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Pharmacokinetic and Pharmacodynamic Evaluation of Transferrin Conjugated Polymeric Nanoparticles of Ziprasidone Hydrochloride

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Abstract

The development of advanced drug delivery systems can significantly enhance the therapeutic efficacy of drugs with limited bioavailability and brain targeting capabilities. This study focuses on formulating transferrin-conjugated chitosan nanoparticles (Tf-CH-NPs) of Ziprasidone Hydrochloride (ZH) to improve brain delivery and bioavailability. Optimization of chitosan nanoparticles using the Box-Behnken Design, surface modification with transferrin, and subsequent in vitro and in vivo evaluations were performed. Pharmacokinetic and pharmacodynamic studies in rat models revealed significantly improved brain targeting efficiency and therapeutic effects of Tf-CH-NPs compared to non-conjugated nanoparticles and ZH solutions. The study provides a robust framework for developing transferrin-based nanocarriers for effective brain delivery of therapeutic agents.

Keywords: Ziprasidone Hydrochloride, transferrin-conjugated nanoparticles, brain delivery, pharmacokinetics, pharmacodynamics, chitosan nanoparticles

Introduction

High-performance liquid chromatography (HPLC) has revolutionized analytical techniques by offering precise quantification of pharmaceutical compounds, thereby ensuring drug quality and efficacy. Ziprasidone Hydrochloride (ZH), an atypical antipsychotic, is widely prescribed for treating schizophrenia and bipolar disorder due to its high efficacy and safety profile. However, its poor bioavailability and limited ability to cross the blood-brain barrier pose significant therapeutic challenges [1,2].

Nanoparticles have emerged as an effective strategy to address these limitations, offering enhanced drug stability, controlled release, and targeted delivery. Chitosan-based nanoparticles, owing to their biocompatibility and mucoadhesive properties, have been extensively studied for intranasal drug delivery [3,4]. Surface modifications, such as transferrin conjugation, can further enhance brain targeting by exploiting receptor-mediated transcytosis across the blood-brain barrier [5,6].

This study aims to develop transferrin-conjugated chitosan nanoparticles (Tf-CH-NPs) for the efficient delivery of ZH to the brain. Using a Box-Behnken Design, the nanoparticles were optimized for size, encapsulation efficiency, and drug loading. Pharmacokinetic studies were performed to evaluate nasal bioavailability, brain targeting efficiency, and direct drug transport percentages. Pharmacodynamic studies assessed the therapeutic efficacy of Tf-CH-NPs in rat models of schizophrenia induced by ketamine.

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The novelty of this research lies in the dual evaluation of pharmacokinetics and pharmacodynamics of Tf-CH-NPs, providing a comprehensive understanding of their therapeutic potential. The findings contribute to the growing body of evidence supporting nanotechnology-based drug delivery systems for enhanced treatment of neurological disorders [7-16].

The main objective of the study was development and evaluation of transferrin conjugated polymeric nanoparticles of Ziprasidone Hydrochloride for enhancing its brain delivery and bioavailability. Hence, the aims of the study were.

- Development of chitosan nanoparticles and their optimization using Box- Behnken Design
- Surface modification of optimized NPs via transferrin to formulate Tf- conjugated chitosan NPs
- *In vitro* drug release and permeability study of chitosan and Tf-conjugated chitosan NPs
- Pharmacokinetics and Pharmacodynamic evaluation of chitosan and Tf- conjugated chitosan NPs

Material and Methods

Pharmacokinetic study

Animal Handling and Dosing:

Rats weighing (200-250 g) were taken and divided into four groups as given in Table 4.5. Each group consisted of 6 animals. The rats were fasted overnight prior to dosing. In first 3 groups, Tf-LH-NPs, LH-NPs and LH-solution were administered intranasally at a dose equivalent to 0.820 mg/kg of LH in each nostril (20 µl) of rats using micropipette while in fourth group LH-solution was given by intravenous route. During intranasal administration rats were kept in slanted position by holding from back. Similarly, 60 µl of LH-solution (10mg/ml LH) administered intravenously by injection through tail vein of rat. Blood samples were collected from the retro-orbital plexuses and at the same time brain homogenates were collected after sacrificing the rats at predetermined time intervals.

Table 1. Grouping of Animals

Group	Treatment	Route
A	Drug Solution	Intranasal
B	LH-CH-NPs	Intranasal
D	Tf-LH-CH-NPs	Intranasal
C	Drug Solution	Intravenous

Extraction of LH from rat plasma and brain homogenates:

From rats collected blood samples, the plasma was separated by centrifugation at 10,000 rpm for 10 minutes at 4° C. Acetonitrile was added to the collected plasma samples and vortexed for 5 minutes and then centrifuged at 5000 rpm for 15 min. Each sample containing brain homogenates in phosphate buffer (pH 6.4) was homogenized at 10,000 rpm for 1 minutes and then centrifuged at 5000 rpm for 15 min. The supernatant collected from both plasma and brain homogenates was injected (20 µL) to HPLC column and analyzed at 235 nm using Acetonitrile: Phosphate buffer (55:45) (Patel et al., 2019).

