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RESEARCH ARTICLE

DMPK studies in rat model for comparative evaluation of bioavailability of alpha-mangostin and its formulated solid lipid nanoparticle using a validated LC-MS/MS method

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Abstract

Garcinia mangostana L., contains the xanthone α-mangostin, which is a bioactive secondary metabolite. The Caco-2 cell line transport of α-mangostin was explored to see whether it could be used to study oral uptake. There has been little *in-vivo* research on the drug metabolism and pharmacokinetics of solid lipid nanoparticles of α-mangostin. The α-mangostin content estimation in plasma of rat was accomplished using a validated LC-MS/MS technique. The P_{app} (permeability coefficient apparent) across the Caco-2 cell monolayer is used to predict the absorption of orally administered α-mangostin and α-mangostin solid lipid nanoparticles (AM-SLNP). In the presence of the solid lipid and emulsifiers, AM-SLNP had 3.72 times higher Papp than α-mangostin after 4 hours of study across the Caco-2 cell line. *In-vivo* rat model study show that formulated AM-SLNP has a 3.3-fold higher bioavailability than pure α-mangostin. High tissue distribution of the AM-SLNP is observed compared to α-mangostin, which may improve the efficacy of the product when compared to pure extract, as the available drug at the site of distribution is high. Because both cell monolayer and animal studies demonstrate the same pattern of drug intake mechanism for SLNP's and as it is almost identical, nanotechnology can be utilized in avoiding hepatic metabolism and improving bioavailability.

Keywords

alpha-mangostin; solid lipid nanoparticles; LC-MS/MS method; Caco-2 cell line study

Introduction

Plant-based products have been used to cure a variety of diseases since decades. Herbal drugs have always depended on traditional knowledge which might or might not be substantiated by empirical evidence. In recent years, the natural products have gained the popularity due to their safety profiles and poly-pharmacological activities compared to the synthetic compounds. Nutraceutical product information and their market are expanding widely, which has boosted their usage in everyday life. There is no common regulatory structure in place in most countries to ensure the safety and efficacy of herbal drugs. The efficacy of the herbal compounds was not proven by a scientifically validated method. However, in recent years the evidences of the therapeutic and harmful effects of herbal medicines are available due to efforts of various researchers across the globe. Many novel active phytochemicals were discovered, and the list is still expanding. The

pharmacological rationale for the herbal medicine's effectiveness was never fully elucidated. The subject of bioavailability is especially interesting since it may be used to determine how much and how quickly chemicals are absorbed following herbal compound administration. The analysis of elimination pathways and their kinetics, as well as the understanding of metabolic pathways, are also of interest. Data from pharmacological tests and clinical outcomes are becoming increasingly essential. A better knowledge of phytochemicals pharmacokinetics and bioavailability can also aid in the development of appropriate dose regimens. Studies for some of the more significant or extensively used herbal ingredients like alpha-mangostin (α-mangostin) are critically examined in this study. In addition, different medication interactions are described, demonstrating the need for caution when using phytopharmaceuticals with chemically produced active pharmaceutical components.

The Clusiaceae family tropical fruit tree *Garcinia mangostana* L., is commonly referred to as mangosteen. It is said to have originated in Southeast Asia with Thailand, Myanmar, Malaysia, Indonesia and Singapore being the most common locations (1). The aerial parts (fruit, leaves, and bark) of the mangosteen tree have been traditionally used in the treatment of a range of ailments including wound healing, arthritis, diarrhoea, inflammation, skin diseases and dysentery (2,3). Extract of mangosteen pericarp is found to have anti-oxidant (4-7), cytotoxic (8), anti-inflammatory (9), anti-bacterial (10), anti-fungal (11), anti-viral (12, 13) and anti-cancer activities *in-vitro* (14, 15). Xanthone derivatives are the primary active components in mangosteen and α-mangostin was the first to be identified (16-18). The tricyclic aromatic ring structure of α- mangostin like that of other xanthone compounds (19) is found in the dried pericarp extract of mangosteena up to 5.5 mg/g concentration (20). All the pharmacodynamic properties of mangosteen extracts have been documented for α -mangostin (21-24). However, *in-vitro* studies were used to obtain the majority of these data. Only a handful of effects have been proven in animal research (21, 25, 26). The degree of therapeutic implications of these pharmacological effects is still the matter of concern. *G. mangostana* extract and pure components obtained have been successfully marketed as herbal supplements due its promising *in-vitro* evidence. Juice of the fruit or pills produced from its extract or ingredients are available from online and other herbal outlets. . Products in different formulations such as juice, extract and in its pure form are available from both online and other herbal outlets . Quantitative data on α-mangostin drug metabolism and pharmacokinetics (DMPK) parameters are needed to assess the purity, tolerability and efficiency of mangosteen pericarp extract. However, there has been only one publication on the bioavailability of α-mangostin in humans following administration of different xanthone derivatives in the extract (27).

The study aims to formulate solid lipid nanoparticles (SLNPs) and evaluate its bioavailability (BA) in comparison with pure extract. Stearic acid and Precirol ATO5 (Solid lipids), Poloxamer 407, and sodium taurocholate (emulsifier and co-emulsifier) are used as additives in the preparation of SLNPs *via* hot melt homogenization followed by ultrasonication. The objective of the research was to determine the pharmacokinetics (PK) of α-mangostin in a rat model following oral administration of both formulated and non-formulated α-mangostin. Because metabolite concentrations in plasma and tissue samples are so low, a precise and accurate method is required to identify them. In the current investigation, a simple and rapid approach with triple quadrupole mass spectrometer (LC-MS/MS) analysis was shown to be a suitable instrument for determining α-mangostin concentrations in rat biological samples. The Caco-2 cell line transport of α-mangostin was explored to see whether it could be used to study oral uptake. In Sprague Dawley rats, PK analysis for the biological content estimation of α-mangostin analyte was done using Sprague Dawley rats by administering α-mangostin extract and AM-SLNP orally and further analysis was carried out using a validated LC-MS/MS technique. α-mangostin SLNPs transfer was also observed to be better than the pure α-mangostin across cell lines (Caco-2). The technique of hot melt homogenization followed by ultrasonication resulted in a 3.33 fold increase in AUC in a PK investigation. Formulation of AM- SLNP's showed better oral BA compared to pure extract of α-mangostin.

