



Comparative Analysis of Paroxetine-Loaded Nanostructured Lipid Carriers vs Paroxetine Dispersion in LPS-Induced Depression Model

Kiran Akbar¹, Masood ur rehman², Ismail Badshah³, Sidra Younas⁴

¹Department of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan; | ²Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan; | ³Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan; | ⁴Department of Pharmacy, Bacha Khan University, Charsadda, Pakistan.

Correspondence should be addressed to Sidra Younas; sidrayounas@bkuc.edu.pk

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ABSTRACT

The current study was intended to investigate the comparative antidepressant activity of Paroxetine-entrapped nanostructured lipid carriers (PAR-NLCs) and Paroxetine dispersion (PAR-Dispersion) in a lipopolysaccharide (LPS) induced depression model. Previously prepared, PAR-NLCs with the optimal acetyl alcohol to oleic acid ratio (76:24 w/w) were used in the study. To compare the release of PAR-NLCs with PAR-dispersion, *in vitro* release was carried out by the dialysis membrane diffusion method. In addition, the *in vivo* antidepressant activity of PAR-NLCs and PAR-dispersion was assessed in an LPS-induced depression model in terms of behavioral studies and histopathological examination using Hematoxylin & Eosin (H&E) staining. Furthermore, antioxidant levels (GSH, GST, and CAT) and TBARS were also assessed. The results showed biphasic drug release of PAR-NLCs with a burst release in the initial 2 hr followed by sustained release up to 6 hrs. Behavioral studies depicted reduced immobility time in rats treated with PAR-NLCs. Furthermore, H & E staining exhibited improved neuronal architecture and survival in the PAR-NLCs group compared to PAR-dispersion. The concentrations of antioxidants were also significantly increased in the PAR-NLCs group. Taken together, the therapeutic effectiveness of PAR was markedly augmented in PAR-NLCs, and they could be potential nanocarriers for effective delivery of antidepressants to the brain.

1. Introduction

Depression is prevalent on a global scale, affecting approximately 5 % of adults. Depression, in its most severe form, can lead to suicidal inclinations, hence ranking as the second most prevalent cause of death among those aged 15–29 (WHO, 2021). It is characterized by low mood, low self-esteem, grief, loss of interest, disturbed sleep, and even suicidal thoughts (Kircanski et al., 2012; Sharma & Dang, 2020). By 2020, the World Health Organization predicted that depression would be the leading cause of disability worldwide. It has been reported that depression is episodic and occurs at different points in life, thus affecting life severely. On the other hand, a relatively less severe form of depression is dysthymia, which is a chronic type that decreases a person's ability to perform functions without making the patient completely disabled (Sharma & Dang, 2020).

Multiple factors lead to the pathogenesis of this disorder, including genetic predisposition and a lack of noradrenaline and serotonin (NA or 5-HT) in some parts of the brain (Brigitta, 2002), decrease in hippocampal function leading to excess cortisol levels (hypercortisolemia) (Gubert et al., 2020; Varghese & Brown, 2001), decreased neurotrophic factors causing neuronal atrophy, loss of cells (glia), and reduced hippocampal neurogenesis (Brigitta, 2002; Fox & Lobo, 2019), inflammation, tumor necrosis factor alpha (TNF- α), and misalignment in the circadian rhythms lead to depression (Peters et al., 2019; Shal et al., 2019; Walker et al., 2020).

Antidepressants are widely accepted and have been used for the treatment of depression and anxiety. Most of the antidepressants are designed as monoamine oxidase inhibitors and tricyclic antidepressants, and later progressed in the form of serotonin and norepinephrine uptake inhibitors known as selective serotonin reuptake inhibitors and serotonin- norepinephrine reuptake inhibitors (SSRIs and

SNRIs) (Karroui et al., 2021). One of the widely practiced FDA-approved drugs, paroxetine, falls under the category of SSRIs and is used for the management of depression, anxiety, and other related psychiatric disorders. Paroxetine is highly lipophilic with a half-life of around 21 hrs. It binds to serotonin and inhibits its reuptake; the amount of serotonin is restored in the synaptic cleft of the depressive brain and cures mood-related problems. Paroxetine achieves a steady-state level in around 7–14 days with regular administration of the drug, and its inactive polar metabolites are excreted through urine (65%) and feces (25%) (Bourin et al., 2001; Pandey et al., 2016).

Presently, antidepressant drugs are available only in the form of oral formulations. The orally administered drugs undergo hepatic metabolism before reaching the target organs. Due to enzymatic degradation in the gastrointestinal tract and liver metabolism, a very small amount of the drug reaches the target organs. According to various reports, paroxetine is easily absorbed in the gastrointestinal tract after oral administration and passes to the liver via the hepatic portal vein. The liver enzymes degrade the drug to an inactive form extensively, and less than 50% of the active molecule reaches the systemic circulation (Hiemke & Härter, 2000).

