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Advances in Bioresearch

ORIGINAL ARTICLE

Acute Intranasal - Inhalational toxicity and Behavioral Assessment of rodent olfaction of Brivaracetam –*Abelmoschus esculentus* nanogels

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ABSTRACT
The aim of this research is to summarize the special concerns in animal research, including Acute Intranasal - Inhalational toxicity and Behavioral Assessment of rodent olfaction of Brivaracetam –Abelmoschus esculentus nanogels. In this study, two fundamental techniques are presented that allow for quick evaluations of albino rats' anosmia (the lack of a sense of smell). The ability to detect volatile odours is evaluated using the hidden food test. In order to determine if an animal can recognise and distinguish between various odours, including social and non-social odours, the olfactory habituation/dishabituation test is used. The tests in this unit can be easily modified and only the most minimal tools are needed. The study of the olfactory system and the right interpretation of a variety of mouse behaviours, particularly learning and memory, emotionality and affect, and sociality, depend on accurate assessment of olfaction defined by food buried test and olfactory parameters. Albino rats use their sense of smell to find food sources, find social and mating partners, and ward off predators. Olfaction is tightly linked to many mouse behaviours, including learning and memory, social interaction, fear, and anxiety, and behavioural studies intended to measure those brain functions may utilize odours as cues.
Keywords *: Acute intranasal, social interaction, anxiety , behavioural studies .*

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INTRODUCTION

Both topical and systemic medications can be administered effectively via the nasal route. The major point of entry for inspired air—which may contain both planned and accidental particles, vapours, and gases—is the nose. Olfaction and lung protection are two significant additional functions of the nose, which also heat, humidity , and filter incoming air.

This latter function allows for the reflex sneezing that can be used to evacuate big or bothersome particles or droplets that would otherwise accumulate in the lungs or absorb into the body. Drugs can, however, have a therapeutic impact when administered properly and can be absorbed locally or through the nasal mucosa.

Small particles that aren't taken in by the nose can be breathed into the lung, where they may act locally, enter the bloodstream, or be eliminated via muco-ciliary action and macrophage absorption. In addition to being absorbed in the nasal cavity, bolus fluids supplied to the nose may also be inhaled or aspirated (intentionally or unintentionally) to transfer medications to the lungs, where absorption and clearance may also take place. The gastrointestinal (GI) tract also clears bigger breathed particles.(1)

The cilia in the nose drive the particles and droplets backwards (mucociliary clearing), where they leak into the nasopharynx before being ingested by the oesophagus and stomach. This method can lead to drug exposure via the GI tract in addition to drug clearance via the nose. In this way, intranasal drug

administration can affect a variety of local and systemic targets, causing both desired pharmacological effects as well as unanticipated harmful ones. (2)

MATERIAL AND METHODS

Brivaracetam was a gift sample from, Haridwar, Uttrakhand, Carbopol-934 was purchase from Sigma-Aldrich Chem (Mumbai, India), Polypropylene glycol, Methyl paraben, Propyl Paraben, was purchased from Thermo Fischer Scientific Ltd. (Mumbai, India). All other chemicals and reagents used in the study were of analytical grade. Okra was extracted and separated in the laboratory premises itself. Sodium phosphate monobasic , sodium phosphate dibasic , Formaldehyde were purchased from the sigma Aldrich chem, Mumbai, India .

Animals

Albino rats (160-180) were purchased from the source of government animal purchasing unit LAR Section IVRI, Izzatnagar, Bareilly,U.P [196/GO/ReBiBtS/ ReBiL/2000/CPCSEA) Laboratory Animal Division ,CSIR, CDRI, Lucknow(CPCSEANo.34/GO/ReSL/BiS/99/CPCSEA)

Animals were housed at an ambient temperature of 25±1°c in polypropylene cages. Then the animals were brought to lab and acclimatized to lab conditions at 12 h/12 h light anddark cycle. Animals were fed with standard pellet diet and with water *ad libitum.* The institutional animal ethical committee Animal House, Moradabad Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad. (CPCSEANo.1867/PO/Re/S/16/CPCSEA, Date of Registration: 23/02/2016 has approved the experimental protocol to be conducted during Jun 2022 to November 2022 at Animal house of Moradabad Educational Trust Faculty Of Pharmacy, Moradabad.