Materials and Methods

Materials

α-mangostin extract was procured from Laila Nutraceuticals (Andhra Pradesh, India). Caco-2 cell lines were purchased from BTL Biotechno lab Pvt. Ltd. (New Delhi, India). All the chemicals and reagents were of analytical grade and obtained commercially.

Method

LC-MS/MS method development and validation

LC-MS method for estimation of A-mangostin in different studies

Water "ACQUITY UPLC" BEH C18 Column chromatography (Milford, USA) and Water XEVO TQ-S, Mass Lynx version 4.1 software, Scan type-MRM (Milford, USA) were used to evaluate the samples. Each sample was injected 5µL of fluid. The analyte was eluted by chromatography on a Kinetex C_8, 1.7µm 100x2.1mm column with a flow rate of 0.3 mL/min and a 5-minute run time. Eluent A (0.1% formic acid with 10 mM ammonium formate buffer solution) and Eluent B (acetonitrile) were mixed in a ratio of 30:70 v/v in the mobile phase. The retention time (RT) for α-mangostin was found to be 1.86 ± 0.3 min. At an ion transition of 411.1595 m/z, the α-mangostin was measured using multiple reactions monitoring (MRM). The electrospray ionisation needle was charged in the positive-ion mode for the analysis. The ionization source temperature was held at 150±1°C. The capillary potential was held at 2.11kV, the nebulizer gas flow was held at 6.49 bars, and the desolvation gas flow was held at 800 L/hr. Collision triggered dissociation with nitrogen gas was used to

fragment α-mangostin molecules. The collision potential was set to 26.0 V and the collision gas flow was found to be 0.15 mL/min. We utilized a 0.200 second dwell time. Linearity was established across the concentration range of 5.66 to 5103.54 ng/mL, and they were determined to be good and reproducible. The current study's accuracy data varied from 98.17 to 101.78 %, indicating that no endogenous plasma or other components interfered with the results. The proposed approach is very precise and accurate, as evidenced by R.S.D. values of less than 10.0 in both inter and intra-day duplicates of α-mangostin.

Preparation of stock solutions, calibration and validation standards

α-mangostin was weighed exactly and mixed with 5 mL of mobile phase in a 10 mL flask. With the help of the mobile phase, a stock standard solution comprising 1000 µg/mL was obtained. Working standard solutions were made by diluting the stock solution with the mobile phase to concentrations ranging from 5.66 to 5103.54 ng/mL. At 5.66, 12.153, 51.709, 290.651, 944.719, 1857.17, 3996.972, and 5103.542 ng/mL, eight calibration standards were created. At 57.60, 288, 1920, 3840, and 4800 ng/mL, validation standards were created in the same way.

Plasma sample preparation

Extracting plasma

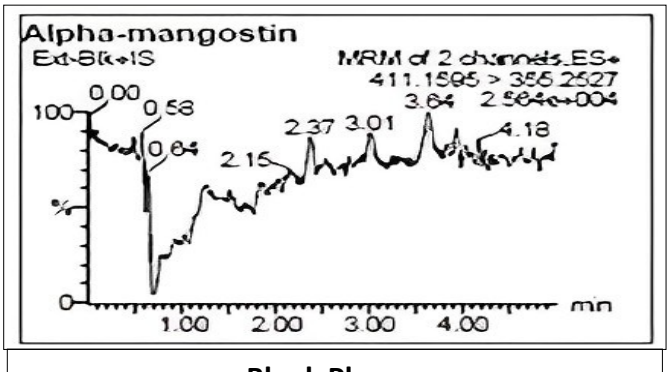
In a vial, 25 µL of plasma sample, followed by 10 µL of formic acid and 0.400 mL of acetonitrile were added. The resultant mixture was vortexed and centrifuged for 10 minutes. Supernatant was collected and transferred to a centrifuge tube, diluted with the mobile phase and then injected into LC-MS for analysis of extracted sample.

The effectiveness of extraction

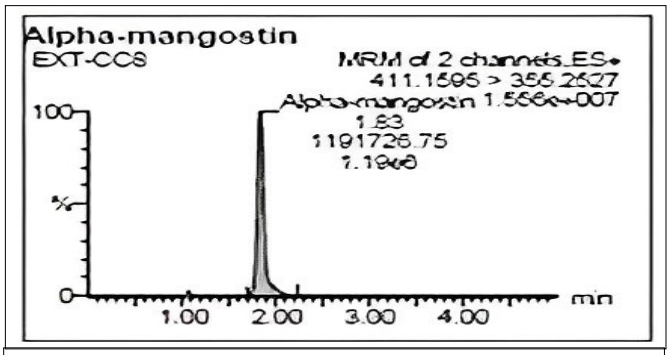
By adding a definite amount of α-mangostin to plasma, the extraction efficiency was determined. In LC-MS, a known concentration of plasma sample was injected, and the peak areas of the samples with and without plasma were evaluated.

Validation

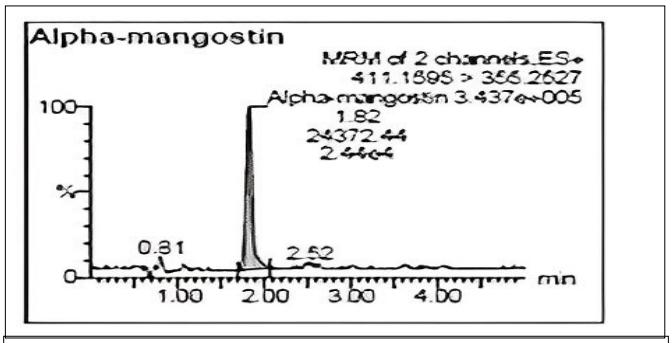
As shown in Figure-1, by comparing chromatograms of blank plasma, reference, α-mangostin, and AM-SLNP samples, the specificity of the devised technique for plasma samples was evaluated. The most important parameter in method validation is the recovery study, which necessitates extra vigilance during the investigation and interpretation of recovery outcomes. As a consequence, the accuracy of study findings was evaluated. Sensitivity is defined as the limit of detection and quantification.



