals

Table 1: Various groups used for pharmacodynamics study

Groups	Route of administration	Treatments	No. of animals
I	Intranasal route	Normal saline	6
II	IV route	Buspirone solution	6
III	Intranasal	Buspirone loaded *in situ* nanoemulsion gel	6

Table 2: Details for conducting a pharmacokinetic study in rats

Groups	Route of administration	Treatments	No. of animals
I	Intranasal route	Buspirone solution (positive control)	6
II	IV route	Buspirone solution	6
III	Intranasal	Buspirone loaded nanoemulsion	6
IV	Intranasal	Buspirone loaded *in situ* nanoemulsion gel	6

have administrated nanoemulsion and *in situ* nanoemulsion gel containing buspirone (intranasal route), respectively (Table 2). The buspirone concentration was determined by the validated HPLC method reported by Ki Taek Kim with few modifications[15].

Animal study formulations: About 15.5 mg of Buspirone Hydrochloride (BH) was dissolved in 5 mL of sterile isotonic saline and filtered through a sterile (0.2 mm) membrane filter to make the Buspirone Hydrochloride Solution (BHS) for intravenous injection. The formulation's pH was adjusted to 7.4 in the end.

By dissolving 15.5 mg of buspirone hydrochloride in 5 mL of sterile 0.5% sodium chloride solution and filtering through a sterile (0.2 mm) membrane filter, a control buspirone hydrochloride solution for nasal administration (BHP) was prepared. The formulation's final pH was 5.5[16].

Preparation and characterization of *in situ* nanoemulsion gel: The aqueous titration method was used to develop buspirone-loaded nanoemulsions (BNEs) containing Tween 80, PEG 400 and Oleic acid as the surfactant, co-surfactant and oil respectively. The NEs were evaluated for the globule size, zeta potential, Polydispersity Index (PDI) value, thermodynamic stability, appearance, viscosity, pH, conductivity and refractive index. *In vitro* drug release from Nanoemulsions (NEs) in phosphate buffer pH 5.5 was studied by using Keshary-Chien cell (KC cell, 25 mL). Optimized formulation with drug release 90±0.39% in 6 hrs, PDI value 0.230±0.01 and mean globule size of 105.4±1.10 nm was used as temperature-sensitive *in situ* nanoemulsion gel formulation with Pluronic F127(17.5 % W/W). The *in situ* nanoemulsion gel was transparent and uniform, with a good bioadhesive strength. The *in vitro* cumulative drug release was reported to be 90.00±0.39% at the end of 6 hrs. The buspirone loaded nanoemulsion gel formulation, showed a significant improvement in *in vitro* drug release, ensuring a significant dose reduction as well as reducing side effects by avoiding first-pass metabolism via the nasal route for the treatment of anxiety disorders[17].

Pharmacodynamic study elevated plus-maze model (EPM): The EPM apparatus (Avani Scientific Co., Haryana, India) consists of a plus shape maze raised above the floor. It possesses 2 open arms fronting one another and is differentiated by a central square and two closed arms of the same measurement but this one is surrounded by walls. The EPM model is used to access the anxiety behaviour of rodents. The EPM builds on the revulsion of rodents for open space and dose struggle between probe and resistance, to an upraised open place. Hence this apparatus is usually mentioned as an unconditioned spontaneous behavioural conflict model.

The rats are placed at the junction of the 4 arms of the maze, fronting to the open arm. They manifest a designed behaviour of turning away from the open space and bent toward closed space. Behavioural reactions in EPM are comfortably evaluated and recorded for 5 min. The sequence of preference or penchant is closed>centre>open. This one expressed their liking for a comparatively more secured section of the maze. Other estimates that can be observed in the rat in this process are fear, head dips, stern, faecal boli, freezing and straighten posture. Such propensity is repressed by anxiolytics. This behaviour study reveals remarkable data as per the ANOVA table by calculating a quotient of time spent on the open arms to the time spent on the closed arms[18].