Method of Preparation

Preparation of Brivaracetam- *Abelmoschus esculentus* nanogels :

Carbopol-934 (0.5, 1, 1.5, 2 % w/w) and purified water were taken in a beaker and allowed to soak for 24 hours. To this required amount of drug (100mg) was dispersed in water and then Carbopol -934 was then added with the required quantity of methyl paraben and propyl paraben in dissolving quantity of propylene glycol to the above mixture. (3) Add precisely weighed concentration of okra polymer with water and added to the above mixture to form a gel base .The distilled water was then added to make up the remaining 100 ml, and the mixture was thoroughly homogenized with the help of high speed homogenizer (REMI ELECTROTECHNIK.LTD) (REMIRQ121) at 4000 rpm to form a uniform gel for about 3–4 hours for neutralizing with sufficient quantity of triethanolamine. The formulations were prepared by following the Box-Behnken design at different concentrations leading to thirteen different formulations which further stored under the necessary storage conditions for further analysis.(4)

[PROTOCOL -1 Acute Intranasal/Inhalational toxicity as per OECD 433]

Numbers of animals and concentration levels:

1. In addition to the pairs of animals used in the sighting study, a total of five animals of one sex (the most sensitive sex as stated in the sighting study, or males only) would typically be used for each concentration level at doses of 5, 1, 0.5, and 0.05mg/L)] examined. (5)
2. The onset, duration, and severity of toxic symptoms dictate the amount of time between exposures at each level. Animals should not be exposed to the next concentration unless it is certain they will survive the ones that were tested. (6)

Observation period:

The animals should be monitored closely throughout the exposure time. (7). The detailed clinical observations should also be made after exposure at least twice on the exposure day, or more often if the animals' responses to the treatment warrant it. After that, for a total of 14 days, clinical observations must be made at least once per day. (8).

Body weight (34)

At least three times during the acclimatization phase—on days 1, 3, and 7 (and every week after that)—as well as at the moment of death or euthanasia if it has been longer than day 1—should individual animal weights be recorded.(9) According to body weight, a crucial indicator of toxicity, animals exhibiting a drop in body weight of more than 10% from the day of exposure indicate evident toxicity has been reached.(10)

Pathology

Gross necropsy should be performed on all test animals, even those that pass away during the experiment or are removed from the investigation for animal welfare reasons.(11)

Preclinical observations (12)

Sign of toxicity are tremors, convulsions, salivation, diarrhoea, lethargy (hypoactivity), irregular respiration, sleep, coma and bodyweight loss.(13). Mortality, Tremors, Convulsions*,Salivation*,(14)

Diarrhea*,Lethargy, Irregular respiration/ (Dyspnea)*, Sleep,* Coma,*, Chromodachryorrhea: (15),Circling movement*, Body weight loss.**(16),Coughing/Sneezing.*,*Epistaxis** Locomotory **(17), Paresis: ***Pinna reflex, Self-mutilation: **(18) Vocalisation: (32,33)

*Clinical signs as per OECD 433 **Clinical signs as per OECD Environmental Health and Safety Publications Series on Testing and Assessment No. (19)

According to the FDA's guideline for industry on nonclinical safety evaluation of reformulated drug products and products intended for administration by an alternate route (herein referred to as the FDA reformulation guidance), intranasal toxicity is toxicology that affects the nasal passages.

