doi.org/10.36721/PJPS.2025.38.2.REG.13843.1

Assessment and evaluation of melatonin loaded PLGA injectable nanosuspension for the treatment of subarachnoid hemorrhage: Preclinical study

Nong Qian[1] and Jun Tian[2]*
[1]Department of Pharmacy, An'kang Traditional Chinese Medicine Hospital, An'kang China
[2]Department of Neurosurgery, The First Affiliated Hospital of Xi'an Medical University, Xi'an, China

Abstract: Oxidative stress has connection in the development of acute brain injury after SAH, according to a large body of research. Strong antioxidant melatonin is non-toxic and readily crosses the blood-brain barrier when converted to nanoparticles with PLGA. Nanosuspension of melatonin was formulated by use of different concentrations of PLGA and other stabilisers with variable concentration. Nano particles of Melatonin were prepared by high pressure homogenization method. Their particle size, PDI, zeta potential, surface morphology, encapsulation efficiency and in vitro release study were evaluated. Animal study was performed on rats on different groups. Out of nine batches, formulation F6 was optimized batch with drug: PLGA concentration was 1:3 and Poloxamer 188 was used as stabilizer. Physicochemical parameters for the nanoparticles were found within limit. Drug release was found in sustain release pattern for F6 batch complete release of the drug was observed in 120 hours and showed sustained release of the drug. A considerable decrease in 24-hour mortality was observed in the optimised batch when 30 mg/kg of melatonin was administered. The melatonin therapy group's brain water content was assessed to determine whether a decrease in brain edema was linked to a lower death rate and the prevention of edema and cerebral water content was discovered. Melatonin in a medication delivery system nanocapsulated could be a promising treatment for subarachnoid haemorrhage.

Keywords: Subarachnoid hemorrhage, nanosuspension, nanoparticles, sustain release

Submitted on 25-09-2024 – Revised on 26-12-2024 – Accepted on 06-02-2025

INTRODUCTION

Subarachnoid haemorrhage (SAH) is a hazardous and occasionally fatal disorder characterised by bleeding into the area between the arachnoid membrane and the pia mater protecting the brain. Cerebrospinal fluid, which cushions the brain and spinal cord, often fills this region. The bleeding usually results from a ruptured aneurysm, but it can also be caused by trauma or other vascular malformations. Frequent reports of an abrupt, intense headache as "the worst headache of my life" vomiting and nausea, rigid neck, Light sensitivity (photophobia), Loss of consciousness, Seizures and Neurological deficits such as weakness, numbness, or difficulty speaking are some of the symptoms of subarachnoid hemorrhage (Lawton and Vates, 2017; D'Souza, 2015) Several risk factors can increase the likelihood of experiencing a subarachnoid hemorrhage (SAH). These risk factors include both modifiable and non-modifiable elements: Hypertension is one of most important risk factor where Prolonged hypertension can erode blood vessel walls, raising the possibility of aneurysm development and burst. Smoking also damages the blood vessels. Tobacco use is a significant risk factor for SAH, as it can damage blood vessels and contribute to the formation of aneurysms. Heavy alcohol consumption can raise blood pressure and contribute to vascular disease (Feigin *et al*., 2005; Qureshi *et al*., 2011). The use of certain recreational drugs, particularly cocaine and amphetamines, can cause sudden increases in blood pressure, increasing the risk of aneurysm rupture. Being overweight or obese can contribute to hypertension and other cardiovascular conditions that increase the risk of SAH (Teunissen *et al*., 1996; Longstreth *et al*., 1985; Andreasen *et al*., 2013). There are some non- modifiable risk which includes, age, sex and family history, previous aneurysms, certain genetic conditions and Race/Ethnicity. Activities that involve heavy lifting or intense physical strain can trigger an aneurysm rupture in susceptible individuals. Although the evidence is inconclusive, some studies point to a potential connection between using oral contraceptives and an increased risk of SAH (Andreasen *et al*., 2013; Choudhury *et al*., 2015).

The incidence of sub arachnoid hemorrhage (SAH) varies globally, but it is estimated to affect approximately 6 to 10 people per 100,000 per year worldwide. This translates to roughly 500,000 cases of SAH annually on a global scale. Approximately 6-10 per 100,000 people annually and Around 500,000 new cases each year globally found. SAH has a high mortality rate, with about 25-50% of patients dying within the first 30 days. of those who survive, a significant portion may experience long-term neurological deficits. Some studies suggest that the incidence may be higher in certain regions such as Japan and Finland, where rates can reach up to 20 per 100,000 people per year. Other

**Corresponding author:* e-mail: shanxitianjun@sina.com

regions, such as parts of the United States and Western Europe, tend to have lower incidence rates. Different genetic predispositions, lifestyle factors, healthcare systems, and diagnostic procedures across different populations can all be blamed for the discrepancies in incidence rates.