Pharmacokinetic analysis:

Calibration graphs between LH concentration versus time

profiles for plasma and brain homogenates were plotted after intravenous and intranasal administration. Pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain view, CA) was used to calculate pharmacokinetic parameters such as Cmax. Tmax, T1/2and AUC0-1440. Nasal bioavailability, brain targeting efficiency, and

direct drug transport % after intranasal administration of CH-NPs and Tf-CH-NPs were calculated by given below equations:

Nasal bioavailability =AUCIN/AUCIV

The time average portioning ratio was represented by drug targeting efficiency (DTE %). The percentage drug transport from nose to brain is calculated as %DTP using following equation:

% DTP = ((BI.N. – Bx)/ BI.N) × 100 Here, Bx= (BIV/PIV) PIN

BIV -AUC0-t (brain) after intravenous administration; BIN -AUC0-t (brain) after intranasal administration;

PIN -The AUC0-t (blood) after intranasal administration PIV - The AUC0-t (blood) after intravenous administration.

Pharmacodynamic Studies

Animals and Dosing

Rats weighing between 180-250 g were used for all pharmacodynamic study. The rats were divided into separate groups as given in Table with five animals in each group and housed in a cage with free access to food and water. LH and LH-NPs equivalent to 0.820 mg/kg LH were administered in rats via intranasal route in all studies.

To establish the schizophrenia animal model for pharmacodynamic studies of NPs ketamine was used. Ketamine act as a non-competitive and non-selective N-methyl-D- aspartate (NMDA) receptor antagonist. In rats it was used to produce schizophrenia-like symptoms. The repeated dosing of ketamine in rodents emulate cognitive, negative and positive symptoms. Abnormal mTOR expressions in rat brain after acute dosing of ketamine is found which are linked to schizophrenia like neurological. LH-solution, CH-NPs and Tf- CH-NPs were given to the Wistar rats for21 consecutive days. Ketamine (30 mg/kg) was given on 7^{th} and 14^{th} day as a single dose via intraperitoneal route. After dosing with ketamine, the animals were assessed for different behavioral i.e., locomotor activity, elevated plus maze test and catalepsy test.

Table 2. Animal Groups for Pharmacodynamic study

Group 1	Control (normal saline)
Group 2	Toxic (ketamine)
Group 3	Per se (LH-NPs without ketamine)
Group 4	Drug solution + ketamine
Group 5	CH-NPs + ketamine
Group 6	Tf-CH-NPs + ketamine

Induced locomotor activity test

The locomotor count was determined by Video path activity analyser. The rats were placed in the activity chamber and the spontaneous locomotor counts were measured for 10 minutes after injecting the ketamine (30 mg/kg) in rats at 7^{th} day.

Elevated Plus Maze Model

In this study a maze model of cross-shaped made of plexiglass having two closed arms (30 x 5x 20 cm) and two open arms **(**30 x 5 cm) with (5 x 5 cm) central platform that is placed above 40 cm from the ground was used. The rats were placed in the central compartment and the time spent by each rat in closed and open arm and number of entries in both arms were noted at day9 and day 11. The apparatus was cleaned after each trail with 70% alcohol.

Catalepsy test

This test was performed using the bar in which the rat forepaws was placed on horizontal bar fixed at 10 cm height above the surface whilst their hind limbs rested on a platform until the rat could grasp it. The amount of time animal remains immobile was calculated after administration of control, LH-solution, LH-CH-NPs and Tf-LH- CH-NPs at 2h, 4h, 6h, 8h and24 h. The time spent in atypical position will be recorded after 1 h dosing.

Histomorphology

Brain was isolated from rats after 21 days dosing with drug solution, and drug loaded NPs and then they were washed with normal saline and kept in 10% formalin solution. The brain was sliced and

embedded in paraffin wax. The isolated slides were cut using slide microtone (5 μm thickness) followed by staining with hematoxylin and eosin (H&E). Fluorescence microscope(Motic AE31) with infinity analyse software was used to observe the histological changes in the brain hippocampus region. The % of degenerated neurons in brain tissues was assessed from pyknotic and necrotic neurons.

Biochemical estimation

Patients suffering with schizophrenia show increased proinflammatory and pro- oxidative status. As, LH have anti-inflammatory and antioxidant effect, so the various antioxidant and neuroinflammatory biomarkers in rat brain were estimated.

Glutathione (GSH)

For estimation of GSH in brain modified Ellman method was used, the brain was weighed and homogenized in 0.1 M phosphate buffer. Then 1 ml of brain homogenate was taken and precipitated by adding 4% trichloroacetic acid and centrifuge at 5000 rpm for 10 minutes. The supernatant was taken and 2 ml of di-sodium hydrogen phosphate and 0.4 ml distilled water was added to it. In the above solution, 0.01 M (5,5- di-thio-bis-2-nitrobenzoic acid in 1% w/v sodium citrate) DTNB (0.25 ml) was added and incubated at room temperature for 10 minutes. The samples were analysed using UV-
VIS spectrophotometer at 412 nm within 30 second after adding DTNB and development of yellow colour. The GSH level was calculated using extinction coefficient 1.36 x 10^4 $M^{-1}cm^{-1}$ and expressed as μmole/mg.