Table:1 Species Comparison of Nasal Volume, Surface Area and Turbinate Complexity

Species	Volume (mL)	Total Surface Area (cm^2)	Turbinate Complexity
Mouse	0.03	2.8	Complex scroll

Table 2: Suggested Volumes and Doses/Day for Commonly Used Toxicology Species

Species	Device	Volume/Nostril (µL)	Nasal Volume (µL)	Dose Volume/Nasal Volume	Maximum Doses/Day
Rats	Micropipette	10	400	3%	3

[PROTOCOL -2 The food buried Test]

This test provides quick evaluations of rats with anosmia (the lack of a sense of smell). The ability to detect volatile odors is evaluated using the hidden food test. (20) .The ability to smell volatile odors is verified using the hidden food test, which depends on the animal's innate propensity to employ olfactory signals for foraging. (31)

The buried food test evaluates an animal's ability to locate a small piece of familiar, palatable food—such as cookies, cereal, chocolate chips, or food pellets—hidden beneath a layer of bedding after an overnight fast. (21) It is presumed that food-restricted rats that are unable to use odour cues to locate the food within a 15-minute window have olfactory impairments. The majority of rats with normal olfaction can locate the concealed food item in a matter of minutes. (22)

Food deprivation

Remove all chow pellets from the home cage's food hopper 18 to 24 hours prior to the test. Examine the cage's interior and take out any pellet pieces that may be hiding there. Keep the water bottle in place. (23) When the animals are given an overnight fast as opposed to mild food deprivation, the buried food test is more sensitive (24) After the hidden food test is over, food will be replaced.

Scoring the latency to find the cookie

The test starts with the subject rat being placed in a clean cage with clean bedding that is 3 cm thick. The subject has five minutes to become used to the cage. (27) Move the subject to a clean, empty cage. Bury the food stimulus in the bedding-containing cage in a random cage corner, about 1 cm below the surface. (35) Place the subject back inside the cage and close the lid.(29)The observer then leaves the cage and returns to the observation station, which should be around 2m away.(25) Start the stopwatch and timer, and when the subject rat discovers the hidden food, stop the stopwatch.When a subject begins to eat, typically while holding the food in its forepaws, it is deemed to have uncovered the cookie. Take note of the time it took to locate the cookie and if it was eaten. After 15 minutes, if the subject is still unable to locate the hidden food, the test should be stopped and the subject's latency grade recorded as 900 sec. (26,28,30)

RESULT AND DISCUSSION

The results showed that over the study period (7 days) the BRA-NG nanogel formulation showed no signs of redness, inflammation, and pre-clinical symptoms compared to the BRA solution , proving the safety of the used excipients to be applied topically in the nasal epithelium. This result was expected based on the small size of the NVs and complete solubility of drug in them. Another explanation is the healing effect of nanogel on mucous tissue which protected the nasal mucosa from any possible irritation caused by okra and drug. These 3 groups are expected as no toxicity conditions showed as in normal saline group no severe preclinical observations were shown as signs of toxicity. In pure drug, no severe preclinical observations were shown as signs of toxicity. In test compound, no severe preclinical observations were shown as signs of toxicity on the basis of body weight and pathological conditions.

ANIMAL	DAY 0	DAY1	DAY3	DAY7
N.S 1	297	294	291	293
N.S.2	302	298	301	299
N.S.3	264	257	260	261
N.S.4	262	259	254	254
N.S.5	279	271	276	274
N.S.6	165	168	169	168
P.D.1	255	254	251	254
P.D.2	261	260	258	259
P.D.3	267	265	262	264
P.D.4	273	271	268	270
P.D.5	287	285	285	283
P.D.6	279	274	274	276
T.C.1	333	329	328	329
T.C.2	310	307	304	305
T.C.3	297	295	287	289
T.C.4	286	284	285	284
T.C.5	278	275	271	273
T.C.6	294	292	291	287

Table- 3 Observation of Body Weight

(N.S. – Normal Saline, P.D.-Pure Drug, T.C.- Test Compound , weight in gms)