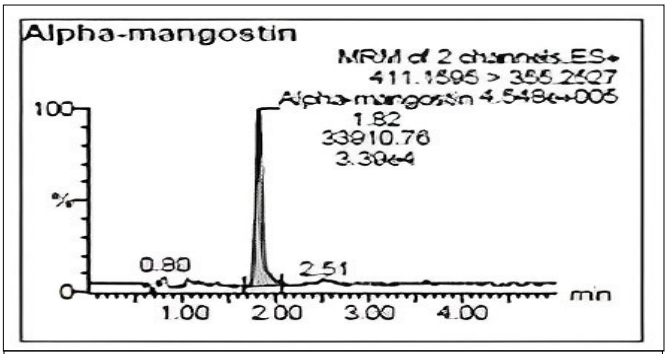
Blank Plasma



Standard Plasma



A—Mangostin Extract Plasma



MSLNP Plasm

Fig. 1. Chromatogram of Blank, Standard A-mangostin, A-mangostin and AM -SLNP Plasma.

α-mangostin solid lipid nanoparticle preparation

To formulate α- mangostin SLNPs, hot melt homogenization followed by ultrasonication was utilized. Briefly, solid lipid (2:3 ratio of stearic acid to Precirol ATO5) was heated to a melting temperature of 60–65°C (approximately 5°C above the solid lipid melting point) in order to produce SLNPs. The liquefied lipid was mixed for 5 minutes at high rpm using a high-speed stirrer (15,000 rpm). The required amount of α-mangostin (1 part) was added to the molten substance while being constantly stirred at the same temperature and the mixture was thoroughly mixed for an additional 5 to 10 minutes. Poloxamer 407-Sodium Taurocholate (1:1) surfactant and co-surfactant were mixed in distilled water, heated to the lipid phase temperature, added to the molten lipid, and homogenised at 15,000 rpm for 30 minutes to create a coarse emulsion.

The pre-emulsion was then sonicated at 50% amplitude for 3 minutes, six times for 30 seconds each, with 1-minute breaks in between. The emulsion was then lyophilized using mannitol as a cryoprotectant for 24 hours in a freeze dryer.

α-mangostin and AM-SLNP Caco-2 cell lines transport

Cell line characteristics

Caco-2 cells are derived from human colorectal cancer cells that were grown *in-vitro* as monolayer epithelia cells. Polarised cells with tight connections and apical brush boundaries were detected after 21 days of differentiation in regular culture media.

Culture medium

Dulbecco's modified eagle's media (DMEM) with *L*-glutamine (without sodium pyruvate), FBS (10%), amino acids (non-essential-1%), glucose syrup (4.5 g/L), Penicillin (10,000 U/mL) and streptomycin (10 mg/mL) were used to cultivate Caco-2 cells.

Transport medium

DMEM with *L*-glutamine (and without sodium pyruvate), sodium chloride (1.987 g/L), HEPES (4.76 g/L), and glucose syrup (4.5 g/L) was used as the transport medium.

Temperature and humidity

At 37°C, 5% CO_2 and 95% O_2 are present.

Cultivation of layers

Spin down the cells after trypsinization. Culture the cell lines in trans-well polycarbonate cell culture inserts with a 12 mm diameter and 3 µm pore sizes. Place the required number of cells and filters by spreading 0.5 mL of re-suspended cell solution on each filter. Incubate the plate with the filter supports in the basolateral chamber filled with transport medium. For complete differentiation, the medium was changed every day for the first seven days, then once every three days. The cells were cultured for 21 days to ensure complete maturity and confluence. On the 21st day, fully polarised cells will be ready with the formation of tight junction and the expression of P-glycoprotein (P-gp) in the cellular monolayer. The detection and quantification limit is a term used to describe the upper limit of detection and quantification. 1.5 and 0.5 mL of culture media was included in the basal and apical compartments, respectively.

Cellular uptake

Labelled fluorescein samples were generated with rhodamine-B using the antisolvent precipitation technique to track the cellular absorption of α-mangostin and AM-SLNP. Cells were exposed to test solutions for 1hr at 37°C in this investigation. After removing the samples from the well after 0, 0.5, and 1 hours, the cell line was rinsed with Hank's Balanced Salt Solution. The cells were subsequently stained with a 5 µg/mL solution of 4, 6-diamidino-2-phenylindole (DAPI) and pictures were captured using an Olympus confocal laser micrograph.

Transport Study

Before and after the transport, the TEER value (Trans epithelial electric resistance) is evaluated to test the integrity of the cell line using Milli cell-ESR. The resistance created due to HBSS media is removed from the TEER value to avoid its background resistance. The resistance of the transport medium (HBSS) was removed from the TEER value and used as the background resistance. The study design eliminated cell line with TEER values of <250 Ω·cm^2. Before commencing the experiment, the cell monolayer was washed with PBS (with Ca^{+2} and Mg^{+2}) at 37±0.5°C followed by addition of transport media (HBSS) to both basal and apical chambers and was equilibrated at 37±0.5°C/60mins (transport studies). The HBSS was withdrawn after 60 minutes, and the sample solutions at 100µg/mL α-mangostin (both pure extract and AM-SLNP) in HBBS were added to the apical compartments. Join the filter and receiver plate; incubate at 37±0.5°C/60rpm shaking in rotary shaker. At various time intervals (30, 60, 90, 120, 240, and 480 minutes), the sample (50µL) was taken from the receiver chamber and replaced with an equal amount (50µL) of fresh HBSS to maintain a consistent volume.

As previously mentioned, the concentration of drug in the samples was measured using a validated LC-MS technique. The tests were carried out in triplicates. (Eqn-1) was used to compute the apparent permeability coefficient (P_{app}, cm/s).