Melatonin shows significant potential as a neuroprotective agent for curing subarachnoid hemorrhage due to its antioxidant, anti-inflammatory and neuroprotective properties (Wang J *et al*., 2022). The pineal gland is the main producer of the hormone melatonin, which is well-known for controlling sleep-wake cycles. It has garnered interest in its potential therapeutic applications for various neurological conditions, including subarachnoid hemorrhage (SAH) (Xu *et al*., 2022).

The development of drug delivery technologies for melatonin is crucial to maximize its therapeutic potential, especially for treating conditions like subarachnoid hemorrhage (SAH). These technologies aim to enhance the bioavailability, stability, and targeted delivery of melatonin to achieve optimal therapeutic effects. Drug delivery technologies includes, polymeric nanoparticles, lipid based nano particles, liposomes, hydrogels, microspheres and transdermal drug delivery systems. Also, intravenous, intra-arterial, oral delivery systems and nasal drug delivery systems are available.

The important aspects of use of melatonin nanosuspensions for curing subarachnoid hemorrhage (SAH) are, it enhances bioavailability and stability, it allows the controlled and sustained release of melatonin, it can be applied for the targeted drug delivery system to the brain. Nanosuspensions can be administered in various forms (oral, intravenous, nasal), offering flexibility in administration routes and improving patient compliance (Mulam TR *et al*., 2021).

MATERIALS AND METHODS

Poly (D,L-lactic glycolic acid) (PLGA) was obtained from Biomer, Germany. Melatonin, PVP K30, Poloxamer 188 was purchased from Sigma Aldrich, USA. Dichloromethane (DCM) was purchased from Shouguang Fukang Pharmacy Factory (Shandong, China). The remaining reagents were all analytical grade.

Preparation of nanoparticles

Nano particles of melatonin were prepared by high pressure homogenization method by using different PLGA with variable concentration. First, 30 mg of melatonin and PLGA was dissolved in 20 mL of dichloromethane. Various concentrations of PLGA (1:1, 1:2, 1:3) were used in this formulation. Different concentration of stabilizers were also used namely PVP K 30, Poloxamer 188 and Tween 80. For fifteen minutes, stabilizers were mechanically stirred into the water. Solutions of stabilizers were prepared. The resulting organic solution of drug and then, using high speed homogenization (Lb 10hsh, Labotronics, USA,) at 10,000 rpm, PLGA was introduced drop by drop into the aqueous phase containing various stabilisers (Omni PDH, USA).

Later, it was placed into high-pressure homogenization at 800 bar pressures for about 3 cycles. To evaporate the organic solvent, the resultant suspension was mechanically stirred at room temperature (25^{o}C). After the suspension was ultracentrifuged (15,000g for 30 min at 10^{o}C), the nanoparticles were recovered and at least three times they were cleaned in distilled water. To obtain dry powder of nanoparticles, DCM was extracted from the suspension of nanoparticles making use of rotor evaporation process at less pressure and 37^{o}C. Until they were needed again, At 4^{o}C, the final dried nanoparticles were stored (Vasava, *et al*., 2015; Zhang *et al*., 2018).

Table 1 lists various batches that include varying concentrations of stabiliser and polymer. Particle size, PDI, drug content, saturation solubility study, and in vitro drug release study were assessed for each batch.

Preparation of nano suspension

The given quantity of PLGA (as shown in table 1) along with nanoparticles (equivalent to 30 milligrams of melatonin) were suspended in water using syringe, then ultrasonicated (Bandelin RK 255 H, capacity 5.7 L, Merck, Tokyo, Japan) for 15-20 minutes. To this resulting suspension, 0.9% sodium chloride and 0.1% HPMC were added and stirred continuously until the sodium chloride gets completely dissolved. The entire system was refrigerated between 2^{o}C-80^{o}C for a day. This suspension was put into transparent, clear vials and autoclaved for 20 minutes at 121^{o}C and 15 psi.