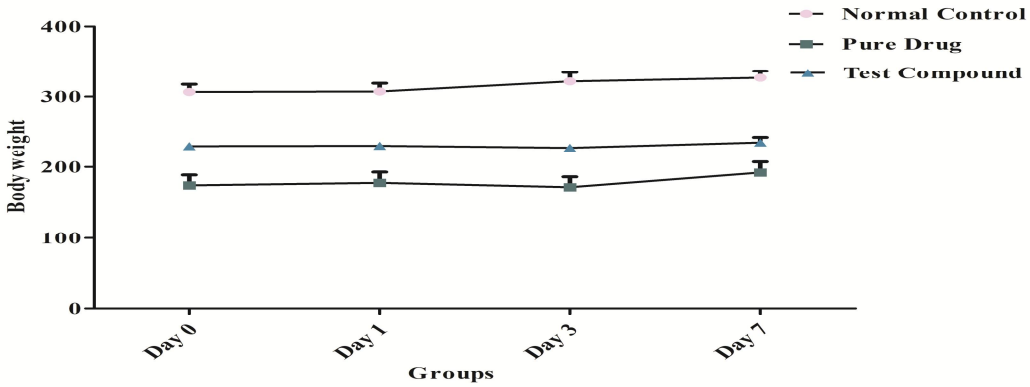

Figure -1 Effects of the standard drugs and their combined treatments on body weight gain in rats after 14 days. Values represent them an ± SEM (n = 6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Pathology

Gross necropsy should be performed on all test animals, including those that pass away during the experiment or are eliminated from the study for animal welfare reasons. the pathological studies showed no animal death during the toxicity studies for 14 days according to the OECD guidelines which showed no toxicity in the formulation and not a single animal were eliminated in the further groups.

Animal	Pathology Result	Necropsy
N.S.1	No death	yes
N.S.2	No death	yes
N.S.3	No death	yes
N.S.4	No death	yes
N.S.5	No death	yes
N.S.6	No death	yes
P.D.1	No death	yes
P.D.2	No death	yes
P.D.3	No death	yes
P.D.4	No death	yes
P.D.5	No death	yes
P.D.6	No death	yes
T.C.1	No death	yes
T.C.2	No death	yes
T.C.3	No death	yes
T.C.4	No death	yes
T.C.5	No death	yes
T.C.6	No death	yes

Table -4 Observation of pathology of animals

PRECLINICAL OBSERVATIONS (SIGNS OF TOXICITY)

The preclinical studies showed the symptoms performed during the 14 days toxicity studies according to the OECD guidelines. In the table -5, the normal group were clearly studied that no preclinical symptoms were showed during the studies.

GROUP -1 (NORMAL SALINE)

S.NO	PRECLINICAL PARAMETERS														
		D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1	Mortality*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	Tremors*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	Convulsions*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	Salivation*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
5	Diarrhea*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	Lethargy (hypoactivity	N	N	N	N	NR	N	N	N	N	N	N	N	N	N
7	(Dyspnea	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
8	Sleep*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Coma*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Chromodachryorrh	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Circling movement**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	Bodyweight loss.**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	Coughing/Sneezing	N	N	N	N	N	N	N	N	N	N	N	N	N	N
14	Epistaxis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	Locomotory activity*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
16	Hyper-reflexia	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
N	Paresis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	Pinna reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N
19	Self-mutilation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	Urine retention**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
21	Vocalisation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Table-5 Pre-clinical observations of sign of toxicity , n- no , y-yes, nr- normal , abr- abnormal

In the studies of group second as the pure drug solution were given to the albino rats for the studies of 14 days of toxicity studies according to the OECD guidelines. This study clearly shown that no preclinical shown of toxicity shown in the animals during the studies as the pure drug are not carcinogenic and teratogenic to the animals as per the studies in table-. 6

