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Research Article

Optimization of Lipid-Based Ketoconazole Nanoformulations Through Stirring Techniques and Ingredient Quantity Adjustments

Muhammad Irfan[1,2], Mir Azam Khan[*1], Aziz Ur Rahman[1], Faiza Naureen[3]

[1]Department of Pharmacy, University of Malakand, Chakdara, Pakistan.
[3]Swat College of Pharmaceutical Sciences, Swat, Pakistan.
[2]Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan.
*Correspondence: mirazam786@yahoo.com

Abstract

The Biopharmaceutical Classification System (BCS) classifies drugs on the basis of solubility and permeability. Ketoconazole belongs to class II drugs of the Biopharmaceutical Classification System (BCS) which are characterized by high permeability but low solubility issues. The current research work aimed to formulate optimized nano-formulation for ketoconazole by altering stirring time, speed, and ingredient ratios. In this study, a stirring speed minimum of 600 rpm and a maximum of 1200 rpm was used. The minimum stirring time of 7 minutes and a maximum of 30 minutes was noted. Olive oil was used as a lipid, Tween 80 was incorporated as a surfactant, and polyethylene glycol (PEG 6000) was used as a co-surfactant. Different ratios of Surfactants and Co-surfactants were used. Formulations with different particle sizes and polydispersity index were obtained. The quantity of surfactant (tween 80) used was 1.5g, the Co-surfactant (polyethylene glycol) was 0.4g, the lipid used was 1g, and the drug quantity was 0.12g in the optimized formulation (KZME 1). The stirring time applied for optimized ketoconazole nano-formulation was 15 minutes and a stirring speed of 800 rpm was applied while keeping the drug quantity at 120 mg. The optimized ketoconazole nano-formulation obtained has a particle size of 50.29nm and a Polydispersity index of 0. 377. It was observed that optimized and stable ketoconazole nano-formulation can be achieved with the help of proper stirring speed and time which can improve the solubility of ketoconazole drug, and can lead to an increase in the bioavailability and therapeutic efficacy of ketoconazole.

Keywords: Ketoconazole, ingredients ratios, surfactants, particle sizes, polydispersity index

1. Introduction

A Several techniques are used to improve the solubility of drugs. Some of these include bottom-up approaches (sol-gel technique, spinning method of nanoparticles synthesis, pyrolysis process, biosynthesis method) and top-down approaches. In bottom-up synthesis, materials are manufactured from the atomic to the cluster and then the nanoparticle level. The sol-gel technique is a highly adjustable soft chemical process that is widely utilized in the synthesis of metal oxides, ceramics, and glasses. One of the several benefits of this method is that it can be used to create metal and ceramic nanomaterial at temperatures between 70 degrees Celsius and 320 degrees Celsius, increasing the range of materials that may be treated (Li, Wang et al. 2003, Jaroenworaluck, Sunsaneeyametha et al. 2006, Vijayalakshmi and Rajendran 2012, Verma, Mantri et al. 2015) attaining atomic-level consistency in the final product, compositional control at molecular-scale, and porosity to get high-surface-area materials. A spinning disc reactor is the equipment which is used in the spinning method of nanoparticle synthesis. A disc rotates inside a chamber/reactor whose physical properties, like temperature, can

Table 1: **Fabrication of blank lipid nanoparticle (blank/unloaded mean without drug).**

Formulation Name	Olive Oil (g)	PEG 6000 (g)	Tween 80 (g)	Stirring time(minutes)	RPM (Revolution Per minute)	Particle Size (nm)
UME -1	1	0.2	1	15	800	37
UME -2	1	0.2	1	10	800	53.3
UME -3	1	0.4	1.5	15	800	47.4
UME -4	1	0.4	1.5	15	900	30.1
UME -5	1	0.4	1.5	10	1200	29
UME -6	1	0.4	1.5	07	1200	35
UME -7	1	0.4	1.5	30	600	46
UME -8	1	0.4	1.5	10	1000	33