Characterization of nanoparticles

Yield of PLGA melatonin nanoparticles

Using the following formula, the percentage yield of nanoparticles for each batch was calculated.

$$\%\text{Yield}\,\frac{\text{Actual weight of NP}}{\text{Weight of PD + Weight of excipients}}\times 100$$

Particle size and PDI

Zetasizer (Nano Series Nano-S90, Malvern Pan analytical, UK) operates on the diffraction concept of laser light Using a technique called photon correlation spectroscopy (PCS), the mean and size distribution of the particles were examined. Its foundation is the measurement of Brownian motion in particles (Gourishetti *et al*., 2020; Mirza-Aghazadeh-Attari *et al*., 2022).

Zeta Potential

For optimised batch, zeta potential was determined by the use of Zetasizer (Nano Series Nano-S90, Malvern Pan analytical, UK). The sample was diluted ten times with distilled water.

Encapsulation efficiency

By calculating the amount of free melatonin in the supernatant, using spectrophotometry, the encapsulation effectiveness of the nanoparticles was measured at 280 nm. To summarize, two distinct microcentrifuge tubes were filled with 0.5 mL of the formulation and blank formulation and diluted with 0.5 mL of Type-I water, and the mixture was underwent centrifugation at 54,200 g for 30 minutes at 4ºC using a cooling centrifuge to determine whether any free drug was present in the formulation. After being collected, the supernatant was filtered using a syringe filter (0.22 µ). Using a UV Spectrophotometer (UV-1601PC, Shimadzu Corporation, Kyoto, Japan), the absorbance of the filtrate was measured at 280 nm. The formula was used to determine the formulation's entrapment efficiency (EE) (Mirza-Aghazadeh-Attari *et al*., 2022).

$$\% \text{ EE} = \frac{\text{Total drug} \;\; \text{free drug}}{\text{Total drug}} \times 100$$

In vitro release study

The instrument used to take the nanosuspension was a modified diffusion cell. Dialysis tube was applied (donor compartment) where it contains 300ml of phosphate buffer pH 7.4 at 37± 10C for two hours was added to a water-jacketed beaker holding the known quantity (10ml) of the nanosuspension, the drug release from the nanosuspension was ascertained. Using a magnetic stirrer, the contents of the beaker were stirred. Samples were taken out in 5 ml increments for 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes at a time and they were then replaced with 5 ml of brand-new phosphate buffer 7.4. After properly diluting the samples, they were filtered via filter paper. The amount of melatonin was measured at 280 nm using the UV technique (Zhang *et al*., 2018).

SEM

The surface characteristics of the generated nanoparticles were investigated using SEM imaging. (FESEM-S 4800, Hitachi, Japan). At several sites, the form, size and surface morphology were noted.

Evaluation of injection

The pH and appearance of the nanosuspension were assessed. A digital pH metre was used to measure the solution's pH. The pH of the sample was measured using about 10 ml. Visual observations were done to see how the solution seemed. Using a T-type helipath spindle and a Brookfield viscometer (RV, spindle no. 21) (AMETEK, USA), the formulation's viscosity was determined. An osmometer (model 3250, version 2.4) was used to measure the formulation's osmolarity. Since particle size is the most critical factor, syringebility is one of the crucial requirements for injections with 18–22-gauge needles, it is guaranteed. To pass, the test sample needs to go through the syringe; those who don't pass will be deemed to have failed.

Animal study

This study was conducted in strict accordance with the recommendations of the animal ethical committee of the Huaian Hospital of Xian Medical College. The research protocol with animal experimentation was approved by the Scientific Ethics Committee of Xian Medical College (Protocol Number: XYLS2020109).To conduct the animal study the species male Sprague Dawley rats (weight ranging from 300 and 380 grams) were used. The animals were housed in a 12-hour cycle of light and dark so they can get acclimated to a temperature of 24°C-26°C. They are also provided with unlimited access to water and a regular diet of chow. From this population, six animals each were assigned to the test and control groups in random manner (Gourishetti *et al*., 2020).

Induction of SAH

The animals were put to sleep and made to lie supine. To open the neck and reveal the left carotid artery, a midline incision was done. To guarantee that the tip of the 5-0 monofilament was positioned close to the point where the ICA and MCA bifurcated, Up until the ipsilateral rCBF fell, it was transferred from the external carotid artery (ECA) into the internal carotid artery (ICA). After that, the filament was pushed farther, and a sudden rise in ICP showed that SAH had been successfully induced. The suture was then removed into the ECA to enable the ICA to be fully perfused. The identical process-introducing the suture in the ICA without resulting in a drop in rCBF or SAH-was carried out in animals that were not subjected to surgery (Mirza-Aghazadeh-Attari *et al*., 2022).

Experimental groups

From the animals who are well adjusted with the testing lab environment, three therapy groups were assessed having six animals in each group as follow:

1) SHAM surgery given vehicle (10% ethanol);
2) SAH given vehicle
3) SAH plus 30 mg/kg melatonin.