Another major factor that limits its brain delivery is the presence of the blood-brain barrier. The blood-brain barrier is a complex and selective system that consists of astrocytes, pericytes, and endothelial cells (Masserini, 2013). The endothelial cells are connected through tight junctions (zonula occludens), which seal the intracellular spaces, and are surrounded by astrocytes and pericyte cells. This anatomical property of the blood-brain barrier prevents the entry of large hydrophobic molecules and some microorganisms, such as bacteria, from entering through plasma into the extracellular fluid of the brain (Dimitrijevic & Pantic, 2014). Only a small number of drug particles

(with molecular weight <400–500 Daltons) and with lipophilic characteristics can pass through this barrier(Katare et al., 2017).

Therefore, scientists and researchers have been trying to develop effective antidepressant formulations and new drug delivery systems for the brain. Nanocarrier-based delivery systems have been reported to enhance drug uptake of drug and increase permeability when delivered through the intranasal route and are considered to be more achievable routes for delivery of drugs.

Lipid nanoparticles, particularly nanostructured lipid carriers (NLC), have been used to improve drug bioavailability, protecting molecules from enzymatic degradation, promoting targeting, and providing prolonged release through different routes of administration. When considering strategies for targeting drugs to the brain, NLC are particularly interesting due to their small size and the possibility of surface functionalization. In addition, the emulsifiers used in the composition of NLC formulations can inhibit the expression of P-glycoprotein, thereby reducing efflux from the brain and consequently improving drug bioavailability in the brain (Correia et al., 2024).

In our previous study, Paroxetine loaded NLCs (PAR-NLCs) were successfully prepared and optimized with respect to the type and ratio of solid lipid and liquid lipid. The developed PAR-NLCs were characterized for physicochemical properties. The current study aims to investigate the *in vivo* antidepressant effect of PAR-NLCs compared to PAR-dispersion/marketed drug. The *in vivo* antidepressant activity in the LPS-induced depression model was evaluated in terms of immobility time, histopathological evaluation, and antioxidant levels in brain tissues.

2. Materials and Methods

2.1. Chemicals and reagents

Paroxetine (PAR), Cetyl alcohol (CA), Oleic Acid, Tween 40, Span 40, Myrj 52, Lipopolysaccharide, Poloxamer 407 (Pluronic F-127), and Poloxamer 188(Pluronic F-68) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of PAR-NLCs

The optimized formulation of PAR-NLCs from our previous study was prepared by nano template engineering technology (Akbar et al., 2024b). Briefly, the drug, cetyl alcohol, oleic acid, and surface-active agents (Span 40, Myrj 52, and Tween 40) were heated to approximately 60°C. Deionized water was gradually added while being kept at the same temperature as the mixture. The mixture was agitated at 800 rpm for 30- 40 min. The heated nano-emulsion was quickly cooled using an ice

bath for 15 to 45 minutes to obtain PAR-NLCs. Untrapped drug was removed using filtration through a 0.45 µm PVDF filter. The filtered PAR-NLCs were stored at 4 °C for further characterization.

2.3. In vitro dissolution

In vitro drug release study of PAR-NLCs was conducted as described earlier (Akbar et al., 2024b). In brief, the dialysis membrane diffusion technique was used to carry out the *in vitro* drug release study using Simulated nasal fluid (SNF) (pH 6.5) as a release medium. PAR-Dispersion and PAR-NLC formulations equivalent to 5 mg of PAR were filled separately in dialysis membranes with a 3500 Da molecular cut-off. A steady stirring speed of 100 rpm was maintained at 34±0.5°C for 6 hrs. 5ml of the sample was taken from each container for analysis at pre-specified intervals (0, 15, 30, 60,120, 240, and 360 minutes) and replaced with an equivalent quantity of SNF to maintain constant volume and sink conditions. The samples were analyzed for PAR contents by UV-visible spectrophotometric analysis at λmax of 295 nm, and the cumulative amount of PAR released (%) was determined and plotted against time (h).

2.4. In vivo activity

2.5. Depression Model

LPS was utilized for the induction of depressive like behavior and was injected at a dose of 500 µg/kg per kg on alternate days for a period of 7 days (Rubab et al., 2021). For *in vivo* studies, rats were divided into four experimental groups, and each group had five rats (n=5). Treatment groups included: Group-1 (saline), Group-2 (control), Group-3 (PAR-NLCs), and Group-4 (PAR-dispersion) (Table I). Group 3 and 4 received the drug (PAR) at a dose of 1.03 mg/kg on alternate days for a week, along with LPS. The volume was determined for each rat separately based on its body weight using the following formula, and the administration was carried out in the supine position.

$$\text{Volume to Administer (}\mu\text{L)} = \frac{\text{Dose (}\frac{\text{mg}}{\text{kg}}\text{)} \times \text{Body Weight of Rat (kg)}}{\text{Concentration of Drug solution (mg/mL)}} \times 1000$$

The volume, in microliters, was delivered intranasally using a micropipette. The rats were not given anesthesia during the process to avoid respiratory distress. Rats received 6–10 µL nose drops with a total volume of 40–100 µL, in accordance with standard intranasal studies. This was a noninvasive technique since the drops are placed close to the nostril every one to two minutes for full absorption, and the animal sniffs them into the nasal cavity rather than sticking a pipette into the nostril. A tuberculin syringe was used to administer the oral dose. Behavioral tests were conducted on the eighth and tenth days following seven days of therapy.