be adjusted. Inert gases such as Nitrogen or others are commonly used to purge the reactor of oxygen and prevent chemical reactions (Hassellöv, Readman et al. 2008). Pyrolysis method is one of the thermochemical processes in which the substance is broken down into smaller particles which involves heating the material to high temperatures in the absence of oxygen. This is a standard technique that is used for mass production of nanoparticles in the industry. Pyrolysis has a high yield and simple, efficient, cost-effective continuous process. However, it is challenging to attain the required size of the nanoparticles using this method because as the nanoparticles leave the hot chamber, they attempt to aggregate and nanoparticle chains are formed (Dhand, Dwivedi et al. 2015). Nanoparticles that are safe for humans and the environment can be manufactured by the biosynthesis method. Compared to the use of chemicals and physical processes, the production of NPs using biological sources (such as bacteria, actinobacteria, yeast, fungi, algae, and viruses) is safer (Singh, Kim et al. 2016, Fariq, Khan et al. 2017, Saravanan, Barik et al. 2018).

In the top-down or destructive approach, the materials are broken down into their atomic building blocks. By using this method, large parts of material can be broken down into smaller, nano-sized pieces. A major disadvantage of this method is the challenge of producing particles with the right size and shape. Common techniques for nanoparticle production in this method include mechanical milling, nanolithography, laser ablation, sputtering, and thermal decomposition. Nano-emulsion approach is one of them.

Nano-emulsions have small droplet sizes and they are kinetically stable colloidal systems. They are thermodynamically stable isotropic systems in which two immiscible liquids are mixed together and form a single phase by means of appropriate surfactant and cosurfactant. They have improved functional properties in comparison to conventional emulsions. The composition and structure of the nano-emulsions can be controlled for the encapsulation and delivery of bioactive lipophilic compounds effectively(Malode, Chauhan et al. 2021). Theoretically, smaller particle size, greater surface area, and higher adhesion to the biomembrane are the key factors, which could enhance the oral bioavailability of nanoparticles through better intestinal permeability(Danhier, Ansorena et al. 2012). To develop nanoparticle formulation, various

Table 2: Effect of Ketoconazole concentration on nanoformulation particle size and polydispersity Index (PDI)

Formulations Name	Olive Oil (g)	PEG 6,000 (g)	Tween 80 (g)	Stirring time minutes	Stirring Speed rpm	Drug quantity (g)	PDI	Particle Size (nm)
KZME 1	1	0.4	1.5	15	800	0.12	0.37	50.29
KZME 2	1	0.4	1.5	15	800	0.1	0.931	64.9
KZME 3	1	0.4	1.5	15	800	0.08	0.672	56.17
KZME 4	1	0.4	1.5	15	800	0.06	0.85	56.05
KZME 5	1	0.4	1.5	15	800	0.05	0.946	72.62
KZME 6	1	0.4	1.5	15	800	0.14	1	57.7

preparative parameters, such as the amount of polymer and surfactant, should be taken into consideration because these parameters affect the resulting particle size, distribution, encapsulation efficiency, and zeta potential, which are critical factors for the dissolution rate, permeability, and stability.

For many drugs low solubility and bioavailability is one of the major problems (Khan and Singh 2016). The stirring rate plays an important role to control and optimize the average diameter of microspheres (Mateovic, Kriznar et al. 2002). The average diameter decreases as the stirring rate increases (Mateovic, Kriznar et al. 2002). This can be explained by the production of a finer dispersion of droplets when higher stirring rates are applied and, consequently, by the formation of smaller microspheres (Freitas, Merkle et al. 2005).

Currently, nanomedicines have been studied extensively for enhancing the bioavailability of poorly water-soluble drugs (Kumar, Dilbaghi et al. 2012). Improvement in nanoparticle formulation of the same shape, composition, and size has brought novelty in the field of Nano-Sciences (Revaprasadu and Khan 2024). For targeting drugs at the secondary and tertiary levels, the drug assimilation into the nanocarrier system gives a new prototype for the delivery of drugs. Drug delivery systems based on lipids have attracted the interest of researchers for the past few years. In the Pharmaceutical nanotechnology field, Lipid-based Nanoparticles(LBN) have gained importance due to their many prospective uses in pharmaceutical drug delivery systems (Swidan, Mansour et al. 2018). For enhancement of bioavailability and targeted drug delivery of Biopharmaceutical Classification System Class II drugs (High Permeability and low solubility), lipid nanoparticles are being extensively used (Food 2014). Lipid-based Nanoparticles promote distinct opportunities for the growth of novelty to cure disorders because of their unique size (Badawy, Ghorab et al. 1996, Pathak and Raghuvanshi 2015). Lipid-based Nanoparticles in oral dosage form reduce hepatic first-pass metabolism by theoretically enhancing lymphatic transport and thus increasing oral bioavailability (Borin and Ayres 1989, Food 2014). Lipid-based nanoparticles are non-biotoxic for *in vitro/in vivo* use and as such active progression has been made in the field of lipid-based DNA/RNA nanocarriers(Duan, Dhar et al. 2020). Most lipids used to formulate lipid nanoparticles are biodegradable and biocompatible having few adverse side effects and chronic toxicity(Xu, Wang et al. 2022). In the