Before conducting experiment animals were anesthetized using intraperitoneal (i.p.) injections of ketamine (100 mg/kg) and xylazine (10 mg/kg), were intubated, and provided with mechanical ventilation during the surgical procedure.

Then experimental animals were subjected to the above-mentioned SAH induction method. To perform this, a sharpened USP 4-0 nylon suture was used to penetrate the cerebral vasculature at the ICA bifurcation. Because this model produces large variations in the bleeding volume, the volume of blood in the basal cisterns was assessed. Animals in the basal cisterns were given a score ranging from 0 (no blood) to 18 (severe bleeding) according on the degree of SAH. Animals having haemorrhages ≥ 15 were determined. Melatonin injection (30 mg/kg) was given intraperitoneal (i.p.) after SAH. After SAH, survival was evaluated every eight hours and then every twenty-four

hours. Following the animal sacrifice, the amount of brain water was assessed 24 hours later to assess the degree of brain edema. In summary, brains were immediately sectioned, weighed and allowed to dry for 48 hours, and then weighed once more to determine the water content of the brain (Ayer *et al*., 2009).
[(wet weight – dry weight)/wet weight] x 100

STATISTICAL ANALYSIS

A SAS statistical program (version 9.0; SAS Institute, Inc., Cary, NC) was used to assess the findings or observations made during the trials. The degree was considered significant if the p-value was less than 0.005.

RESULTS

Melatonin loaded PLGA nanoparticles prepared high pressure homogenization method showed the following physico-chemical characteristics as given in table 2. In all batches, the nanoparticles exhibited a production yield of over 90%, suggesting that there was little loss of excipients and other nanoparticle constituents. It was discovered that the percentage of nanoparticles ranged from 90.65% to 96.56%. It was discovered that Formulation F6 had a greater percentage yield—96.56 percent. In formulation F1 to F9 different ratios of PLGA were used along with different ratios of stabilizers. The findings indicate that the lowest size across all batches was determined to be 108 nm with PDI 0.10, while the largest size across every batch was discovered to be 267 nm with PDI 0.71.

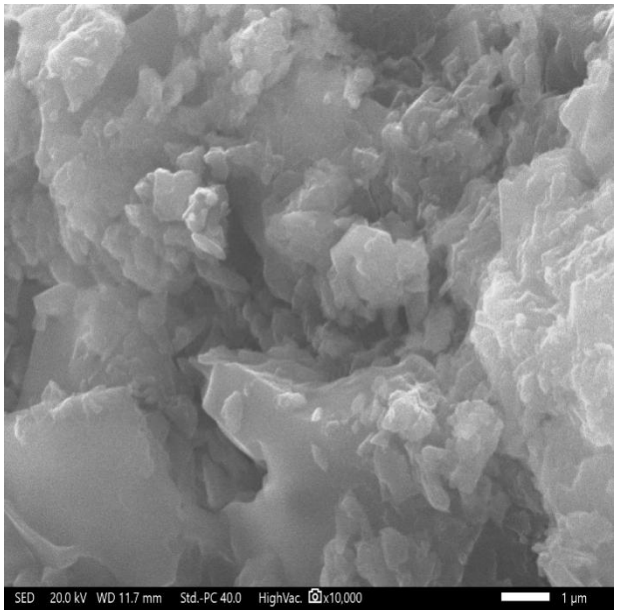

Fig. 1: Melatonin nanoparticles under scanning electron microscopy

Encapsulation efficiency of the melatonin nanoparticles was found in the range of 77.89 % to 98.95%. With high concentration of PLGA, entrapment efficiency was reduced. Optimum concentration of polymer is necessary for the maximum EE. F6 formulation was found to stable and EE was found to be 98.95%. As seen in fig. 1, the surface morphology analysis demonstrated that the dried nanoparticles were smooth, spherical and freely flowing. pH of the injection was found to be between 7-8 and viscosity, osmolarity and syringe ability was found within acceptable limits and passed the test

Excellent sustained release behaviour was seen by all nanoparticle batches over a period of 12 to 32 hours. Treatment for subarachnoid haemorrhage might benefit from such regulated and prolonged release behaviour. When compared to low concentrations of PLGA, melanin-NPs made from greater concentrations of PLGA demonstrated better regulated release of melatonin. After 24 hours of submerged activity, almost 50% of the melatonin in the Mel-NPs made from a 1:3 PLGA ratio was released. About 50 % of the drug was released from the optimized formulation (F6) (fig. 2).