Table 1. Experimental groups along with the dose.

Experimental groups	Treatment
Group 1-Control	Normal saline only (2ml/kg) I.n. daily
Group 2-Disease	LPS only (0.5mg/kg I.p on alternative days
Group 3-PAR-Dispersion	LPS only (0.5mg/kg) I.p on alternative days+PAR-dispersion (1.03mg/kg) orally daily
Group-PAR –NLCs	LPS only (0.5mg/kg) I.p. on alternative days+PAR NLCs (1.03mg/kg) I.n. daily

2.6. Behavioral Analysis

2.6.1. Tail suspension test and Forced swimming test

Tail suspension and forced swim tests were utilized as behavioral despair tests to evaluate the antidepressant potential of PAR-NLCs. Armario's method for conducting the Tail Suspension Test (TST) was followed. At a distance of 2 cm from the tips of their tails and roughly 50 cm above the ground, the rats were suspended. The test lasted for 5 min. A video camera was used to record the immobility period. Complete motionless state and passive hanging were interpreted as immobility (Armario, 2021). The method provided by (Planchez et al., 2019) was used to conduct the forced swim test. Rat was placed in a 21 × 21 × 50 cylindrical glass container with a water level of up to 10 cm. The temperature was set between 23 and 25°C. Rats were allowed to swim for 5 minutes after being individually placed in the container. Rats that were floating and making only a few forced movements to stay above the water's surface were considered immobile. The container's water was swapped out for a new animal due to the buildup of odor.

2.7. Hematoxylin & Eosin (H&E) Staining

In medical diagnosis, hematoxylin and eosin (H&E) stain is used as a primary contrast technique. There are many different cytoplasmic, nuclear, and extracellular matrix structures that it is used to differentiate (Aicher et al., 2003; de Haan et al., 2021). The brain sections were examined under a light microscope for morphological changes following hematoxylin and eosin (H&E) staining (Silva et al., 2022). Because of this, the brain tissue sections (cortex and hippocampus) that were prepared were deparaffinized using 100% absolute xylene, rehydrated using gradually increasing amounts of ethanol (100, 95, 90, 80, and 70%), and then washed with distilled water. After being cleaned with PBS, the rehydrated tissue segments were submerged in hematoxylin for ten minutes and in water for 5 min. They were then inspected under a microscope to confirm nuclear staining.

The tissue segments were retreated with hematoxylin if they were not stained as expected. Later, these slides were kept for 5 min in HCl solution (1%) and ammonia water (1%) and afterwards rinsed with water. At last, the tissue segments were treated with eosin for 5–10 min, washed with water, and dried in air. The tissues were dried out by successive treatment with ethanol in reverse order (70, 80, 90, 95, and 100 percent), followed by fixation in xylene and arrangement of cover slips. Lastly, the histological micrographs of brain tissues were taken with the help of a light microscope (Olympus, Japan) and examined with ImageJ software. These micrographs were studied for neuron morphology, vacuole formation, and cellular infiltration. The cytoplasm and extracellular network are stained pink by eosin, while the nuclei are stained blue or purple by hematoxylin. This system's basic components include graded alcohols, xylene, eosin, and hematoxylin. During the staining process, these materials are used in the intermediate stages of rehydrating and dehydrating tissue sections. The micrographs were taken using an Olympus light microscope (Olympus Japan), and ImageJ software was used for analysis. Each group's images were captured, and they were subsequently examined for cellular infiltration, neuron morphology, and vacuole formation.

2.8. Antioxidant analysis

2.8.1. Estimation of GSH And GST

The isolated brain tissue of rats was homogenized and centrifuged at 1500 rpm for 30 min to collect the supernatant. The supernatant was analyzed for glutathione (GSH) and glutathione S-transferase (GST) content assessment. The GST activity was determined by estimation of CDNB conjugate formation using 0.1 M Phosphate Buffer and tissue homogenate. Finally, the absorbance was measured at 340nm using a microplate reader for 5 mins at 23°C. The activity of GST was

calculated as μmol of CDNB conjugate/min/mg of protein. For GSH estimation, GSH was oxidized with DTNB in 0.2 M Phosphate buffer with 0.2 mL of tissue supernatant for 10 min, producing 2-nitro-5-thiobenzoic acid, which is yellow in color. Consequently, the absorbance of the reaction mixture was measured at 412 nm. Phosphate buffer was used as a control, while DTNB solution without tissue lysate was kept as a blank. GSH activity was expressed as $\mu\text{mol}/\text{mg}$ of protein (Badshah et al., 2023).