MDA Malonaldehyde or Thiobarbituric Acid (TBARS)

The brain was weighed and homogenized in 0.1 M phosphate buffer. In 0.5 ml of brain homogenate, tris-hydrochloric acid pH 7.4 (0.5 ml) was added and incubated for 2 hours at 37℃. To the above mixture 1ml trichloroacetic acid was added and centrifuge for 10 minutes at 1000 rpm. To 1 ml supernatant, 1ml of 0.67% thiobarbituric acid was added and kept on water bath for 10 minutes. The mixture was cooled and analysed at 532 nm using UV-Vis Spectrophotometer. The MDA level was calculated using extinction coefficient 1.50x 10^5 $M^{-1}cm^{-1}$ and expressed as n mole/mg.

Superoxide Dismutase SOD

To 10 μL of brain homogenate 970 μL of 0.05 M (ethylene diamine tetra-acetic acid (EDTA) in sodium carbonate buffer pH 10.2) was added. To the above mixture 30 mM epinephrine (20μL) was for commencement of reaction. The SOD activity was assessed at 480 nm using 4020 M-1cm-1 as molar extinction coefficient and expressed in units/mg protein.

ACHE Activity

Brain homogenate was prepared in 0.1 M phosphate buffer. To 0.4 ml of brain homogenates, 0.1 ml (5,5-di-thio-bis-2-nitrobenzoic acid in 1% w/v sodium citrate) DTNB and 2.6 ml of 0.01M sodium phosphate buffer was added. The samples were analysed at 412 nm using spectrofluorometer. Then, 0.02 ml of acetylcholine was added and after 15 min the samples were again analysed at 412 nm. The absorbance changes were noted and results expressed in mmol/minute/gram.

Biochemical estimation of neuroinflammatory markers using ELISA

The neuroinflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1-β (IL-1-β) quantitative estimation was done in rat brain. The kits were purchased from Krishgen Biosystems. The assay was done as per the provided method with the specific cytokine kit. The standard solution was prepared and both the
standard and test solutions were added to the pre-coated antibody wells and were kept for binding. The wells were incubated at mentioned conditions and washed with wash buffer to remove the unbound sample. Cytokines specific enzyme-linked polyclonal antibody was added to the wells. The wells were incubated at mentioned conditions and then washed to get rid of unbound antibody. Substrate solution was added to each well that generates blue colour which changes to yellow after addition of stop solution. The samples were analysed at 450 nm using spectrophotometer. The quantity of TNF-α, (IL- 6) and IL-1-β was calculated from the standard plot.

Results and Discussion

Pharmacokinetic Study

The concentration of ZP in Wistar rat plasma and brain homogenates determined after intranasal administration of ZP suspension,

ISSN: 0976-7126
Ganesh & Malviya, 16(4):1-13, 2025

ZP-CH-NPs and Tf-conjugated ZP-NPs are presented in Tables 5.39 and 5.40 and Figures 5.32 and 5.33. The pharmacokinetics parameters calculated for ZP-solution IV and IN, ZP-CH-NPs and Tf-ZP-CH-NPs IN, are given in Table 5.41 in rat plasma and in Table 5.42 for rat brain homogenates.

Table 3: Concentration of ZP in plasma after and Intranasal administration of ZP-solution, ZP-CH-NPs and Tf-ZP-CH-NPs and intravenous administration of ZP- solution. Data expressed as mean ±SD (n = 3)

Time (h)	ZP concentration in Plasma (ng/mL)			
	ZP-solution IV	ZP-solution IN	ZP-CH-NPs IN	Tf-ZP-CH-NPs IN
0.5	652.26 ± 26.58	70.56 ± 5.39	85.36 ± 3.34	71.59667 ± 13.80
1	596.53 ± 38.79	192.81 ± 6.84	149.04 ± 5.25	129.2833 ± 5.27
2	468.1 ± 41.69	190.87 ± 7.70	197.33 ± 11.89	163.11 ± 5.45
4	405.8 ± 6.73	199.22 ± 5.0	173.4 ± 8.60	159.5 ± 6.28
8	293.16 ± 18.38	118.56 ± 5.2	117.66 ± 7.09	83.78667 ± 8.73
24	197.7 ± 44.62	97.35 ± 4.19	94.50 ± 6.26	99.38333 ± 10.23