Marble burying test: In the present study, after selecting a strain of rats are kept individually in transparent plastic boxes (30×30×28 cm) carrying 25 glass marbles (1.5 cm in diameter) are arranged at an equal distance on sawdust deep down 5 cm. without food and water, for 30 min. The rat will start burying marbles. This etiquette is considered to, interconnected with anxiety and Obsessive Compulsive Disorder (OCD). A marble is scrutinized

Table 3: Various pharmacokinetic parameters obtained by different routes in Wistar rats

Formulation	Organ/tissue	C_{max} (ng mL^{-1})	T_{max} (hrs)	$AUC_{0-480\ min}$ (ng min mL^{-1})	Brain/blood ratio
BHP (IN)	Blood	486.72±10.54	2	2274	0.889
	Brain	432.57±12.22	2	1548	
BNE (IN)	Blood	293.31±8.26	2	1097	2.398
	Brain	703.23±17.21	2	2232	
BNG (IN)	Blood	332.70±11.24	2	1260	2.779
	Brain	924.57±24.03	2	3018	
BHS (IV)	Blood	2732.42±28.6	0.5	5852	0.102
	Brain	278.42±15.24	2	981.9	

BHP: Buspirone hydrochloride plain, BNEs: Buspirone-loaded nanoemulsions, BNG: Buspirone loaded *in situ* nanoemulsion gel, BHS: Buspirone hydrochloride solution

buried if 2/3 of the marble is enveloped with sawdust. The outcome of marble buried is decreased with the effect of anxiolytic drugs[19].

Locomotor activity (light-dark test): The light-dark test builds on dissension between the search of a novel surrounding and escape away from brightly lit open areas. The light-dark box has 2 compartments, one is light and another is dark. The light compartment is 2/3 part (opened and illuminated with bright light) and the dark one is 1/3 part (close and illuminated with dim light) of the boxes. Rats exhibit more locomotor activity interchanging the compartments. Rats prefer darker areas above lighter areas. When the rat is allowed for activity and kept into a brightly lit portion, will speedily proceed for a darker area. If the rat is administrated with anxiolytic drugs, then the percentage of time that expands in the darker compartment will decrease. Thus 5 major considerations are now available to evaluate the treatment of anxiolytic drug profile: The delay during the first crossing from light compartment to dark one, a total transformation between compartments, the movement in an individual compartment and the time consumed in every compartment. Rearing and grooming is also a sign of exploration, hence sometimes this is as well considered[20]. Rats are remarked for the locomotor activity that was assessed by an Actophotometer (Avani Scientific Co., Haryana, India). The important consequences are manifested by the animal with the treatment of BNG formulation via nasal route.

Pharmacokinetic study: After completion of the behavioural study, a multiple-time point pharmacokinetic study was performed. The rats were divided into four groups (Table 2). Group I (positive control) comprised of control animals receiving BHP as a vehicle through the intranasal route. Group II animals (Standard) were administrated BHS (intravenous route) while group III and IV (test) animals have administrated BNE and BNG (nasal route), respectively. Animals were treated with test formulations. About 20 µL of test formulations of BHP, BNE and BNG was instilled in both the nostrils (n = 6). After 30, 60, 120, 240 and 480 min the animals were sacrificed and the brain was isolated and kept on ice. Blood was withdrawn by cardiac puncture and plasma was isolated. The brain was homogenized in Phosphate Buffer Saline (PBS). Brain and plasma samples were extracted with methanol and analyzed on HPLC. The analysis of buspirone was carried out by the validated HPLC method reported by Ki Taek Kim with few modifications[15].

The data in Table 3 were used to calculate pharmacokinetic characteristics for buspirone loaded formulations. Pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA) was used to analyze the plasma concentration-time profiles of BH after (IN) and (IV) administration. Individual brain and plasma concentration-time profile studies were used to calculate specified pharmacokinetic parameters following (IN) and (IV) administration. Various parameters such as the Area Under the Curve (AUC), C_{max} and T_{max} were calculated by using pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA). Equations 1 and 2[10] were used to calculate the efficiency of brain targeting i.e., Direct Transport Percentage (DTP) and Drug Transport Efficiency (DTE).

$$DTE = \frac{(AUC\,brain / AUC\,blood)\ (IN)}{(AUC\,brain / AUC\,blood\ (IV)} \quad (1)$$

$$DTP\ (\%) = \frac{(B_{in} - B_x)}{B_{in}} \times 100 \quad (2)$$

$$Bx = \frac{B\ (IV)}{P\ (IV)} \times P\ (IN)$$

where, Bx is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration, B (IV) is the AUC0-480 (brain) following intravenous administration, P (IV) is the AUC0-480 (blood) following intravenous administration, B (IN) is the AUC0-480 (brain) following intranasal administration, P (IN) is the AUC0-480 (blood) following intranasal administration, AUC is the area under the curve[21].