The transport rate is dQ/dt, the initial α-mangostin concentration on the apical compartment is C_0, and the surface are of the membrane filter's (A) is 1.12 cm^2.

$$Papp(cm/sec) = \left(\frac{dQ}{dt}\right)/AC_0 \quad \text{Eqn-1}$$

Estimation of cell viability

Viability of cells in the cell line was assessed at the end of the study by using tryphan blue staining procedure. There is no statistical difference observed after the evaluation of cell vitality for the cells treated with pure extract and AM-SLNP. Cell vitality was not beyond 90% for the product and control cell lines. The negatively charged chromophore (trypan blue) does not react with the viable cells. So, we can ensure the cell vitality as they do not contain dye.

In-vivo PK studies in rat model

Animal details for in-vivo studies

Table-1 shows the animal details of an *in-vivo* PK research conducted on male rats. The studies were conducted as per the guidelines of committee for purpose of control and supervision of experimental animals (CPCSEA) with Institutional animal ethical committee (IAEC) registration number, LN/IAEC/DMPK/LN220302. Food and water were made available to the rats at all times. Prior to the studies, the rats were given unrestricted access to water and feed.

Table 1. Animals detail used for PK studies

Species/Strain	Sprague Dawley rats
Weight(g)/ Age(weeks)/ Sex	230-260g/8-10 weeks/Male
Number of animals used for BA studies	12
Animal source	Mahaveer Enterprises

Dosing and sampling

Rats were split into two groups in PK studies (6 per group). At a dosage of 75 mg/kg (dose volume–10 mL/kg), the α-mangostin and equivalent dose of AM-SLNP were given orally as a suspension (dispersed in water) using a gavage needle to an approximate graduated syringe under continuous vortexing. At 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after the dose via the retro-orbital venous-plexus with an anesthesia, blood samples of 250µL were taken. Every metabolic cage was washed with 5 mL of water at the end of 24 hours in the rat urine collector.

α-mangostin tissue distribution

The surgical processes and α-mangostin treatment were carried out in accordance with a previously published technique (28). As much blood as possible was obtained via the carotid artery at 3 hours following oral administration of α-mangostin at a dosage of 75 mg/kg, and each rat was subsequently killed by cervical dislocation. Each liver and lung were removed, weighed, and homogenised with water equal to four times the organ's size. The supernatant was collected after centrifugation for 10 minutes, and all samples were kept at –80°C until needed.

Analysis of plasma samples

LC-MS was used to evaluate the α-mangostin concentrations in plasma samples. Protein precipitation was used to obtain data for the sample analysis. All of the samples underwent the same extraction technique (Standard validation samples). 400 µL acetonitrile was added to 100 µL plasma samples, which were vortexed for 5 minutes. The materials were centrifuged for 10 minutes after vortexing at 1,470g. Separate supernatant in a separate centrifuge, diluted with mobile phase, analyzed using validated LC-MS method and estimated the % drug concentration in the collected plasma.

Pharmacokinetic and statistical analysis

PK parameters were calculated using concentration of drug form plasma samples analyzed in LC-MS apparatus. Maximum drug plasma concentration (C_{max}) at its T_{max} (time for max. drug plasma concentration), area under the curve (AUC), and other parameters were determined with the help of the PK solver. Microsoft Excel 2013 was used for all of the other mathematical computations

Results and Discussion

The LC-MS method for evaluation of α-mangostin

Method Development and Optimization

The most important stage is to optimize the chromatographic settings with the goal of achieving symmetrical peaks with a faster analysis time, good specificity, and sensitivity. Alpha-mangostin was quantified using ion transitions at m/z 411.1595.

Validation parameters

The suggested approach uses a linear regression model to create calibration curves from the responses of varied concentrations. Regression coefficient (r^2) of 0.9994; was selected for further calculation of validation and BA samples. Method trueness was justified by calculating the relative percentage bias, which was determined to be confined within [-3.27 percent to +2.50 percent], as shown in Table-2, indicating that the method is appropriate. The precision and repeatability of the procedure were confirmed by a precision study, which revealed that the RSD for both reproducibility and intermediate levels was less than 3%, as shown in Table-3. Sample matrices were integrated in the validation process, which involves recovery experiments, after the correctness of all parameters connected to the system and the established technique were confirmed. In sample matrices, recovery investigations were carried out using the usual addition method. The overall inaccuracy of test findings is taken into consideration in these recovery investigations, which is indicated by the accuracy results of 98.17-101.78% and 98.24-99.71%, respectively, for intraday and interday. The results of the accuracy experiments are shown in Table-4. With a LOD of 5.76 ng/mL, the LOD data suggest that this approach is sensitive enough to test plasma.

Table 2. Results of Trueness in terms of relative bias (%)

Nominal concentration (ng/mL)	Obtained concentration (ng/mL)	S.D (ng/mL)	Relative bias (%)
57.6	56.14	1.31	-2.53
288	290.65	3.16	0.92
1920	1857.17	52.29	-3.27
3900	3950.61	46.81	1.30
4800	4920	107.44	2.50

NOTE: Results are expressed as mean±S.D., where n=6. S.D = Standard deviation.

Table 3. Inter and intraday precision results

Concentration (ng/mL)	Intra-day Precision		Inter-day Precision	
	Mean	S.D (ng/mL)	Mean	S.D (ng/mL)
57.6	57.59	0.01	57.47	0.61
288	282.33	7.53	283.80	4.58
1920	1913.17	53.16	1915.67	51.33
3900	4000.00	63.97	3892.00	105.62
4800	4900.00	65.53	4773.33	123.48

NOTE: Results are expressed as mean±S.D., where n=6. S.D = Standard deviation.

Table 4. Inter and intraday accuracy results

Concentration (ng/mL)	Intra-day Accuracy (%)		Inter-day Accuracy (%)	
	Mean %	S.D (ng/mL)	Mean %	S.D (ng/mL)
57.6	98.72	1.10	98.24	1.02
288	98.3	3.38	99.16	3.95
1920	101.78	32.77	98.35	28.26
3900	98.17	133.40	99.71	138.91
4800	99.61	129.25	99.45	115.99

NOTE: Results are expressed as mean±S.D., where n=6. S.D = Standard deviation.