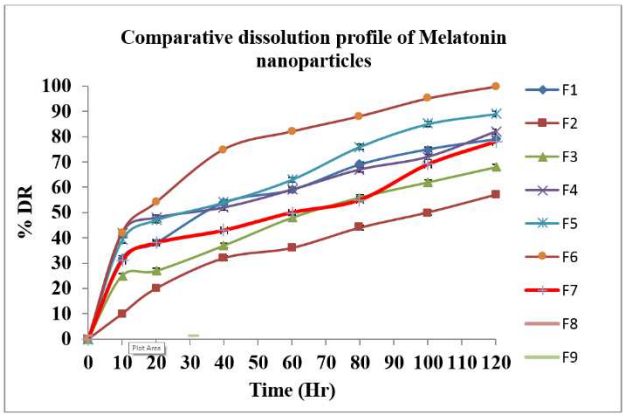

Fig. 2: Comparative release from batches of nanoparticles exhibiting superior sustain release characteristics

Animal study

In all, 35 rats were employed in this investigation. A total of 20 animals received endovascular SAH, 10 underwent SHAM surgery and 5 experienced cardiac arrest following anaesthesia induction, which prevented them from being included in the analysis. Animals with SAH grades below 15 were not included in the model since the smaller bleeds do not result in any mortality. The SAH grade distributions were identical for every group assessed at 24 hours. (table 3). Thirty milligrams per kilogramme of melatonin dramatically increased the 24-hour survival rate in rats that had made it through the first two hours of therapy. To find out if less brain edema was linked to a lower death rate, the brain water content of the 30 mg/kg group was assessed. A significant melatonin dose reduced increases in brain water content (table 3).

Table 3 presents the mean ± standard deviation (SD) values of brain water content in the right hemisphere, left hemisphere, and cerebellum of rats subjected to subarachnoid hemorrhage (SAH) or sham surgery.

Table 1: Formula for nanosuspension with variable concentration of polymer and stabilizer

	Drug (mg) (Melatonin)	Drug: PLGA ratio	Drug : Stabilizer ratio
F1	30	1:1	1:0.5 (PVK 30)
F2	30	1:2	1:1 (PVK 30)
F3	30	1:3	1:1.5 (PVK 30)
F4	30	1:1	1:0.5 (Poloxamer 188)
F5	30	1:2	1:1 (Poloxamer 188)
F6	30	1:3	1:1.5 (Poloxamer 188)
F7	30	1:1	1:0.5 (Tween 80)
F8	30	1:2	1:1 (Tween 80)
F9	30	1:3	1:1.5 (Tween 80)

Table 2: Physicochemical parameters for melatonin loaded PLGA nanoparticles

Batch Code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Yield (%)	91.11	92.14	90.65	94.15	95.16	96.56	93.56	93.56	93.76
Particle size (nm)	119	124	135	129	115	108	155	267	198
PDI	0.35	0.29	0.25	0.11	0.15	0.10	0.12	0.71	0.21
Zeta potential (mV)	18.11	21.16	25.23	20.58	20.33	20.02	20.31	20.01	20.87
EE (%)	77.89	89.18	81.20	93.21	94.20	98.95	93.71	93.11	91.21

Table 3: Brain water content in animals 24 hr after SAH

Group	n	Right Hemisphere	Left Hemisphere	Cerebellum	Mortality
SHAM surgery+ Vehicle	3	0.719±0.0442	0.711±0.0222	0.755±0.0154	0
SAH + vehicle	6	0.819±0.0112*	0.809±0.0455*	0.8419±0.2342	4
SAH+30 mg/kg	1	0.710±0.1242	0.743±0.0467	0.709±0.0321	3

Data are expressed as mean ± SD. Statistical comparisons were performed using one-way ANOVA (*p < 0.05 vs. SHAM + Vehicle).

Mortality data are also provided per group. Statistical comparisons were performed using one-way analysis of variance (ANOVA) to evaluate group-wise differences in brain water content. Comparisons were only made between the SHAM + Vehicle group (n = 3) and the SAH + Vehicle group (n = 6), as the SAH + 30 mg/kg group included only one animal (n = 1), precluding statistical analysis for that group. Statistically significant differences ($p < 0.05$) between the SHAM and SAH + Vehicle groups are indicated in the table by an asterisk (*).