Statistical analysis: The data from experiments are expressed as Mean±Standard deviation for each treatment of the group. Standard error of the mean has been used to specify the statistical uncertainty. The data obtained from each response measure were subjected to a one-way analysis of variance (ANOVA). Statistical analysis was carried out using GraphPad Prism 8 software. Statistical analysis was performed applying a significance level of 0.05.

RESULTS

In the EPM model, it has been observed that the control group reveal an inclination to persist in the closed arm, which proposed an anxiogenic effect. The outcome of standard and BNG formulation of familiar anxiolytic has come across a significant rise in entrance and draining time in the open arm. When correlating it with the control group, the relative control group showed a decrease in such activity of entry and spending time in the open arm. Dunnett's multiple comparison test, control vs standard and control vs. test exhibit remarkable differences. When the result of closed arm entry is put in one way ANOVA test then there is a significant outcome of the intranasal treatment of BNG formulation (Mean difference = -17.67, P = 0.002) as compared to the control group. Likewise, BNG treated group manifest an increase in the entries and time consumed in the open area as compared to the vehicle group. Thus when applied one-way ANOVA to open arm entries the quotient shows a notable effect of the intranasal treatment of BNG formulation (mean difference = 52.83, p = <0.0001) as compared to the control group (Fig. 1a-b).