The effectiveness of extraction of a sample of plasma

For plasma samples, the new method's extraction efficiency was calculated and determined to be between 94.48 and 104.05%. These findings support the use of the described approach to plasma sample analysis. Table-5 displays the results of α-mangostin extraction from plasma samples.

Table 5. Plasma extraction efficiency results

Extraction sample		Plasma result	
Concentration spiked	226.72ng/ml	2497.30ng/ml	4185ng/ml
Mean extraction (n=3)	94.48±2.18%	104.05±1.96%	98.64±2.64%
% Extraction average	99.05%		

α-mangsotin solid lipid nanoparticles evaluation results

The particle size and zeta potential of the formulated α-mangostin solid lipid nanoparticles were 173.6 nm and -42.3 mV, respectively. The results of particle size and zeta potential are given in Figure-2&3 respectively.

α-mangostin and AM-SLNP Caco-2 cell lines transport

Cell Viability estimation

Viability studies carried out for blank, α-mangostin, and AM-SLNP showed greater than 80% cell viability at all concentrations ranging between 10 and 200 µg/mL. These results showed no significant decrease in values was observed between the pure extract (α-mangostin) and AM-SLNP, which confirms no toxicity associated with the excipients or the process adopted during the preparation of SLNP. The results of the study are given in Figure-4.

Cellular uptake

Figure-5 depicts the cellular uptake of α-mangostin. In comparison to the α-mangostin SLNP, the micrograph clearly shows that relatively few individual particles have been detected in the α-mangostin. As a result, it appears that the absorption of α-Mangostin from the formulation has increased. Additional transportation studies were conducted to determine the quantitative effect.

Transport Study

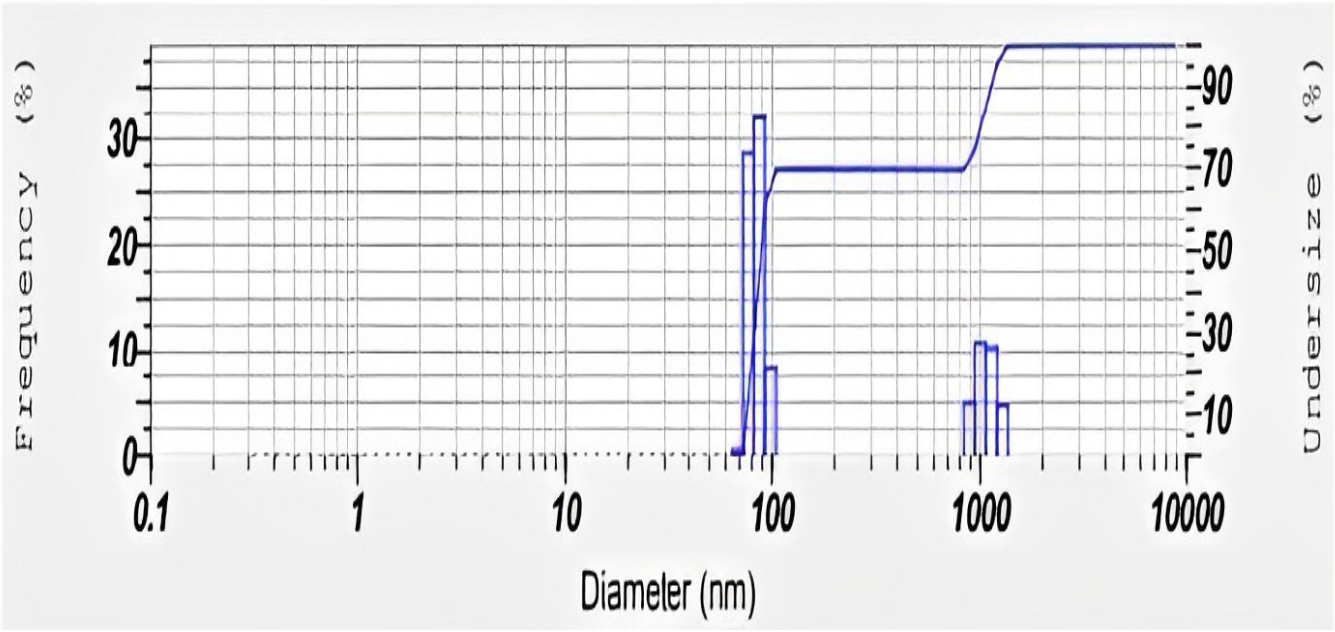


Fig. 2. Particle size of AM-SLNP.