GROUP-2 PURE DRUG

S.NO	PRECLINICAL PARAMETERS														
		D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1	Mortality*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	Tremors*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	Convulsions*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	Salivation*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
5	Diarrhea*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	Lethargy (hypoactivity	N	N	N	N	NR	N	N	N	N	N	N	N	N	N
7	(Dyspnea)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
8	Sleep*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Coma*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Chromodachryorrh	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Circling movement**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	Bodyweight loss.**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	Coughing/Sneezing	N	N	N	N	N	N	N	N	N	N	N	N	N	N
14	Epistaxis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	Locomotory activity*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
16	Hyper-reflexia	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
N	Paresis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	Pinna reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N
19	Self-mutilation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	Urine retention**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
21	Vocalisation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Table-6 Pre-clinical observations of sign of toxicity , n- no , y-yes, Nr- normal , abr- abnormal

In the group -3 as the test compound or the BRA-NG formulation were given to the animals to measure the toxicity studies according to the OECD guidelines. The studies clearly shown that no acute and severe toxicity were sho in 14 days toxicity studies which were shown by the preclinical symptoms during the toxicity studies. This studies clearly shown that the formulation having no toxicity parameters and further evaluated for the antidepressant activity to the animals .

GROUP-3 TEST COMPOUND

S.NO	PRECLINICAL PARAMETERS														
		D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
1	Mortality*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	Tremors*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	Convulsions*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	Salivation*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
5	Diarrhea*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	Lethargy (hypoactivity	N	N	N	N	NR	N	N	N	N	N	N	N	N	N
7	(Dyspnea)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
8	Sleep*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Coma*	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Chromodachryorrh	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Circling movement**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	Bodyweight loss.**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	Coughing/Sneezing	N	N	N	N	N	N	N	N	N	N	N	N	N	N
14	Epistaxis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	Locomotory activity*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
16	Hyper-reflexia	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
N	Paresis	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	Pinna reflex	N	N	N	N	N	N	N	N	N	N	N	N	N	N
19	Self-mutilation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	Urine retention**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
21	Vocalisation:	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Table-7 Pre-clinical observations of sign of toxicity , n- no , y-yes, nr- normal , abr- abnormal

All the olfactory tests required the animals to be singly housed and handled for at least 1 week prior to the test. After this preparation, all the cotton tip– based olfactory tests can be finished within a few days, with times required varying from 5 min for the olfactory preference test to 3 to 5 days for the olfactory detection threshold test. The result showed no olfactory loss of smell and mucosal degradation of the animal showed no symptomatic effects on nasal mucosa of the formulation based on latency of sniffing, finding and eating. In the test compound the latency of sniffing and finding will not alter the time and latency of eating, increases without any changes compared to normal group. This shows that the formulation showed no alteration in olfactory test for smell and other olfactory behaviors shown in table **-8,9,10.**

Group -1 NORMAL SALINE

	ANIMAL 1	ANIMAL 2	ANIMAL 3
Food Deprivation			
W_{PRE}	345gm	304gm	332gm
W_{PRO}	320gm	314gm	320gm
W_{LOSS}	25gm	10gm	12gm
Final W	335gm	309gm	314gm
Food Finding Test			
Latency of Sniffing	2	2	3
Latency Of Finding	2	2	3
Latency Of Eating	1	1	2

Table :8 Percentage latency of albino rats towards feed approach and food finding and eating during buried test

GROUP 2 PURE COMPOUNDS

	A1	A2	A3	A4	A5	A6
Food Deprivation						
Wpre	224gm	130gm	201gm	148gm	188gm	163gm
Wpro	220gm	137gm	200 gm	143gm	192gm	165gm
%wW loss	04 gm	07 gm	01 gm	05gm	03 gm	02 gm
Final w	220gm	137gm	200gm	143gm	192gm	165gm
Food finding test						
Latency of sniffing	4	3	4	3	3	4
Latency of finding	2	2	3	3	2	4
Latency of eating	2	2	3	2	1	3

Table :9 Percentage latency of albino rats towards feed approach and food finding and eating during buried test