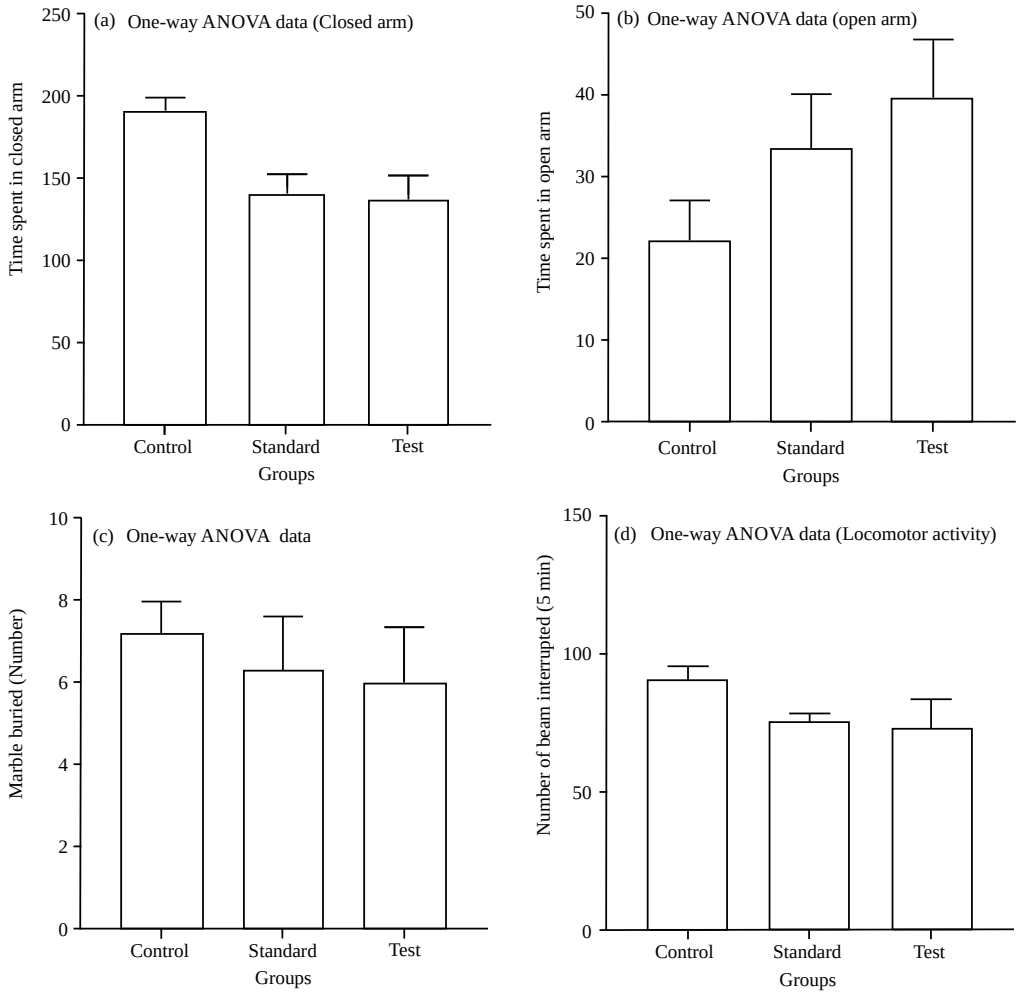

Fig. 1 (a-d): Behavioral evaluation of buspirone loaded *in situ* nanoemulsion gel in rats, (a) Effect of BNG on EPM (Time spent in the closed arm), (b) Effect of BNG on EPM (Time spent in the open arm), (c) Effect of BNG on marble buried and (d) Effect of BNG on locomotor activity

Table 4: Drug targeting efficiency and direct nose to brain transport

Groups	Route of administration	Drug targeting efficiency (DTE %)	Direct nose to brain transport (DTP %)
BHP (IN)	Intranasal route	4.057117	75.35
BHS (IN)	IV route	12.1262	91.75
BNG (IN)	Intranasal	14.27532	93.00

BHP: Buspirone hydrochloride plain, BHS: Buspirone hydrochloride solution, BNG: Buspirone loaded *in situ* nanoemulsion gel

Marble burying behaviour in rats is reduced with BNG formulation via nasal route and the reduction was significant relative to the control group. The standard group also exhibits a remarkable lowering in the number of buried marbles (Fig. 1c). When rats are treated with BNG formulation via nasal route, the outcome shows a remarkably higher locomotor score in comparison to the control group. When this result is placed in one way ANOVA, that reveals a significant effect of Dunnett's multiple comparison test (Mean difference =16.75, p =0.0005) as compared to the control group (Fig. 1d).

The brain blood ratio of BH formulations was determined in Wistar rats following (IN). (BNG, BNE and BHP) and (IV) (BHS solution) administration and concentration were estimated at different intervals up to 8 hrs. As compared to blood, the BH concentration in the brain following BNG administration was considerably higher at all-time points. At t_{max}, the brain blood ratios for BHP (IN), BNE (IN), BNG (IN) and BHS (IV) formulations were 0.889, 2.398, 2.779 and 0.102, respectively, confirming direct nose-to-brain transport without crossing the blood-brain barrier (Table 3).

As shown in Table 3, the brain concentration achieved after (IN) administration of BNG (924.57±24.03 ng mL^{-1}, t_{max} 120 min) and BNE (703.23±17.21, t_{max} 120 min) was substantially greater than that achieved after (IV) administration of BHS (278.42±15.24 ng mL^{-1}, t_{max} 120 min) (Table 3). At all-time points, the brain concentration of BNG and BNE after (IN) the administration was higher than that of BHS after (IV) administration. The assessed pharmacokinetic parameters showed a significant difference between (IN) and (IV) administration ($p<0.05$). The AUC in the brain through BNG (IN) at BHS (IV) was found to be almost 3.074 times greater when compared to BHS (IV) as reported in Table 3 (IV). This finding revealed that (IN) administration of pharmaceuticals into the brain provided a noninvasive alternative for CNS delivery (Table 3). The DTP and DTE indicate, how much of the drug is delivered directly to the brain via the olfactory pathway. In the rats, BH from three distinct formulations (BHP, BNE and BNG) was administered by the intranasal route, while BUH solution was given intravenously. Equations 1 and 2 were used to calculate DTP and DTE. While compared to the (IV) administered BHS solution, BNG revealed the highest DTE value and DTP (%) of (14.275) and (93.00%), respectively (Table 4).