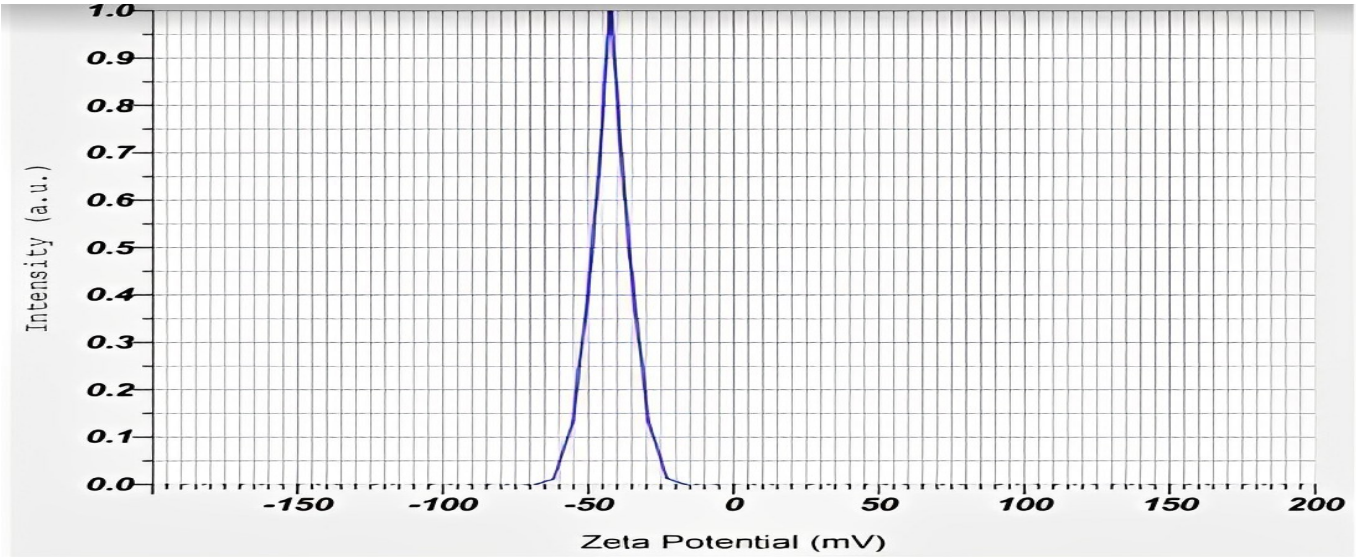


Fig. 3. Zeta potential of AM-SLNP.

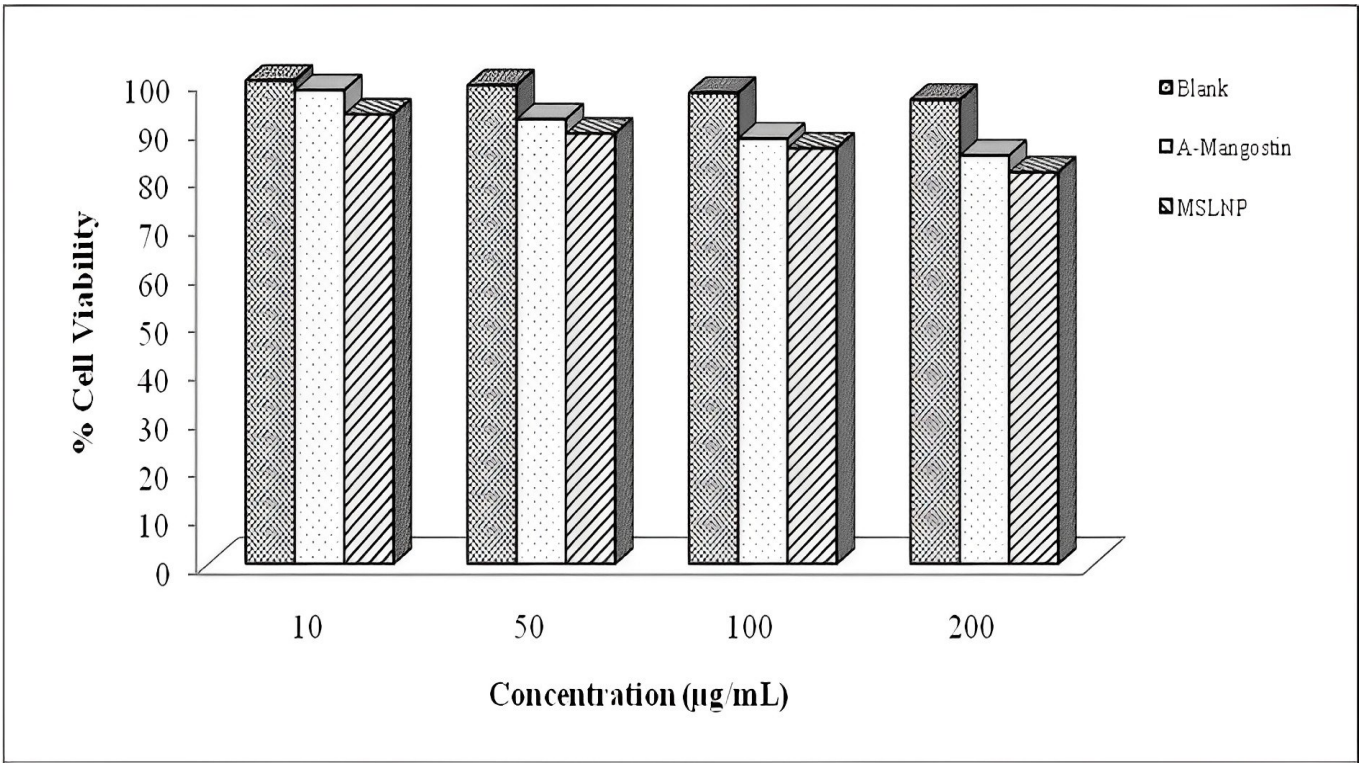


Fig. 4. Cell viability results for Blank, A-mangostin, and AM-SLNP.