GROUP-3 TEST COMPOUND

	A1	A2	A3	A4	A5	A6
FoodDeprivation						
Wpre	254gm	239gm	215gm	238gm	216gm	215gm
Wpro	243gm	225gm	202gm	220gm	205gm	200gm
%Wloss	11gm	14gm	13gm	18gm	11gm	15gm
Final W	243gm	225gm	202gm	220gm	205gm	200gm
Food finding test						
Latency of sniffing	3	3	3	4	3	4
Latency of finding	3	3	3	4	3	4
Latency of eating	2	2	2	3	2	3

Table :10 Percentage latency of albino rats towards feed approach, food finding and eating during buried test

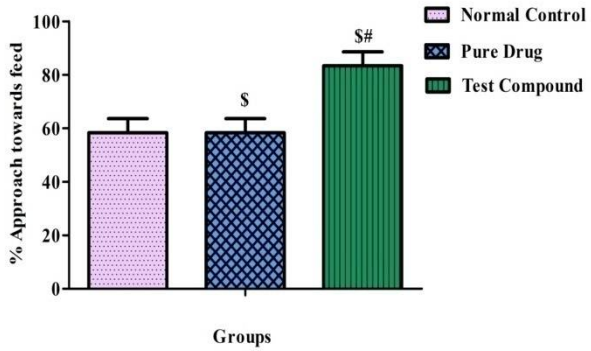

Figure -2 Percentage latency of feed eating of albino rats during food buried test

In the graphical presentation showed that the test compound have higher food eating approach without any alteration in the nose as the animals are able to eat the food as it increases the latency of eating as per normal groups . It clearly shows the increases in appetite of the animals after the dosing of test compound without any alteration in the olfactory behavior of animals.

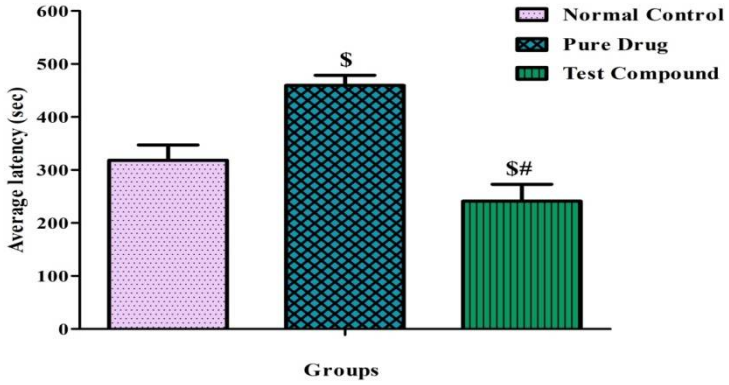

Figure -3 Percentage of average latency of feed finding and sniffing of albino rats in time

In the graphical presentation showed that the test compound have little lower food finding approach without any alteration in the nose as the animals are able to sniff the food but increases the latency of eating as per normal groups in figure 2 . It clearly shows the increases in appetite of the animals after the dosing of test compound without any alteration in the olfactory behavior of animals. The latency of food finding and sniffing were little lower than the normal groups in figure 3 but increases the food eating without the alteration in olfactory behavior of animals.

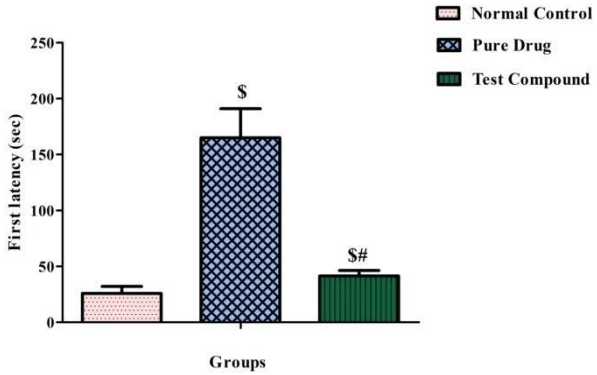

Figure -4 :Percentage of first latency of food buried test in albino rats

This study of food buried test clearly indicates that the test compound given to the albino rats for the behavioral changes in animals after dosing of nasal nanogel shown that the animals did not shown any olfactory behavioral changes in food finding , sniffing and eating as compared to the normal group . It clearly show the activity were increase after the dosing of test compound as compared to the normal group shown that no olfactory behavioral changes during the test compound studies.