2.9. Estimation of CAT

The rate of H_2O_2 degradation was measured in the presence of catalase. The absorbance was measured at 240 nm with the use of a microplate reader. Catalase activity was measured in moles $\text{H}_2\text{O}_2/\text{min}/\text{mg}$ of protein (Razzaq et al., 2022).

2.10. TBARS

200 μL of supernatant was added to 580 μL of 0.1 M Phosphate buffer with pH 7.4, 200 μL of 100 mM ascorbic acid, and 20 μL of ferric chloride. The mixture was incubated for 60 min in a water bath at 37°C. Then the reaction was stopped by adding 10% trichloroacetic acid (TCA) and 1000 μL of thiobarbituric acid (0.66% TBA). The mixture was kept in tubes in a water bath for 20 min. Afterwards, the tubes were cooled in an ice bath and then centrifuged at $3000 \times g$ for 10 min. For both the blank and the supernatant, the absorbance was measured at 535 nm. The results were expressed as TBARS-nmol/mg protein (Jesse et al., 2010).

2.11. Statistical analysis

The results are shown as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was used for statistical analysis, and Tukey's multiple comparisons post hoc test was used to identify group differences. Statistical significance was defined as $P \leq 0.05$, and high significance was defined as $P \leq 0.01$ and $P \leq 0.001$.

3. Results

3.1. *In vitro* drug release

The *in vitro* drug release profile from the optimized PAR-loaded-NLCs shows a release of around 30 % in the first 2 hrs, followed by sustained release up to 6 hrs, whereas PAR-Dispersion showed around 70% release in the first 2 hrs and complete release in 3 hrs (Fig.1)

3.2. *In vivo* evaluation in depression model

3.3. 3. Tail Suspension Test (TST)

A behavioral assay in mice/rat widely used to evaluate antidepressant activity. Reduced immobility time shows improved coping behavior and antidepressant-like effects. In the tail suspension test, it was observed that the immobility time was successfully reduced by both treatment groups compared with the control, indicating enhanced antidepressant-like activity compared to simple dispersions. However, the results of the NLC-containing group were better than the dispersion group. (Fig. 2A).

3.4. Forced Swim Test (FST)

Mobility served as a main sign of depressive-like behavior in the tail suspension test (TST) analysis. Both PAR-NLCs (Paroxetine-loaded nanostructured lipid carriers) and PAR-dispersion cut down on immobility when compared to the control group, showing antidepressant-like effects (Fig. 2B). The PAR-NLCs group really stood out, likely due to better bioavailability, a steady release, and more effective brain targeting from the nanostructured lipid carriers.