Table 3: Effect of various parameters for Ketoconazole loaded nanoformulations on polydispersity index.

Formulations Name	Olive Oil (g)	PEG 6,000 (g)	Tween 80 (g)	Drug quantity (g)	Stirring time minutes	Stirring Speed rpm	PDI	Particle Size (nm)
KZME 7	1	0.2	1	0.12	*15*	800	1	49.4
KZME 8	1	0.2	1	0.12	*10*	800	1	65.7
KZME 11	1	0.4	1.5	0.12	10	*1200*	0.4	39
KZME 13	1	0.4	1.5	0.12	10	*1000*	0.08	44
KZME 9	1	0.4	1.5	0.12	*15*	*900*	0.9	40
KZME 10	1	0.4	1.5	0.12	*30*	*600*	1	57
KZME 12	1	0.4	1.5	*0.1*	*07*	*1200*	1	48

current investigation, we used several stirring speed and time combinations and tried to formulate an optimized nano-formulation for ketoconazole by altering stirring time, speed, and ingredient ratios.

2. Materials & Methods

2.1. Chemicals/Drugs

Active drug (Ketoconazole) was a kind gift by Atco Laboratories Limited Karachi, Pakistan, Tween 80, Polyethylene glycol 6,000 were supplied by Scientific traders, and manufactured by VMR International Belgium. Olive oil was obtained from Buraq Scientific traders. Deionized water, ultraviolet light UV-254 treated for laboratory use only was purchased from Haqq Chemicals.

2.2. Equipment

2.2.1. Zeta Sizer

Malvern Zeta sizer (UK) Nano ZS-90, was used to determine Particle Size, Polydispersity Index (PDI), and Zeta Potential.

2.2.2. Digital Weighing Balance

Electronic digital balance by WELAB was used to measure accurately the required weight for formulations.

2.2.3. Centrifuge

Centrifuge Model 5415 D Made in Germany, was used for Centrifugation at 10,000 rpm.

2.2.4. Vortex Mixer

The vortex mixer that was used for redispersion was from Thermo-Scientific USA. It is used for mixing precipitate after centrifugation.

2.2.5. Magnetic Stirrer

Magnetic stirrer uses centrifugal force for the fabrication of Lipid Nanoparticles. The GR BIOTEK magnetic stirrer was used.

2.3. Research Methods

Different formulations were formulated through the micro-emulsion method by using different concentrations of surfactant, co-surfactant), drug, and olive oil by changing stirring time and speed. While preparing the blank formulation organic phase, Olive oil(lipid) was warmed and PEG 6,000(Co-surfactant) was melted at 75 °C, 5°C above its melting point. For the preparation of the aqueous phase, tween 80(surfactant) was dissolved in water by stirring. The organic phase was then mixed with the aqueous phase, stirring was started and cold water in small quantities was added dropwise to obtain nano-emulsion. The best formulation in terms of particle size and PDI was selected from blank formulations and was loaded with ketoconazole drug by using the same method. The ketoconazole-loaded nano-formulation centrifugation was done at 10,000 rpm for 15 minutes. The supernatant was filtered and the precipitate was then redispersed in water with the help of a vortex mixer for Particle size, PDI (Polydispersity Index), and Zeta Potential, and the optimized nanoformulation obtained