From these studies, the novel preparation nanogel have high dissolution rates as the particle sixe considers in nano form yet releases fastly and exhibits therapeutic properties without any toxicity rate.

CONCLUSION

The purpose of this study is to provide information on the toxicity studies on intranasal route of administration of the composition of *Abelmoschus esculentus* – briavracetam nanogel preparation In this study, animals should be monitored closely throughout the exposure period. Additionally, after exposure, detailed clinical observations should be performed at least twice on the exposure day, or more frequently if the animals' reactions to the treatment suggest it. After that, for a total of 14 days, clinical observations must be made at least once per day. The observation of toxicity parameters on body weight shows as there were no evidence of fatal toxicity in albino rats in the test compound. The body weight becomes constant hence no elevation of weight parameters shown in the test compound as compared to the pure drug.

In the pathology no death occurred after the toxicity parameters of drug on low dose and high dose shows highly therapeutic efficiency .Tremors, convulsions, salivation, diarrhea , lethargy (hypoactivity), irregular respiration, sleep, coma and bodyweight loss. In normal saline group no severe preclinical observations were shown as signs of toxicity. In pure drug, no severe preclinical observations were shown as signs of toxicity. In test compound, no severe preclinical observations were shown as signs of toxicity.

In food buried test, food-deprived albino rats with normal olfaction typically found the cookie within 3 minutes shows no immobility after dose considerations. No behavioral responses such as immobility (sometimes observed in animals with high levels of anxiety) or vigorous bedding shoveling 1occurs. As latency of sniffing and finding also increase in test compound when compared with normal shows no behavioral changes occur after dosing through nasal route.

DISCUSSION

The characterization of brivaracetam nanogels was systematically investigated in the experiment. The preliminarily toxicity effects of brivaracetam nanogel were also assessed in animal models. The results showed that brivaracetam-okra nanogels were successfully prepared by a reverse microemulsion method and presented excellent therapeutic behavior. Finally, the preliminary effect of brivaracetam –okra on behavioral despair in mice and the CUMS rat model suggested that compared with nasally administered impramine at a low dose and high dose showed no toxicity behaviour and any olfactory changes in

behavior by food buried test. The complex drug delivery method of the administered intranasally nanogel loaded self-assembled thermosensitive hydrogel system increases the antidepressant-like activity of brivarcetam . Brivarcetam –okra nanogel as a novel administration system, is a new traditional Chinese medicine preparation to treat depression in further studies and acts as anti-depressant agents.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION
Each of the mentioned authors contributed significantly, directly, and intellectually to the work, and they all gave their consent for it to be published.

DATA AVAILABILITY
All datasets obtained or studied during this study are incorporated in the manuscript.

ETHICS STATEMENT
Albino rats (160-180) were purchased from the source of government animal purchasing unit LAR Section IVRI, Izzatnagar, Bareilly, U.P (196/GO/ ReBiBtS / ReBiL / 2000/ CPCSEA) Laboratory Animal Division, CSIR, CDRI, Lucknow (CPCSEANo.34/GO/ReSL/BiS/99/CPCSEA)
The institutional animal ethical committee Animal House, Moradabad Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad. (CPCSEANo.1867/PO/Re/S/16/CPCSEA, Date of Registration:23/02/2016 has approved the experimental protocol to be conducted during Jun 2022 to November 2022 at Animal house of Moradabad Educational Trust Faculty Of Pharmacy, Moradabad.

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