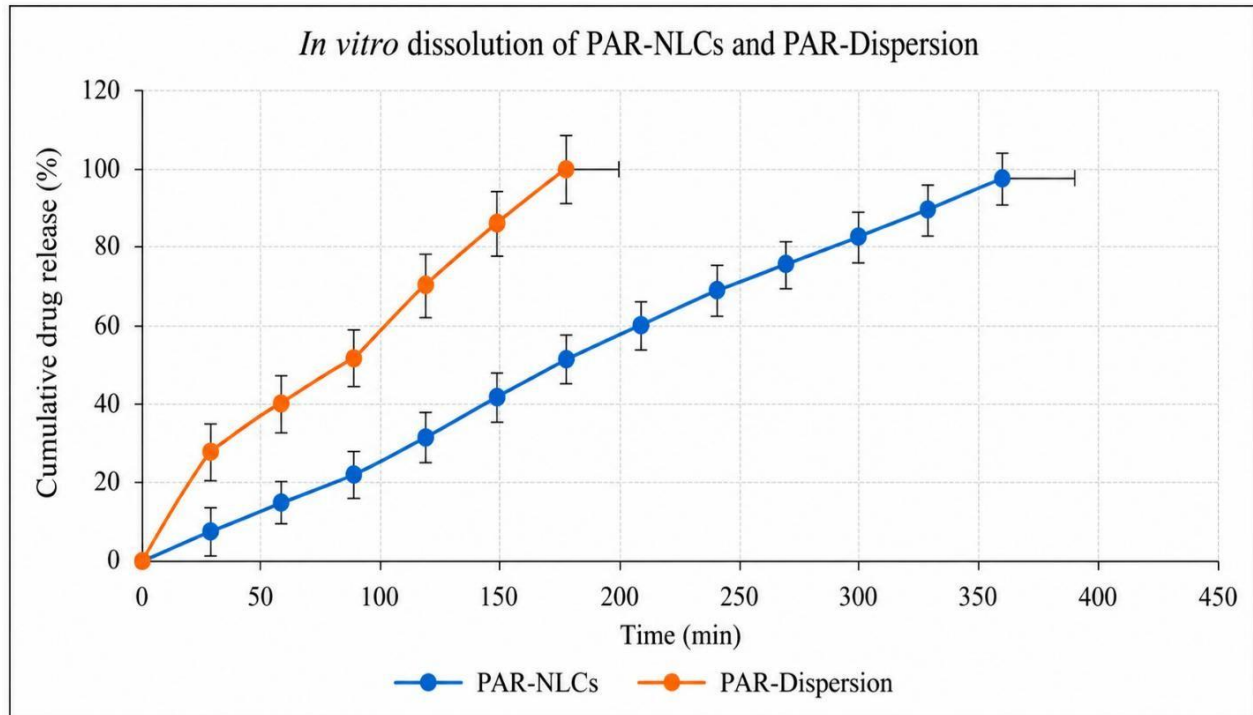


Fig 1. *In vitro* release profile of PAR-NLCs and PAR-Dispersion in SNF (pH 6.5) at 34°C for 6 h. Data is expressed as mean±S.D (n=6).

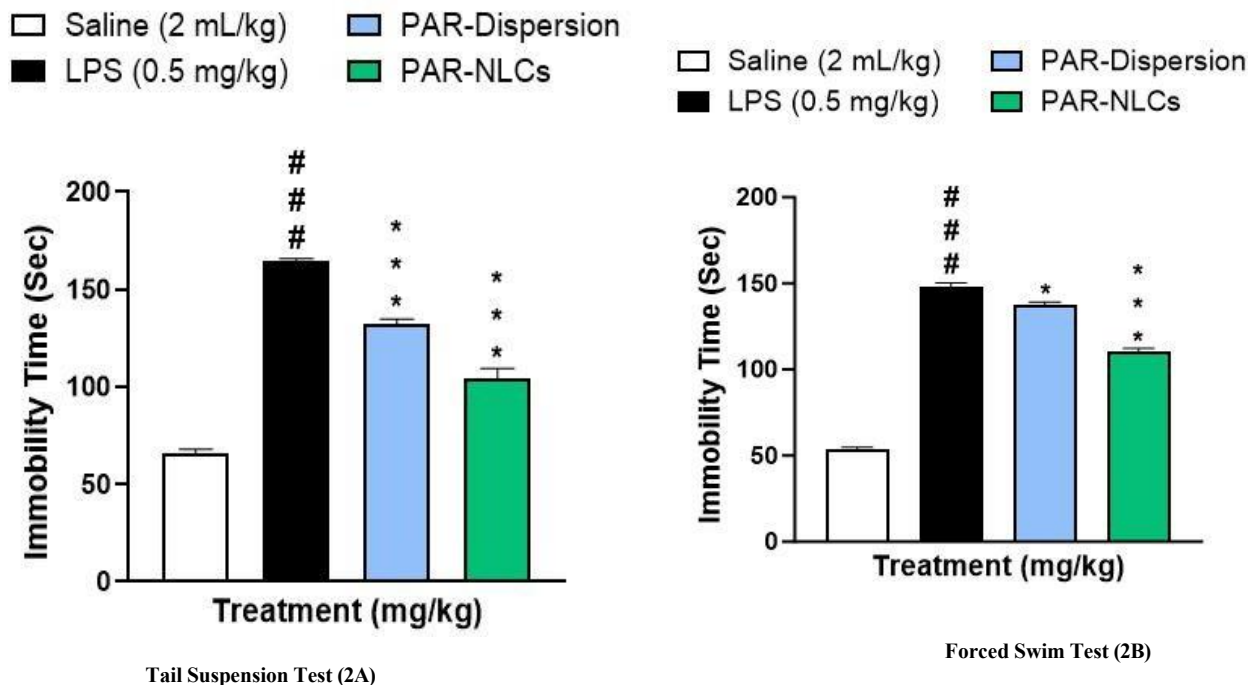
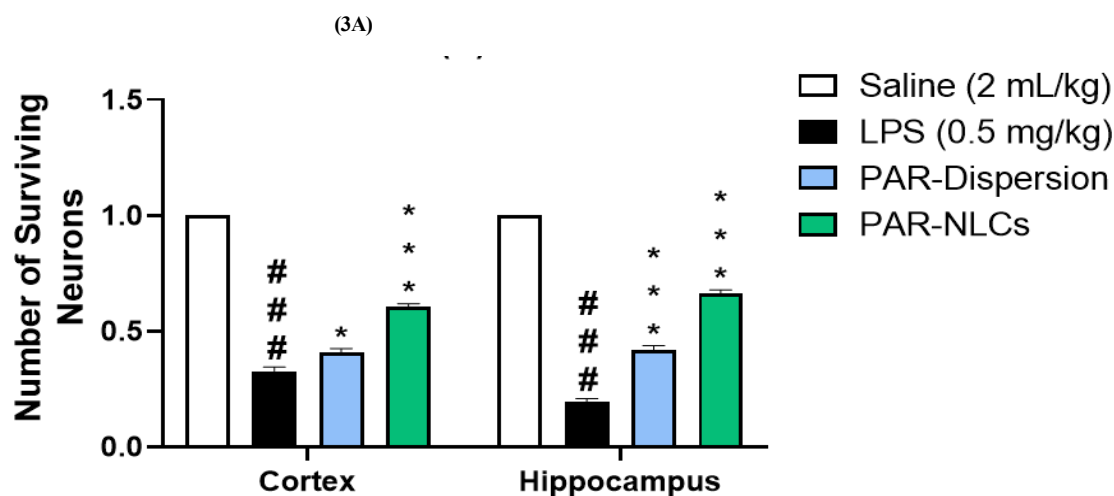
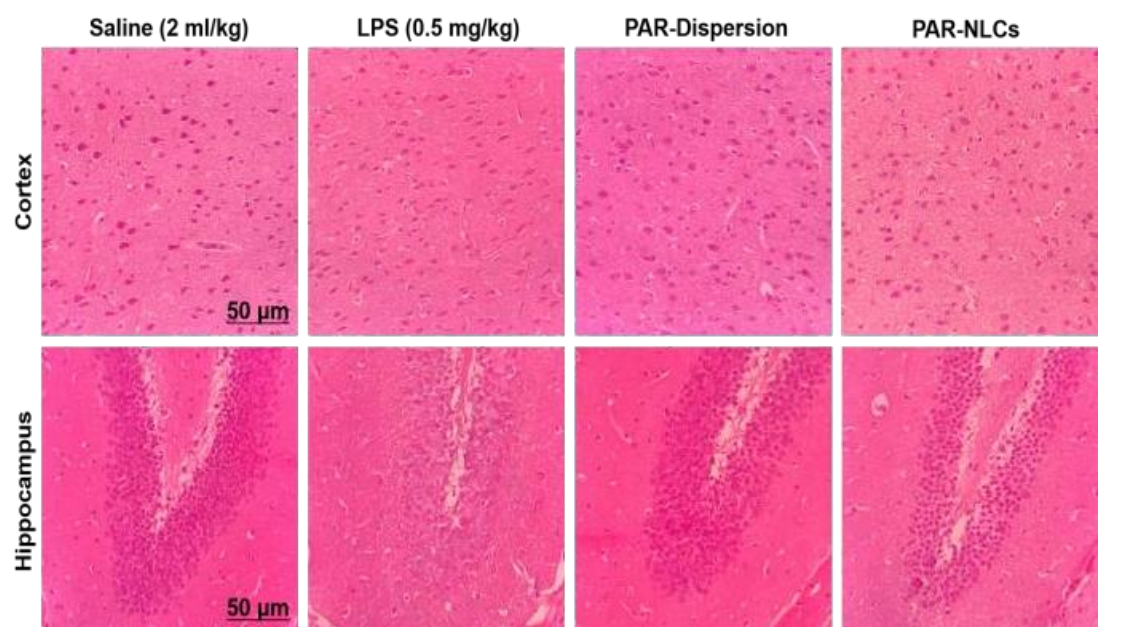