DISCUSSION

The main finding of present research indicates that buspirone containing thermoreversible nanoemulsion gel can deliver the drug through nasal route and the present investigation is supported by behaviour and multiple time points pharmacokinetic studies. These studies were carried out by employing animal models like the EPM model, marble-burying behaviour and locomotor activities followed by multiple time points pharmacokinetic studies which reflect the distribution of a drug in brain tissue and plasma. The neurobehavioral profile of animals under the influence of an anxiolytic medication was assessed in present investigations. The EPM is a well-known model for evaluating anxiolytics and it was used to assess rats' psychomotor performance and behavioural characteristics. This concept, which is based on rodents' inherent fear of heights and open spaces, has been verified in rats and mice and is bidirectionally responsive to pharmacological interventions. The BNG produced a significant increase as compared to the control group (anxiolytic effect) which is confirmed by movement preference profile as open>centre>closed, indicative of the effect of *in situ* nanoemulsion gel formulation. However, the opposite effect was shown by the control group[1-22].

The present study revealed that the test group of animals reduced marble-burying behaviour and the effect was comparable with the standard and control groups. However, the effect is not significant as per ANOVA analysis but behavioural response indicates successful release of formulation via nasal route. The optimized BNG showed, a significant increase in the movement in the individual compartment and the time consumed in every compartment, the number of squares crossed, rearing and ambulation compared to the control group because the light-dark test was used to evaluate the animal emotional state and anxiety-related behaviour. It is characterized by the normal version of the animal to a brightly light area. Thus, animals removed from their acclimatized cage and placed in an environment express anxiety and fear, by showing alteration in all or some parameters. Standard and test groups showed a significant increase in the number of squares crossed, rearing and ambulation compared to control which indicates spatial delivery of buspirone loaded formulation via nasal route[23].

A multiple-time point pharmacokinetic study demonstrated that the BNG administration by intranasal route leads to a higher concentration of buspirone in brain tissues (Table 3), suggesting a possible role that absorption of molecules takes place at the olfactory and respiratory epithelial leads to increase the nasal permeation of therapeutic moiety to the brain, improve delivery drug, protect therapeutics from degradation along the pathway, increase mucoadhesion and facilitate overall nasal transport[24]. It is assumed that a direct nasal pathway to the brain exists if the concentration of a drug is significantly higher by nasal route than that of (IV) administration or if the DTE value is more than one henceforth data obtained from present research supports direct nose to brain delivery of buspirone[16].

Buspirone is extensively metabolized in the liver to active metabolites which are further metabolized and excreted by the kidney so the risk of toxic reaction to this drug may be greater in patients. The report showed a statistically significant difference in multiple time point pharmacokinetic studies and behaviour studies and the finding of this study has significance in terms of reduction in dose and toxicity. The statistical analysis for the present study revealed that *in situ* nanoemulsion gel manifests significant improvement in drug release by avoiding first-pass metabolism via nasal route for treatment of anxiety disorders. The present study indicates that nose-to-brain delivery of therapeutic moieties (who do not pass the BBB) by controlled release novel formulation is advantageous, compared with the classical delivery routes.

CONCLUSION

Buspirone-loaded thermoreversible nanoemulsion gel was successfully formulated. *In vivo*, multiple time point pharmacokinetic and behaviour studies in Wistar rats indicated the superiority of the developed formulation for brain targeting when compared with the results of the oral and nasal solutions of the drug. The study, however, still requires further neurotransmitter studies to evaluate its efficacy based on the risk benefit ratio but based on these research findings, it was concluded that buspirone-loaded thermoreversible nanoemulsion gel could be a promising delivery system for the treatment of anxiety disorder via intranasal administration.

SIGNIFICANCE STATEMENT

The current study revealed that controlled release novel formulations for nose-to-brain administration of therapeutics (that do not penetrate the BBB) are superior to traditional delivery systems. Through this study's finding, it may be possible to approach that buspirone *in situ* nanoemulsion gel may have the potential for the treatment of anxiety disorder via intranasal administration.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Pharmaceutics, SHEAT College of pharmacy, Varanasi, India for providing support to conduct research work. The authors are grateful to Deshpande Laboratories limited, MP (India) for providing a facility for animal studies. For infra structural support, Dr. APJ Abdul Kalam Technical University, Lucknow, India is gratefully acknowledged. The authors acknowledge the support received from Dr. Rajeev Shukla and Dr. Abhishek Kumar, SHEAT College of Pharmacy, Varanasi, Dr. APJ Abdul Kalam Technical University and Ms. Garima Tripathi, Department of science, Banaras Hindu University for their support and encouragement in carrying out this research study.

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