DISCUSSION

Nano particles of Melatonin were prepared by high pressure homogenization method. Physicochemical parameters for the nanoparticles were evaluated and found within limit. Their particle size, PDI, zeta potential, surface morphology, encapsulation efficiency and in vitro release study were evaluated. The PDI is the most important assessment test for figuring out the homogeneity and dispersibility of nanosuspension. PDI for batches F4, F5 and F6 was found to 0.11, 0.15 and 0.12 amongst them, F4 and F5 was found to be unstable physically. Whereas, F1, F2 and F3 batches which are formulated with PVP K 30 showed milky solution and batches F7, F8 and F9 showed crystal formation that might result from Ostwald ripening (Vasava *et al*., 2015). Therefore, poloxamer188 in the ratio of 1:1.55 was chosen for additional research out of the three stabilisers that were being tested. One physical characteristic of nanosuspension is zeta potential. By measuring the particle velocity in an electrical field, the zeta potential can be found. Double layer theory states that there is a net balance of both repulsive and attractive forces in a colloidal system. But there won't be as much stability if the particles have low zeta potential levels. The optimised formulation (batch F6) was found to have a zeta potential value of 20.02 mV. Long-term storage of the suspension revealed additional signs of instability, such as sedimentation and particle agglomeration, even though the suspension's stability could be increased by adding stabilizers. The lyophilized powder was more physically stable than the suspensions and enhanced the transportability of the mixture (Zhang *et al*., 2018).

In vitro drug release study showed excellent sustained release behaviour all nanoparticle batches over a period of 12 to 32 hours. About 80% of the drug was released after three days. And complete release of the drug was observed in 120 hours and showed sustained release of the drug. Comparing the lower particle dimension from low polymer concentration to the substantially bigger particle dimensions from higher concentrated solution, where the reduced surface area of the bigger particle slows down drug diffusion, this raises surface area and promotes melatonin diffusion, which accounts for melatonin's rapid release.

The quick release of Melaonin can be explained by contrasting the lower particle dimension from low concentration of polymer, which results in higher surface area and facilitates Melatonin diffusion, drug diffusion is slowed down because of the larger particle's reduced surface area when it comes from a higher concentrated solution because of its comparably larger particle size (Pandey *et al*., 2015).

Animal study was performed on 35 rats, A significant melatonin dose reduced increases in brain water content. Melatonin treatment dramatically decreased mortality, and this was correlated with a decrease in brain water content 24 hours later. After SAH, brain edema is detected on CT in 6–8% of cases, and it is a reliable indicator of a bad prognosis and death. In the acute phase following SAH, reports of both cytogenic and vasogenic edema have been made (Claassen *et al*., 2002., Doczi, 1985). Development of edema after the worldwide cerebral ischemia that develops right after aneurysm rupture is what causes SAH. Treatment with melatonin has been associated with decreased cerebral edema after global ischemia (Kaur *et al*., 2006). Research has demonstrated that the administration of melatonin alleviated cerebellar edema by down regulating the expression of the proteins vascular endothelial growth factor (VEGF) and astrocytic aquaporin 4 (AQP4). Hypoxic environments cause an increase in VEGF gene and protein production, which is connected to the BBB's disintegration after SAH. Cerebral edema development has been linked to the perivascular astrocyte protein, AQP4, a water transport molecule; these proteins are also high in SAH (Yatsushige, *et al*., 2007). Brain edema was reduced by melatonin before as well as after middle cerebral artery occlusion (MCAO), with the biggest decreases occurring in regions with higher astrocyte densities. Melatonin administration after a cold-induced infarction was also observed to improve cerebral edema and the integrity of the blood brain barrier (Görgülü, *et al*., 2001). When taken as a whole, these studies show that melatonin helps to reduce SAH and cerebral edema. According to this study, melatonin may be useful in lowering brain edema after SAH.

Nano suspensions using nanoparticles enhance bioavailability and pharmacokinetics by improving solubility, absorption and systemic drug availability, particularly for poorly soluble drugs. They enable rapid action, targeted distribution, and controlled release while reducing first-pass metabolism and plasma fluctuations. These benefits result in prolonged therapeutic effects, optimized dosing, and improved patient compliance, making them highly effective for challenging drug formulations (Wang W *et al*, 2024). Melatonin when delivered via nano-suspension offers neuroprotective benefits beyond regulating SAH, with antioxidant, anti-inflammatory, and anti-apoptotic effects that mitigate brain injury. It protects the blood-brain barrier, reduces excitotoxicity, and promotes neurogenesis and synaptic plasticity, aiding recovery in conditions like TBI, stroke, and neurodegenerative diseases. Its safety, affordability, and broad potential make it a promising adjunct therapy, requiring further research for optimal dosing and clinical validation (Chuffa LG *et al*, 2021; Narang JK *et al*, 2025). When comparing melatonin and nimodipine for treating subarachnoid hemorrhage (SAH), both agents demonstrate unique mechanisms of action and similar therapeutic efficacy. Melatonin, an antioxidant and anti-inflammatory agent, reduces oxidative stress and neuroinflammation, critical factors in brain injury following SAH.