Fig 2. Effect of PAR-NLCs and PAR-Dispersion against LPS-induced depression model in Tail suspension test and Forced swim test. Data were expressed as mean±S.D (n=6). For statistical analysis, one-way ANOVA with Bonferroni's test was applied. ### p< denotes a significant difference against the control group. *P<0.05, ***P<0.001 denote a significant difference against the LPS group.

3.5. H & E staining

For the evaluation of morphological changes and neurodegeneration, H & E staining was performed. The micrographs of the normal saline group revealed regular morphology with regularly shaped cells and well-defined round nuclei. In contrast, the LPS-treated group showed

shape deformation, multiple vacuole formation, decreased cell size, and a reduction in the number of cells. PAR-Dispersion significantly reversed LPS-induced morphological damage and restored normal tissue architecture. However, the effect of PAR-NLCs was more significant than PAR-Dispersion (Figures 3A and 3B).



(3B)

Fig 3. Effect of PAR-NLCs and PAR-dispersion against LPS induced morphological changes in mouse brain tissues (3A). Representative histopathological changes in the cortex and hippocampal regions of Saline, LPS, PAR-NLCs and PAR-Dispersion treated mice brain visualized by hematoxylin and Eosin stain (H&E) (Scale bar: 50 µm). Fig 3B depicts number of surviving neurons in the cortex and hippocampus region with Saline, LPS, PAR-Dispersion and PAR-NLCs treatment