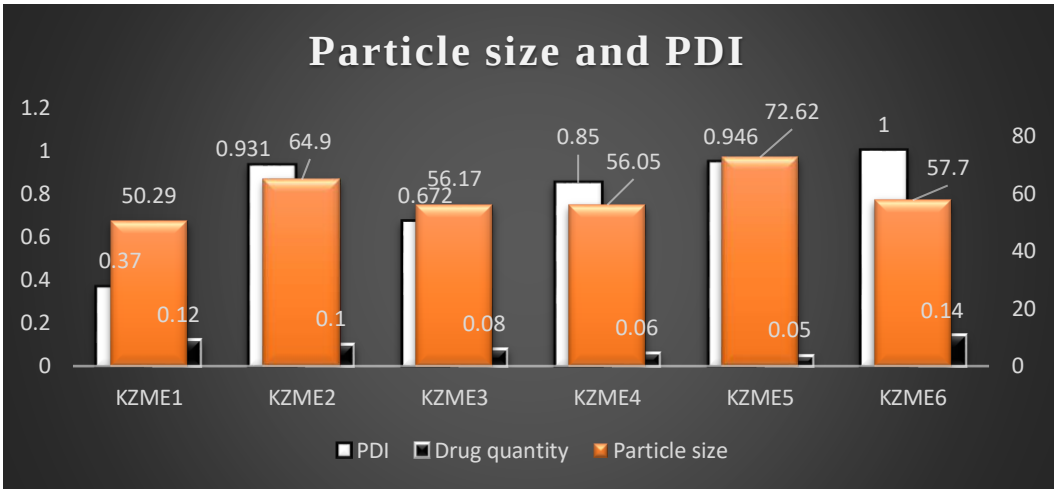

Figure 1: ketoconazole nanoformulation drug concentration effect on polydispersity index and particle size.

from loaded formulation was subjected to further studies(characterization).

2.4. Preparation & Optimization of Nanoemulsion

For the fabrication of lipid nanoparticles, Olive oil was used as a lipid, Tween 80 as a surfactant, and PEG 6,000 as a cosurfactant(Cannon and Long 2018). Olive oil in specific concentrations provides long-term stability to nanoemulsion in terms of particle size(Ren, Dong et al. 2018). During the fabrication process, different parameters were varied for optimization i.e., amount of drug, surfactants (Tween 80), stirring time/speed, and amount of co-surfactants (PEG 6000)(Midha, Nagpal et al. 2017). While comparing the two formulations, one parameter from the above was changed in most formulations and the rest were constant. After selecting the best blank formulation (Table 1), ketoconazole-loaded lipid nano-formulations were prepared and optimization of the formulation was done by determining particle size, Polydispersity index (PDI) (Table 2), and finally by Zeta Potential.

3. Results & Discussion

3.1. Particle Size Analysis

Different blank nanoformulations were formulated as mentioned in Table 1. After selecting the best blank formulation (UME-3) in terms of particle size and polydispersity index, it was loaded with ketoconazole drug (KZME1), which showed low particle size with a size range of 50.29 nm, Poly dispersity index PDI 0.377, and zeta potential of -17.2mv as shown in Table 2 and (figure 1, 2 and 3).

3.2. Surfactant Amount

To stabilize nanoformulation, a specific quantity of stabilizer (surfactant) is required which prevents aggregation of nanoformulation (Pathak, Pattnaik et al. 2018). The surfactant (Tween 80) is a non-ionic surfactant and known for its safety, non-toxic nature, and non-irritating chemicals(Hvattum, Yip et al. 2012). The ratio of Tween 80 was increased from (1 to 1.5) for different formulations but the optimized formulation was achieved at 1.5gm (KZME-1).

3.3. Co-surfactant Concentration

Polyethylene glycol (PEG 6000) was selected as a co-surfactant which has both the properties of a polymer and a surfactant. Ultralow IFT(interfacial tension) value at the oil-middle emulsion phase proposes the appropriateness of PEG 6000 for emulsion preparation (Kumar and Mandal 2020). The amount of co-surfactant (PEG 6000) for the optimized formulation was 0.4 gm. It was observed that with a decrease in concentration of co-surfactant i.e., 0.2gm, the particle size tends to increase UME-1 and UME-2.