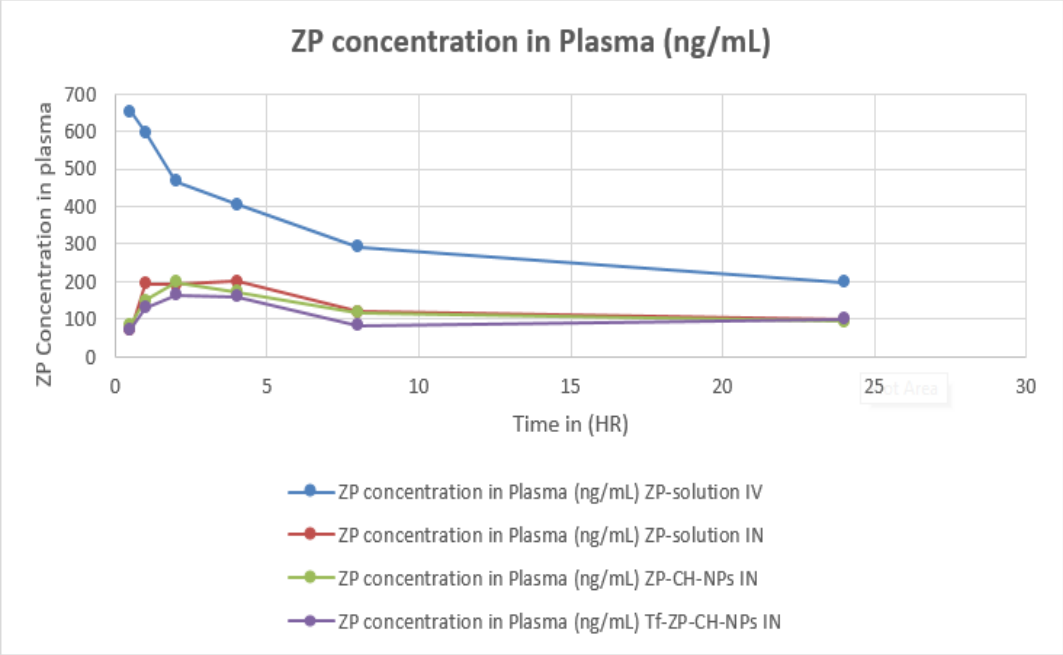

Figure 1: Plasma drug concentration profile of ZP after IV and IN administration of ZP-solution, ZP-CH-NPs and Tf-ZP-CH-NPs respectively.Data expressed as mean ±SD (n = 3)

ISSN: 0976-7126
Ganesh & Malviya, 16(4):1-13, 2025

Table 4: Concentration of ZP in brain after intravenous administration of ZP- solution, and Intranasal administration of ZP-solution, ZP-CH-NPs and Tf- ZP-CH- NPs. Data expressed as mean ±SD (n = 3)

Time (h)	ZP concentration in Brain (ng/mL)			
	ZP-solution IV	ZP-solution IN	ZP-CH-NPs IN	Tf-ZP-CH-NPs IN
0.5	560.26 ± 26.58	91.54 5 ± 8.45	298.09 ± 8.37	374.43 5 ± 21.60
1	673.2 3 ± 22.39	290.3 ± 20.05	698.5 ± 14.2	698.4 ± 14.78
2	571.433 ± 36.98	397.48 ± 14.18	1325.15 ± 49.67	1458.3 ± 37.12
4	419.13 ± 24.71	320.16 ± 11.65	968.2 ± 25.9	859.23 ± 9.54
8	349.83 ± 42.28	261.46 ± 7.88	896.8 ± 30.70	789.8 ± 9.55
24	191.03 ± 33.06	161.2 ± 16.89	365.5 ± 15.05	298.8 ± 9.2

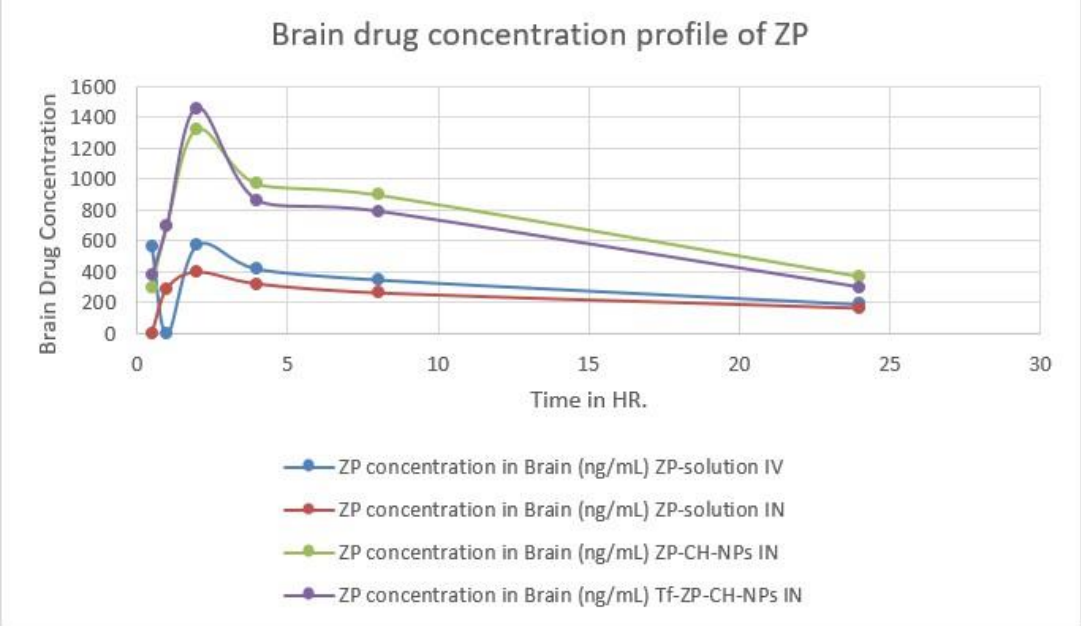

Figure 2: Brain drug concentration profile of ZP after IV administration of ZP- solution and IN administration of ZP-solution, ZP-CH-NPs and Tf-ZP-CH- NPs respectively. Data expressed as mean ±SD (n = 3)