3.6. Anti-oxidant analysis

After the administration of LPS, levels of oxidative stress were evaluated in terms of GST, GSH, CAT, and TBARS in the cortex and hippocampus. Compared with the control group, the LPS-treated group showed a significant increase in TBARS as well as a significant decrease in GST (Fig. 4A), GSH (Fig. 4B), and CAT (Fig. 4C) in both brain regions ($P < 0.001$). It is proposed that LPS increased lipid peroxidation and impaired the endogenous antioxidant defenses, causing oxidative imbalance. On the other hand, treatment with both PAR-NLC formulations markedly decreased TBARS in the cortex and hippocampus and restored GST, GSH, and CAT levels, indicating

attenuation of LPS-induced oxidative damage. These results are in line with earlier research demonstrating that paroxetine can reduce inflammatory signaling and microglial activation after LPS exposure. Additionally, paroxetine has shown neuroprotective effects linked to decreased oxidative damage and ROS production. Additionally, paroxetine has been shown in experiments to improve antioxidant defenses and lower lipid peroxidation in brain tissue. Overall, the current research indicates that, in addition to their anti-inflammatory properties, PAR-NLCs may have a neuroprotective effect by reducing oxidative lipid damage and restoring endogenous antioxidant defenses.

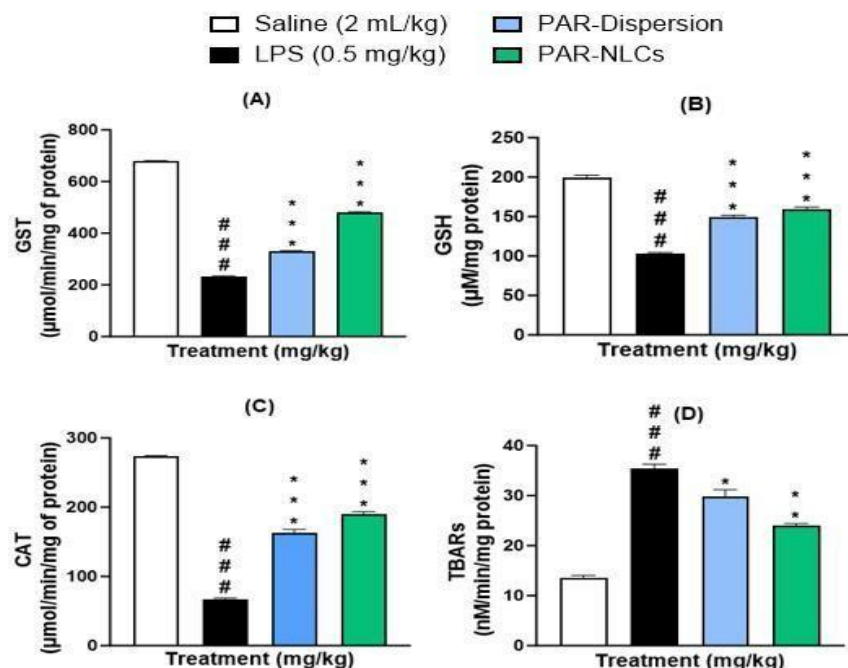


Fig 4. Effect of PAR-NLCs and PAR-Dispersion against LPS induced oxidative stress evaluated by antioxidant assay. (A) GST, (B) GSH (C) CAT and (D) TBARS. Data were expressed as mean±S.D (n=6). For statistical analysis, one way ANOVA with Bonferroni's tests was applied. ### p< denotes a significance difference against control group. *P<0.05, ***P<0.001 denotes a significance against LPS group.

4. Discussion

Because of their high drug-loading capacity, biocompatibility, biodegradability, and ability to improve drug stability and release characteristics, lipid-based nanoparticles have attracted considerable attention for brain drug delivery. Among these systems, nanostructured lipid carriers (NLCs) are composed of a mixture of solid and liquid lipids stabilized by surfactants. The incorporation of liquid lipids into the solid lipid matrix creates structural imperfections that can accommodate greater amounts of drug and reduce drug expulsion during storage, while also allowing modulation of drug release (Ghasemiyeh & Mohammadi-Samani, 2018). It has been reported that drug retention, protection, solubility, and brain targeting can all be enhanced by encasing medications in nanocarriers like NLCs (Akbar et al., 2024a).

NLCs have therefore been investigated as promising carriers for improving drug solubility, bioavailability, stability, and brain targeting. The intranasal route is especially attractive for delivering drugs to the central nervous system because it offers a pathway that can move substances from the nasal cavity toward the brain through olfactory and trigeminal routes. This cuts the need to cross the blood-brain barrier. Moreover, intranasal delivery can raise drug levels in the brain while lowering levels elsewhere in the body and reducing side effects because it bypasses first-pass metabolism (Costa et al., 2021). It offers a unique way to deliver drugs to the brain without systemic exposure.

The intranasal route provides a pathway that can transport substances from the nasal cavity toward the brain through olfactory and trigeminal routes. This eliminates the need to pass through the blood-brain barrier. Furthermore, intranasal delivery avoids first-pass metabolism; it can increase drug levels in the brain while decreasing levels elsewhere in the body and minimizing side effects (Costa et al., 2021). Hence, PAR-NLCs were designed to be administered intranasally and deliver the drug directly to the brain.