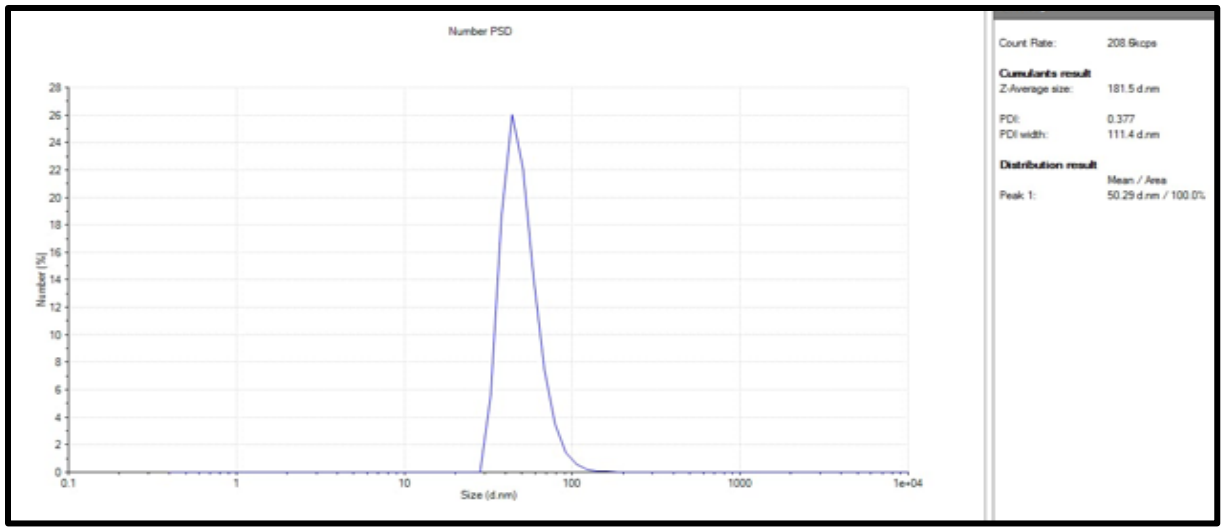

Figure 2: Zeta size of optimized Nano formulation (KZME1)

3.4. Stirring Time and Speed

The stirring time applied for optimized ketoconazole nano-formulation was 15 minutes and a stirring speed of 800 rpm was applied while keeping the drug quantity at 120 mg. With an increase in the stirring speed, the droplet size decreases. The homogenization technique is a method that uses an overhead stirrer. In this homogenization technique, the nanoformulation was only exposed to a simple stirring procedure. The droplets are not subjected to a high shear stress, which could break them down significantly. Stirring helps to homogenize and increase nano formulation spreadability. This results in a small droplet size for the nanoformulation when the stirrer speed increases (Roselan, Ashari et al. 2020).

While changing stirring time all other parameters were kept constant for blank formulations, except in two formulations UME -7 and UME-8 as given in Table 1, where both stirring time and speed were changed. The 15-minute stirring time was best for achieving optimized nanoformulation as shown in Table 1 and Table 2. The best optimized blank formulation (UME-3) obtained has Olive oil (1g), Tween 80 (1.5g), and PEG (0.4g), stirring time of 15 minutes, and stirring speed =800 rpm. In Table 2, from KZME1 to KZME 6, all other parameters were constant but only drug quantity was changed from i.e., 0.12g, 0.1g, 0.08g, 0.06g,0.05g, and 0.14 respectively. Table 2 shows that with drug quantity 0.12g, the particle size is 50.29nm having desirable PDI, while increasing or decreasing the drug quantity from 0.12 g, the particle size was changed in a non-uniform manner and the PDI was disturbed i.e., increased with decreased drug quantity, as shown in figure 1. Therefore, KZME1 is our optimized loaded nano formulation having desirable PDI =0.37 and Particle size =50.29.

The formulation of nanoemulsions involved careful consideration of the ingredients and method used (Visht, Sirwan Salih et al. 2024). In this formulation, we used Tween 80, as a surfactant. Tween surfactants are widely used in oil dispersants, emulsifiers, solubilizers, and protein-blocking agents (Li and Fu 2024). We use a co-surfactant PEG-6000 to further stabilize the nanoformulation. PEG-6000 is a suitable stabilizer both alone and in combination (Taher, Al-Kinani et al. 2022). The stirring method is one of the simple methods for particle size reduction and for any optimized formulation a suitable time and speed of stirring is required(Liao, Wei et al. 2024). It is confirmed from the above study as given in Table 2 (KZME 1) that both a stirring time of 15