Table 5: Pharmacokinetic parameters obtained after IV and IN dosing of drug solution and drug loaded NPs in Plasma. Data expressed as mean ±SD (n = 3)

Parameters	Plasma			
	DS IV	DS	CH-NPs	Tf-CH-NPs
AUC 0-24 (ng. h/mL)	5034.62 ± 945.12	4123.56 ± 867.71	9458.42 ± 672.37	9253.78± 572.07
C max (ng/mL)	560.26 ± 26.58	150.8 ± 7.70	157.3 ± 11.89	133.11± 5.45
T max (h)	30 min	2 h	2 h	2 h
AUC 0- ∞ (ng. h/mL)	6173.62 ± 1205.08	4743.06 ± 2907.01	9758.12 ± 1532.19	9523.08± 1492.09

Table 6: Pharmacokinetic parameters obtained after IV dosing of DS and IN dosing of DS and drug loaded NPs in Brain. Data expressed as mean ±SD (n = 3)

Parameters	Brain			
	DS IV	DS	CH-NPs	Tf-CH-NPs
AUC 0-24 (ng. h/mL)	5152.12±1035.11	3557.42± 726.22	14776.15±1021.24	15682.87±1078.42
C max (ng/mL)	471.433± 36.98	327.48± 14.18	1257.15 ± 49.67	1479.3 ± 37.12
T max (h)	30 min	2 h	2 h	2 h
AUC 0- ∞ (ng. h/mL)	5480.67±1678.98	3707.42± 926.22	15096.65±1965.24	15910.76 1708.02
% DTP	-	109.14 ±15.35	374.93 ± 15.02	396 ± 16.32
% DTE	-	44.08 ± 9.89	76.32 ± 10.12	84.65 ± 11.22
Nasal bioavailability	-	45.67 ± 7.86	175.4 ± 9.89	189.6 ± 10.02

The results obtained from pharmacokinetic study showed that the drug concentration in brain after intranasal administration from Tf-CH-NPs were significantly higher ($p < 0.01$) at each time point followed by CH-NPs (IN), DS (IV) and DS (IN). The Cmax of CH-NPs in brain was estimated to be (1257.15 ± 49.67) followed by (1479.3 ± 37.12) for Tf-CH-NPs, (660.26± 26.58) for DS IV, and (327.48 ± 14.18) for DS IN. Therefore, the results concluded a 4.52-fold increase in ZP concentration in brain from Tf-CH- NPs and a 3.84-fold increase from CH-NPs as compared to ZP-DS when administered via nasal route. The Tmax remained 2 h for nasal groups and 30 min for IV administration. The Tf-CH-NPs and CH-NPs showed a significant ($p < 0.005$) increase in AUC0-24 in comparison to drug solution. The relative bioavailability of Tf-

CH-NPs was found to be 4.40-fold and CH-NPs 4.12-folds higher in comparison to drug suspension in rat brain and a 2.86, and 3.04-folds increase in relative bioavailability of nano formulations respectively in comparison to drug solution given IV. In addition, an elevation in AUC0-24 of CH-NPs and Tf-CH-NPs of ZP was observed in brain (14776.15 ± 1021.24 and 15682.87 ±1078.42 ng.h/mL) in comparison to DS (IN) (3557.42 ±726.22 ng.h/mL) and DS IV (5152.12 ± 1035.11 ng.h/mL) were found to be statistically significant ($p < 0.01$). The CH-NPs and Tf-CH-NPs of ZP consisted of CH that holds mucoadhesive nature and adhere to the nasal mucosa for longer time thus act as permeation enhancer and prevents the clearance of NPs by mucociliary clearance which was not in case of ZP-DS. The above results were found in agreement with other
findings which showed the mucoadhesive agent like CH plays a key role in enhancing drug uptake in brain. The enhanced bioavailability of NPs to brain may be contributed due to smaller size, larger surface area, prolonged residence time and targeted delivery of NPs in Tf-conjugated.

Similarly, the drug concentration of CH-NPs was found to be higher in plasma followed by Tf-CH-NPs, and DS (IV) and DS (IN). However, DS administered IV showed the higher Cmax in plasma. The Tmax remained 2 h for nasally administered formulations whereas 30 min for IV administered DS. The Cmax in plasma was estimated to be 157.3
± 11.89 ng/mL for CH- NPs and 133.11± 5.45 ng/mL for Tf-CH-NPs that was found to be significant ($p < 0.005$) as compared to drug solution I.N. (150.8 ± 7.70) and (560.26
± 26.58) drug solution IV respectively. AUC0-24, and AUC0-∞ in plasma for NPs were also found to be higher than DS after nasal administration. At all times points ZP concentrations in brain for NPs was found to be superior than corresponding plasma concentrations after IN administration.