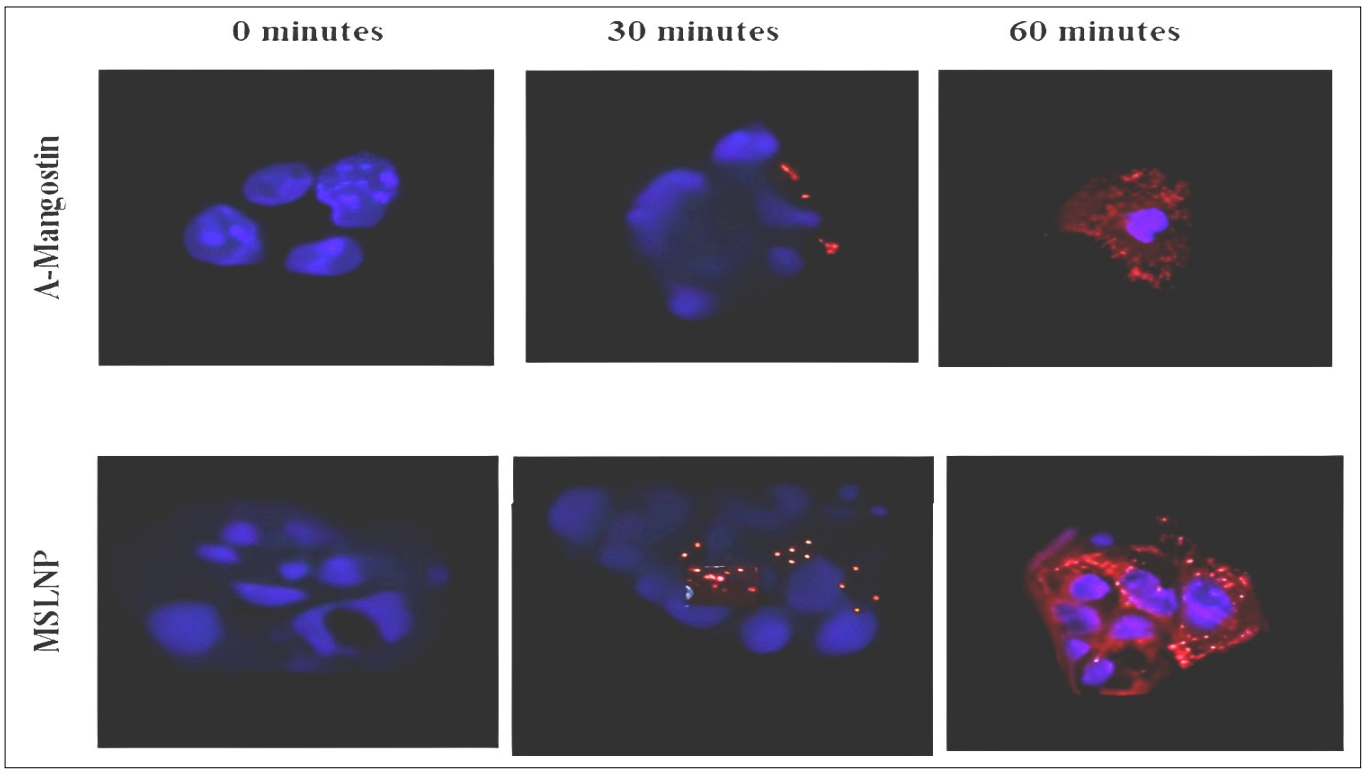


Fig. 5. Cellular uptake of A-mangostin and AM-SLNP at different time intervals across Caco-2 cell line.

To investigate the penetration of formulations and pure extracts into Caco-2 cells, a transport study utilizing trans well inserts was conducted. AM-SLNP was found to facilitate greater transport when compared to pure extract, as evidenced by P_{app} values of $2.12*10^{-6}$ cm/sec and $0.57*10^{-6}$ cm/sec, respectively. Because of their tiny size, broad surface area, and presence of emulsifiers, AM-SLNP had 3.72 times higher drug penetration than α-mangostin at 4 hours.

Pharmacokinetic studies of α-mangostin in rats

In rats, the PK of α-mangostin was investigated to see if it improved the absorption efficiency of α-mangostin when formulated as SLNP. Figures-6 show the plasma drug concentration versus time profiles for α-mangostin and AM-SLNP. There was a considerable variation in the PK profile after oral administration of the α-mangostin and AM-SLNP's. A plot of log concentration versus time profile was established for the plasma results. Figure-7 demonstrates the absorption and elimination phases of the Ln concentration time profile for α-mangostin and AM-SLNP. There is a 3.33 fold increase in the AUC of formulated AM-SLNP compared to α-mangostin. Table-6 shows the PK characteristics for α-mangostin and AM-SLNP. The

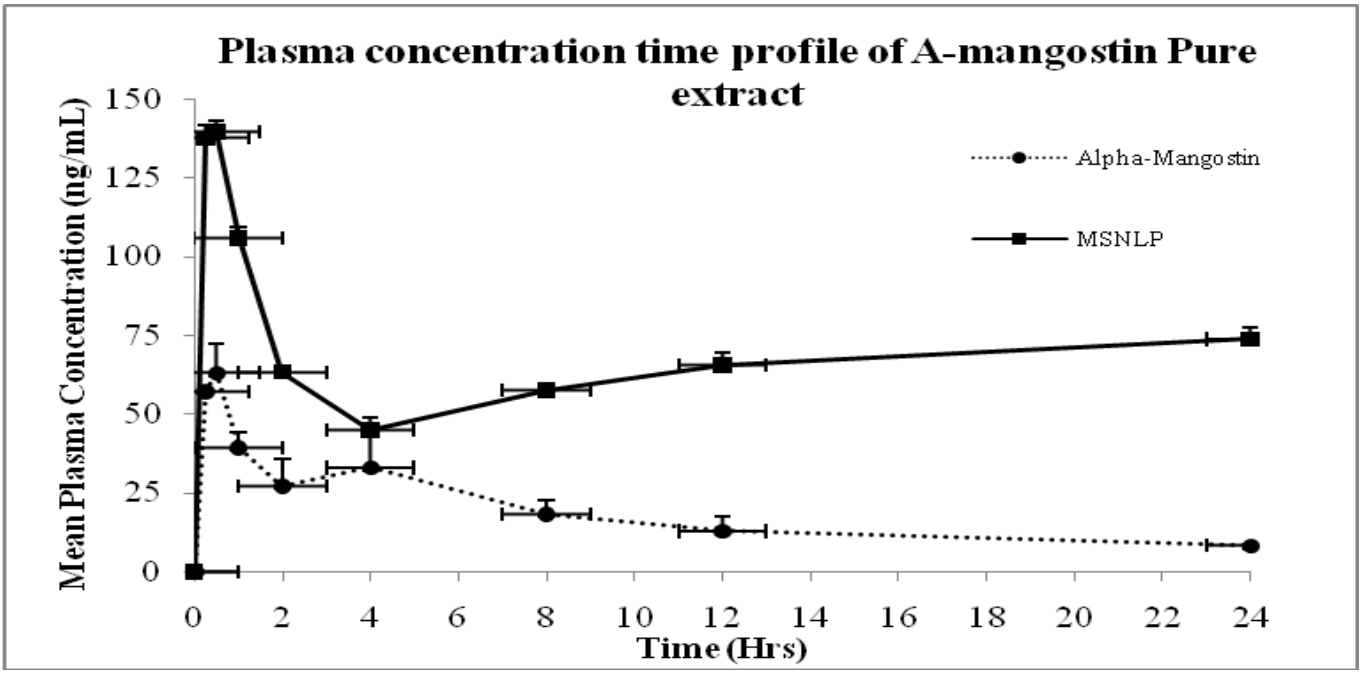


Fig. 6. Plasma concentration-Time profile for A-mangostin and AM-SLNP.