In vitro dissolution study of PAR-NLCs and PAR-dispersion was carried out using the dialysis bag method using phosphate buffer pH 6.5 as the release medium, stirred at 100 rpm at 37±2°C. At predetermined time points, samples were withdrawn and replenished with equal volumes of fresh diffusion medium. Absorbance of samples was analyzed spectrophotometrically at 295 nm to determine the % drug release. It was observed that PAR-dispersion showed 100% release in the first 3 hrs whereas 98% drug was released from PAR-NLCs in 6 hrs. The initial faster drug release from PAR-NLCs is caused by surface-adsorbed drug or loosely attached drug in the outer shell of NLCs. After 2 hr. PAR located deeper in the NLC core was released in a sustained manner due to longer path of diffusion (Das et al., 2012; El-Helw & Fahmy, 2015). The results were aligned with a previous study (Gul et al., 2022) that also reported biphasic release of drug from NLCs.

A well-established LPS-induced depression model in rats was used to determine the antidepressant potential of PAR-NLCs. LPS is an integral component of Gram-negative bacterial membranes and, at low doses, causes behavioral alterations in rodents (Zhao et al., 2019). The behavioral analysis was conducted using the forced swim test and tail suspension tests after LPS administration and completion of the treatment period. The forced swim test and tail suspension tests are common techniques for behavioral analysis in rats and are used to evaluate the antidepressant potential of drugs. In behavioral analysis, LPS causes the animal to behave passively and abort its struggle. On the other hand, antidepressants improve the animal's choice between active and passive behaviors. The reduced immobility time in the PAR-NLCs group demonstrates their enhanced antidepressant potential, as also reported by (Ali et al., 2020; Rubab et al., 2021).

LPS administration in rodents has been reported to induce neuronal damage by augmenting the generation of reactive oxygen species (ROS) and production of proinflammatory cytokines, leading to oxidative stress and neurotoxicity (Hassanzadeh-Taheri et al., 2021). LPS triggers innate immune signaling, particularly through Toll-like receptor 4 (TLR4), causing microglial activation and increased production of

proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6. Persistent inflammatory signaling can promote the generation of reactive oxygen and nitrogen species, increase lipid peroxidation, and disrupt the antioxidant defense system, thereby contributing to oxidative stress and neuronal dysfunction (Beheshti et al., 2020). In the present study, histopathological examination revealed evident attenuation of neuronal damage in the PAR-NLC-treated group, as supported by improved cellular morphology and restoration of cellular integrity compared with the LPS-treated group. The observed preservation of neuronal architecture may show a protective effect of PAR-NLCs against LPS-associated inflammatory and oxidative injury. Likewise, neuroprotective effects have been reported in LPS-induced models, where attenuation of inflammatory and oxidative responses was accompanied by reduced neuronal injury and improved histological appearance. For example, treatment with rosmarinic acid in LPS-induced neuroinflammation reduced TNF- α , IL-1 β , and IL-6 levels, enhanced antioxidant status, and attenuated histologically assessed neuronal injury in the hippocampus. (Batista et al., 2019). The results are also supported by our previous study (Akbar et al., 2024a) that reported neuronal shrinkage, vacuole formation, and nuclear condensation by LPS induction. However, these effects were reversed by nanostructured lipid carriers, demonstrating preserved neuronal morphology when compared to dispersions, due to improved drug delivery

The presence of reactive oxygen species (ROS) can damage lipids of the cell membrane. GSH (Glutathione) is an endogenous antioxidant that regulates various metabolic processes and is crucial in DNA repair. A low level of GSH has been found in individuals with depression. (Joshi et al., 2010; Kodydková et al., 2009; Maes et al., 2011). Catalase (CAT) is an important endogenous antioxidant enzyme that converts hydrogen peroxide into oxygen and water; excessive oxidative stress decreases catalase activity, as demonstrated by clinical trials. According to the findings of the current study and aligned with a previous study (Mandpe et al., 2013), both treatment groups increased GST, GSH, and CAT, whereas TBARs activity decreased.

5. Conclusion

In this study, the previously optimized PAR-NLCs were assessed for *in vitro* release, *in vivo* antidepressant and antioxidant activity in comparison with PAR- dispersion. The formulation of PAR in NLCs successfully augmented its *in vivo* antidepressant effects in behavioral, histological, and antioxidant analyses in rats. These findings suggest that NLCs could be promising nanocarriers for PAR and other antidepressants with improved potential for brain delivery and combating depression.

Declarations

Ethics approval and consent to participate

All animal studies were performed as per standard NIH policies approved by the Institutional Research and Ethics Committee of Riphah International University (Approval # REC/RIPS/2022/024)

Ethical consideration

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author's Contribution

Kiran Akbar: conceptualization, investigation, data curation, writing the original draft. Masood ur Rehman: supervision, resources, editing final draft. Ismail Badshah: reviewing and editing final draft. Sidra Younas: formal analysis, methodology, software

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Data Availability

All data supporting this review are available within the published articles cited herein.

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