Encapsulated nanoparticles of melatonin offers improved bioavailability and sustained release, optimizing its effects with fewer side effects (Tozihi M.*et al*. 2023). Nimodipine, a calcium channel blocker, prevents vasospasm and enhances cerebral blood flow by reducing calcium overload in neurons, mitigating secondary brain injury (Liu J *et al*.,2022). While nimodipine is the standard treatment, melatonin has shown similar effectiveness in preclinical studies by targeting oxidative stress and inflammation. Pharmacokinetically, melatonin-loaded nanoparticles provide controlled release, reducing dosing frequency and enhancing brain delivery, whereas nimodipine requires frequent dosing due to its short half-life and limited bioavailability. Although nimodipine is generally safe, it can cause side effects like hypotension and dizziness with prolonged use. While nimodipine remains the first-line treatment for SAH, melatonin offers potential as an adjunctive therapy, addressing additional aspects such as oxidative stress and neuroinflammation. Both agents have demonstrated similar efficacy in preclinical models, with melatonin-loaded nanoparticles presenting a promising approach. Further clinical trials are needed, but early evidence suggests melatonin could be equally effective as nimodipine, especially when used in combination therapies (Vinge E *et al*, 1986; Morvaridzadeh M *et al*., 2020).

CONCLUSION

Our study's findings have indicated a new course for the management of SAH. When it comes to the viability of introducing medications with more carrier capacity, longer half-lives, reduced size, and improved cellular absorption in biological systems, nano capsulation technology is a very persuasive method. Therefore, melatonin in a medication delivery system nanocapsulated could be a promising treatment for subarachnoid haemorrhage.

ACKNOWLEDGEMENT

Authors are thankful to Department of Neurosurgery, The First Affiliated Hospital of Xi'an Medical University, Xi'an, China, 710000 for providing best of the facility to conduct this research work

REFERENCES

Andreasen TH, Bartek Jr J, Andresen M, Springborg JB and Romner B (2013). Modifiable risk factors for aneurysmal subarachnoid hemorrhage. *Stroke,* **44**(12): 3607-3612.

Ayer RE, Sugawara T and Zhang JH (2009). Effects of melatonin in early brain injury following subarachnoid hemorrhage. In Acta Neurochirurgica Supplements. Springer Vienna. pp.327-330

Choudhury MJH, Chowdhury MTI, Nayeem A and Jahan WA (2015). Modifiable and non-modifiable risk factors of stroke: A review update. *J Natl Inst Neurosci Bangladesh,* **1**(1): 22-26.

Chuffa LG, Seiva FR, Novais AA, Simão VA, Martín Giménez VM, Manucha W, Zuccari DA, Reiter RJ. Melatonin-loaded nanocarriers: New horizons for therapeutic applications. *Molecules.,* **26**(12): 3562.

Claassen J, Carhuapoma JR, Kreiter KT, Du EY, Connolly ES and Mayer SA (2002). Global cerebral edema after subarachnoid hemorrhage: Frequency, predictors and impact on outcome. *Stroke,* **33**(5): 1225-1232.

D'Souza S (2015). Aneurysmal subarachnoid hemorrhage. *J Neurosurg Anesthesiol* **27**(3): 222-240.

Doczi T (1985). The pathogenetic and prognostic significance of blood-brain barrier damage at the acute stage of aneurysmal subarachnoid haemorrhage. Clinical and experimental studies. Acta neurochirurgica, **77**: 110-132.

Feigin VL, Rinkel GJ, Lawes CM, Algra A, Bennett DA, van Gijn J and Anderson CS (2005). Risk factors for subarachnoid hemorrhage: An updated systematic review of epidemiological studies. *Stroke,* **36**(12): 2773-2780.

Gorgulu A, Palaoglu S, Ismailoglu O, Tuncel M, Surucu MT, Erbil M and Klnc K (2001). Effect of melatonin on cerebral edema in rats. *Neurosurgery,* **49**(6): 1434-1442.

Gourishetti K, Keni R, Nayak PG, Jitta SR, Bhaskaran NA, Kumar L, Kumar N, Krishnadas N and Shenoy RR (2020). Sesamol-loaded PLGA nanosuspension for accelerating wound healing in diabetic foot ulcer in rats. *J. Nanomedicine,* pp.9265-9282.