The direct % transport of drug to brain after IN administration of CH-NPs and Tf-CH-NPs of ZP was estimated to be 374.93 ± 15.02 and 396 ± 16.32 respectively that was found to be higher and statistically significant ($p < 0.01$) than ZP-solution 109.14 ±
15.35. Similarly, the drug targeting efficiency for both CH-NPs and Tf-CH-NPs of ZP was higher (76.32 ± 10.12), (84.65 ± 11.22) respectively in comparison to (44.08 ± 9.89) drug solution after IN administration. The nasal bioavailability of optimized nanoparticles was found to be 3.88 and 4.23-folds higher when compared to drug solution (IN).

Pharmacodynamic Study

Locomotor Activity

Figure illustrates the locomotion activity counts in schizophrenia induced models of rats after intranasal administration of normal saline, ZP-solution, ZP-Per se, CH-NPs and Tf-CH- NPs of ZP. The results clearly suggested the high locomotor counts in toxic group animals, as ketamine is found to increase ataxia and locomotion. However, the rats treated with nano formulations (CH-NPs and Tf-CH-NPs) groups indicates significant ($p < 0.001$) decrease in locomotor counts induced by ketamine in comparison to ZP-solution and Toxic group. The control and ZP-per shows almost similar locomotor counts. Thus, the results
demonstrated the antagonist effect of ZP-CH-NPs and Tf-ZP-CH-NPs in ketamine induced hyperlocomotion and could prove to be clinically beneficial as antipsychotic.

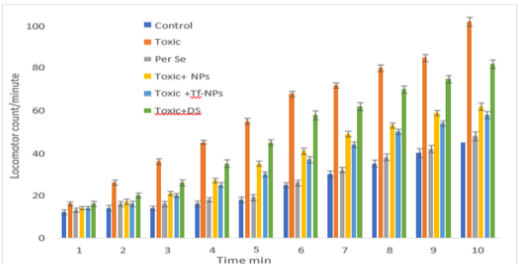

Figure 3: Locomotor activity count in different treatment groups of rats

Elevated Plus Maze Model for Cognitive and Anxiety related behavior

Table illustrates the time spent by rats in open and closed arm after treatment with nano formulations, drug solution, toxic and per se. The rats in ZP-CH-NPs group and Tf-CH-NPs group showed a reduction in the anxiety-related behaviors in the elevated plus maze test. The animals in this group spent more time in the open arm than in the closed arm while the toxic group (administered with ketamine) showed animals in the closed arm for the maximum duration. A significant ($p < 0.01$) reduction in time spent by rats in closed arm was observed with ZP-CH-NPs and ZP-Tf-CH-NPs in comparison to ZP-solution. It showed that ZP act as an anxiolytic compound by decreasing anxiety and increase the open arm entries for exploration time.

Table 7: Time spent by rats in open and closed Arm of different treatment groups

Groups	Time Spent in Seconds			
	Day 9		Day 11	
	Open Arm	Closed Arm	Open Arm	Closed Arm
Control	192 ± 5.48	298 ± 6.05	122 ± 9.45	288 ± 5.05
Ketamine (Toxic)	32 ± 3.21	398 ± 10.16	48 ± 9.87	352 ± 7.98
ZP Per Se	162 ± 6.58	298 ± 6.76	183 ± 5.36	287 ± 6.42
Ket+ DS	99 ± 5.12	291 ± 9.93	99 ± 9.87	292 ± 3.78
Ket+ CH-NPs	189 ± 8.45	281 ± 4.42	184 ± 6.54	276 ± 8.78
Ket+ Tf-CH-NPs	196 ± 7.79	274 ± 6.05	152 ± 4.87	178 ± 9.12

Catalepsy study

Catalepsy is characterized by loss of consciousness and sensation accompanied by body rigidity and is a measure of extrapyramidal side effects (EPS). The cataleptic study was evaluated in schizophrenia induced rat model to evaluate the effect of ZP nanoparticles on ZP-associated EPS as shown in Table 7.23 and Figure 7.27. Results evinced a significant ($p< 0.05$) reduction in cataleptic behaviour in rats treated with CH-NPs and Tf-CH-NPs in comparison to drug solution. The rats treated with ZP per se group did not show any cataleptic effect and behaved like control group thus indicating minimal toxicity of nanoparticles formulation. The enhancement in cataleptic effect with nanoparticles formulation might be credit to the controlled release of ZP from nanoparticles formulations and lower fluctuation in drug concentration thus contributing to antipsychotic activity and reduction of extra-pyramidal side effects.

ISSN: 0976-7126
Ganesh & Malviya, 16(4):1-13, 2025

Table 8: Duration of catalepsy of different test compounds in ketamine- induced schizophrenia rat model. Data expressed as mean ±SD (n = 3).