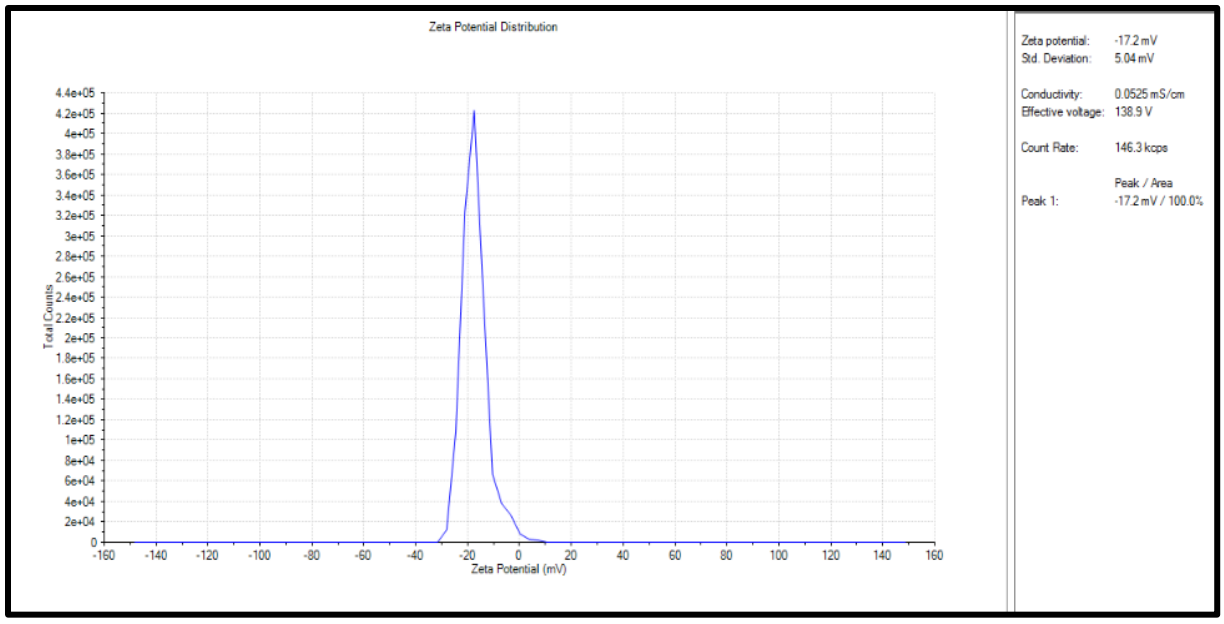

Figure 3: Zeta Potential of optimized Nano formulation (KZME1).

minutes and a stirring speed of 800 rpm are effective for optimized and stable ketoconazole nanoformulation in terms of particle size, PDI, and Zeta potential. It is also concluded from this study that PEG and Tween 80 have an important role in PDI because the formulation (KZME 7) as given in Table 3 has PEG 0.2g and Tween 80, 1g, which were not effective for desirable PDI. However, formulation KZME1 in Table 2 which has PEG 0.4 and Tween 80= 1.5 g showed desirable PDI. It also showed that olive in a specific concentration reduces Ostwald ripening and stabilizes the nanoemulsion along with PEG further. Hence specific quantity of Surfactant Tween 80, Co-Surfactant PEG 6,000, drug, stirring time, and speed is necessary for Ketoconazole nanoparticle production.

4. Conclusion

Ketoconazole nanoformulation was fabricated by using a stirring method which can improve the solubility of ketoconazole drug. The optimum stirring time for ketoconazole is 15 minutes while maintaining stirring speed at 800 rpm. Tween 80, 1.5 g and PEG 6000, 0.4 g are necessary to stabilize the ketoconazole nanoformulation while keeping the drug quantity 120mg and olive oil 1g. Further studies are required to fabricate an optimized nanoformulation for ketoconazole drug using a minimum quantity of drug with improved solubility and bioavailability while maintaining specific parameters.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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Study Approval

There are no animal/human subjects involved so, this study requires no institutional or ethical review board approval.

Consent Forms

NA.

Authors Contributions

MI conceptualized the study and wrote the final manuscript, AUR, and FN helped with the

literature search and analysis and writing the first draft, MI did the literature search and review of the studies, and MAK supervised the whole project.

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