Kaur C, Sivakumar V, Zhang Y and Ling EA (2006). Hypoxia induced astrocytic reaction and increased vascular permeability in the rat cerebellum. *Glia,* **54**(8): 826.

Lawton MT and Vates GE (2017). Subarachnoid hemorrhage. *N. Engl. J. Med,* **377**(3): 257-266.

Liu J, Sun C, Wang Y, Nie G, Dong Q, You J, Li Q and Li M (2022). Efficacy of nimodipine in the treatment of subarachnoid hemorrhage: A meta-analysis. *Arquivos de Neuro-Psiquiatria.,* **80**: 663-670.

Longstreth Jr WT, Koepsell TD, Yerby MS and Van Belle, G (1985). Risk factors for subarachnoid hemorrhage. *Stroke,* **16**(3): 377-385.

Mirza-Aghazadeh-Attari M, Mihanfar A, Yousefi B and Majidinia M (2022). Nanotechnology-based advances in the efficient delivery of melatonin. *Cancer Cell Int,* **22**(1): 43.

Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, Persad E and Heshmati J (2020). Effect of melatonin supplementation on oxidative stress parameters: A systematic review and meta-analysis. *Pharmacol. Res,* **161**: 105210.

Mulam TR, Kshirsagar SJ and Kakad SP (2021). Formulation and Optimization of Ritonavir Nasal Nanosuspension for Brain Targeting. *Ind. Drugs,* **58**(4): 28-41

Narang JK, Dogra A, Kaur T, Narang RS and Singh AP (2025). Antioxidants Against Neurological Disorders. *Antioxidants: Nat. Def. Dis,* **27**: 285-367.

Pandey SK, Haldar C, Vishwas DK and Maiti P (2015). S ynthesis and in vitro evaluation of melatonin entrapped PLA nanoparticles: A n oxidative stress and T ‑ cell response using golden hamster. *J. Biomed. Mater. Res. Part A,* **103**(9): 3034-3044.

Qureshi AI, Suri MFK, Yahia AM, Suarez JI, Guterman LR, Hopkins LN and Tamargo RJ (2001). Risk factors for subarachnoid hemorrhage. *Neurosurgery,* **49**(3): 607-613.

Teunissen LL, Rinkel GJ, Algra A and Van Gijn J (1996). Risk factors for subarachnoid hemorrhage: A systematic review. *Stroke,* **27**(3): 544-549.

Tozihi M, Shademan B, Yousefi H, Avci CB, Nourazarian A, Dehghan G. Melatonin: A promising neuroprotective agent for cerebral ischemia-reperfusion injury. *Front. Aging Neurosci,* **15**: 1227513.

Vasava SS, Chotai NP and Patel HK (2015). Formulation And Evaluation of Nanosuspension Drug Delivery System Of Etoricoxib. *Pharma Science Monitor,* **6**(1):

Vinge E, Andersson KE, Brandt L, Ljunggren B, Nilsson LG and Rosendal-Helgesen S (1986). Pharmacokinetics of nimodipine in patients with aneurysmal subarachnoid haemorrhage. *Eur. J. Clin. Pharmacol,* **30**: 421-5.

Wang J, Gao S, Lenahan C, Gu Y, Wang X, Fang Y, Xu W, Wu H, Pan Y, Shao A and Zhang J (2022). Melatonin as an antioxidant agent in stroke: An updated review. *Aging Dis,* **13**(6): 1823.

Wang W, Yang C, Xue L and Wang Y (2024). Key Challenges, Influencing Factors and Future Perspectives of Nanosuspensions in Enhancing Brain Drug Delivery. *Curr. Pharm. Des,* **30**(32): 2524-2537.

Xu C, He Z and Li J (2022). Melatonin as a potential neuroprotectant: mechanisms in subarachnoid hemorrhage-induced early brain injury. *Front. Aging Neurosci,* **14**: 899678.

Yatsushige H, Ostrowski RP, Tsubokawa T, Colohan A and Zhang JH (2007). Role of c-Jun N-terminal kinase in early brain injury after subarachnoid hemorrhage. *J. Neurosci. Res,* **85**(7): 1436-1448.

Zhang Y, Fei S, Yu M, Guo Y, He H, Zhang Y, Yin T, Xu H and Tang X (2018). Injectable sustained release PLA

microparticles prepared by solvent evaporation-media milling technology. *Drug Dev. Ind. Pharm,* **44**(10): 1591.