Groups	Duration of Catalepsy (Seconds)					
	0 h	2 h	4h	6h	8 h	24h
Control	2.0 ± 0.17	2.8 ± 0.36	3.0 ± 0.26	3.2 ± 0.20	3.5 ± 0.55	3.1 ± 0.8
Ketamine (Toxic)	2.0 ± 0.3	145 ± 6.08	178 ±5.29	255± 7.0	225± 3.6	200 ± 7.0
ZP Per Se	3.2 ± 0.43	3.4 ± 0.26	3.6 ± 0.26	3.2 ± 0.36	3.8 ± 0.2	3.6 ± 0.2
Ket+ DS	3.4 ± 0.10	36 ± 5.0	69 ± 3.0	100 ± 6.24	89 ± 10.81	68 ± 8.0
Ket+ CH-NPs	3.7 ± 0.2	37 ± 4.0	47 ± 2.64	55 ± 3.0	39 ± 5.0	27 ± 3.46
Ket+ Tf -CH-NPs	4.2 ± 0.40	28 ± 3.0	40± 3.6	50± 2.0	36 ± 5.0	27± 3.0

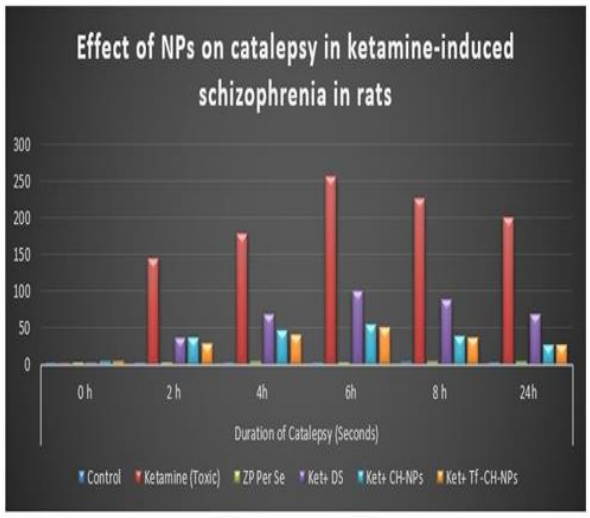

Figure 4: Effect of NPs on catalepsy in ketamine-induced schizophrenia in rats. Data are expressed as mean ± SD (n=3)

Histomorphology

Histopathological examination of Wistar rat brain tissues was performed by staining the brain hippocampus and frontal cortex region with eosin and haematoxylin (E& H)
staining dye. The study was done to ascertain any structural and toxicological variation in the brain after administering of the nano formulations and drug solution. Therefore, basically this study was performed to evaluate the safety of nano formulations after nasal administration. Figure 7.28 depicts the photomicrographs of rat hippocampus whereas Figure shows the photomicrographs of rat frontal cortex after treatment with different groups. In figure the neurons stained with deep blue colour indicates toxicity that corresponds to the toxic (ketamine) groups. Figures revealed the light blue colour of both hippocampus and frontal cortex in nano formulations (CH-NPs and Tf-NPs of ZP) groups suggesting lower toxicity to neuronal cell, oligodendrocytes and glial cells. Both control and per se groups showed similar photomicrographs indicating no sign of toxicity. In addition, there was no sign of toxicity seen with ZP- solution group both in hippocampus and frontal cortex region. The photomicrographs results evinced no neuronal damage, chromatolysis, vacuolization, and pyknosis. Overall, the study concluded the safety of optimized nano formulations (CH-NPs and Tf-CH-NPs of ZP) after nasal administration with no sign of toxicity in rat brain

ISSN: 0976-7126
Ganesh & Malviya, 16(4):1-13, 2025

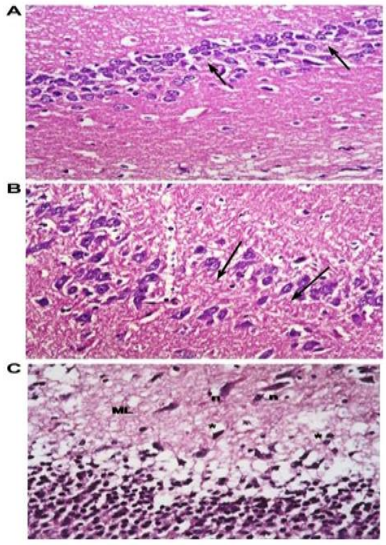

Figure 5: Histopathological micrographs of the rat hippocampus treated with different treatment groups.

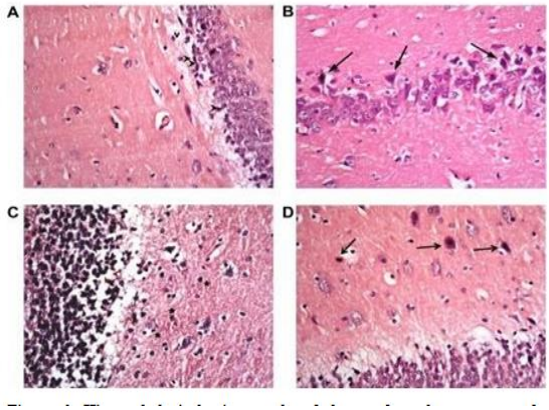

Figure 6: Histopathological micrographs of the rat frontal cortex treated with different treatment groups.

Biochemical Estimation of oxidative stress markers

Table depicts the effect of NPs on oxidative stress markers like glutathione (GSH), Malondialdehyde or thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and Acetylcholinesterase activity. Results indicated a significant ($p< 0.01$) reduction in endogenous antioxidant enzymes (GSH and SOD) activity in toxic (ketamine induced) model and a significant ($p < 0.005$) increase in MDA level in the rat brain in comparison to the control group. This results in degenerative and oxidative stress effects due to accumulation of oxidative free radicals. Conversely, treatment of ketamine induced mice with Tf-ZP-CH-NPs and ZP-CH-NPs significantly ($p < 0.01$) enhanced the activity of antioxidant enzymes (GSH and SOD) and reduces the MDA activity. However, the animals treated with ZP per se group suggested no changes in the level of these endogenous markers level and behaved likely to the control group. The acetylcholinesterase activity was found to be significantly ($p < 0.005$) higher in ketamine induced model in comparison to the control group. The Tf-CH-NPs and CH- NPs treated group showed reduction in acetyl cholinergic activity in ketamine induced model and the per se group revealed the activity similar to the control group. Hence, the results indicated the potential of NPs in restoring the antioxidant enzymes to the normal indicating neuroprotective activity of ZP and thus its antioxidant effect.

Table 9: Effect of Tf-CH-NPs and CH-NPs on different oxidative stress markers in ketamine-induced schizophrenia in rats. Data expressed as mean ±SD (n = 3).

Groups	Brain			
	GSH µmol/mgof protein	MDA nmol / mg protein	SOD U/mg Protein	ACHE mmol/min/g protein
Control	4.0 ± 0.12	0.314 ± 0.024	17.43 ± 1.14	29 ± 4.61
Ket (Toxic)	1.5 ± 0.16	0.867 ± 0.056	4.63 ± 1.51	56 ± 5.32
ZP Per Se	3.6 ± 0.09	0.407 ± 0.036	23.05 ± 0.98	31 ± 3.56

Ket+ DS	4.3 ± 0.24	0.815 ± 0.045	12.73 ± 1.74	46 ± 2.89
Ket+ CH-NPs	5.4 ± 0.45	0.578 ± 0.028	18.09 ± 1.85	34 ± 3.89
Ket+ Tf-CH- NPs	5.6 ± 0.51	0.502 ± 0.041	22.08 ± 3.12	37 ± 5.21

Neuroinflammatory Bio-markers study using ELISA

Neuroinflammation is associated to Schizophrenia. The various cytokines like IL-6, IL- 1-β, and TNF- α were determined in schizophrenia induced model of rats that are summarized. Results concluded the increased in level of neuroinflammatory bio- markers in ketamine treated Wistar rats, however a substantial attenuation of these biomarkers was found in schizophrenic induced rats treated with CH-NPs and Tf-CH- NPs of ZP. The results evinced marked increase in anti-inflammatory effect of ZP-CH- NPs and ZP-Tf-CH-NPs in schizophrenia induced rat brain as compared to drug solution given intranasally found to be statistically significant ($p < 0.005$). However, the drug formulation per se administer in rats without ketamine treatment showed activity like control group. The above results signify the anti-inflammatory potential of ZP nanoparticles.

Table 10: Effect of Tf-CH-NPs and CH-NPs in ketamine-induced Schizophrenia in rats. Data expressed as mean ±SD (n = 3).

Groups	TNF-α (pg/mg protein)	IL-1-β (pg/mg protein)	IL-6 (pg/mg protein)
Control	153.2 ± 5.02	97.5 ± 5.42	84.7 ± 4.67
Ketamine (Toxic)	408.5 ± 10.58	345 ± 4.53	278.5 ± 11.42
ZP Per Se	165.2 ± 7.51	101.5 ± 5.79	91.3 ± 4.19
Ket+ ZP suspension	306.4 ± 12.90	298.8 ± 6.31	252.1 ± 7.76
Ket+ ZP-CH-NPs	202.5 ± 7.89	198.7 ± 4.89	125.2 ± 8.45
Ket+ Tf-ZP-CH-NPs	194.8 ± 5.76	195.4 ± 5.54	118.6 ± 5.01

Conclusion

The study successfully developed and evaluated Tf-CH-NPs for the delivery of ZH. Pharmacokinetic analyses demonstrated enhanced brain targeting and bioavailability of Tf-CH-NPs compared to non-conjugated nanoparticles and ZH solution. Pharmacodynamic studies corroborated these findings, highlighting superior therapeutic efficacy in mitigating schizophrenia-like symptoms and oxidative stress. This research underscores the potential of transferrin-based nanocarriers in advancing treatment strategies for neurological disorders.

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