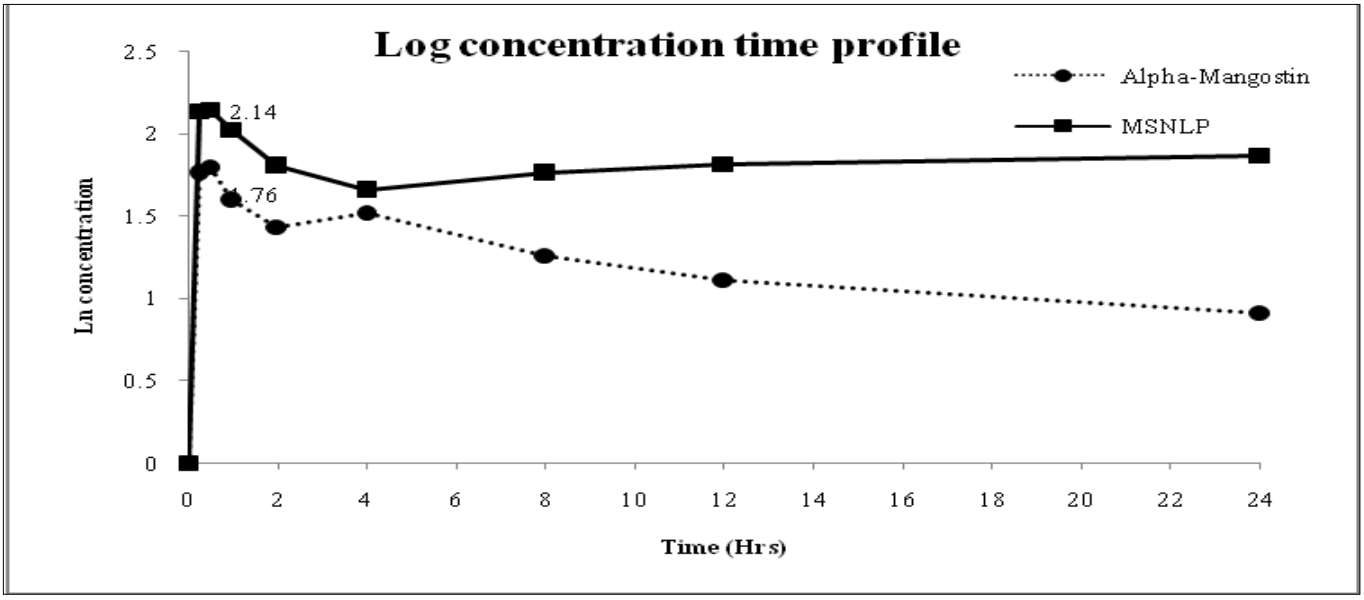


Fig. 7. Ln concentrations versus time profile for A-mangostin and AM-SLNP.

results of the urine confirm that there is greater elimination of α-mangostin compared to formulated AM-SLNP. The results of urine analysis are represented in Figure-8. Figure-9 shows the amount of α-mangostin distributed to the tissues 180 minutes following oral administration of 75 mg/kg each α-mangostin extract and AM-SLNP. Curve fitting revealed a minor shift in the elimination pattern in these figures, but the absorption phase remained essentially unchanged. These findings suggest that the formula-

Table 6. PK Parameters estimated from plasma concentration-time profile of A-m

PK Parameter	PK Units	α-mangostin	AM-SLNP
T_{max}	min	30	30
C_{max}	ng/mL	50.546	111.816
$AUC_{0\text{-}t}$	ng*h /mL	403.75	1344.76
$AUC_{0\text{-}inf\_obs}$	ng*h /mL	671.48	6940
MRT	hours	24.62	92.39
Relative Bioavailability	%	1	3.33

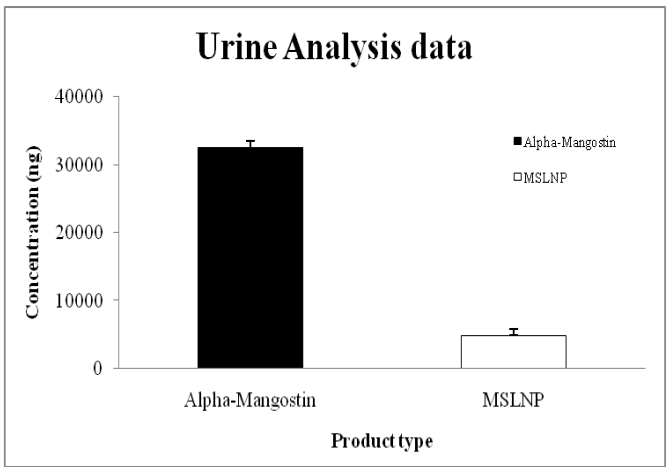


Fig. 8. Urine analysis data of A-mangostin and formulated AM-SLNP.

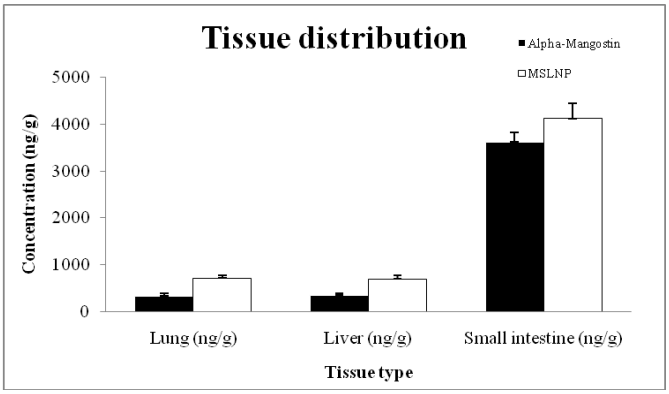


Fig. 9. Tissue distribution data of A-mangostin and formulated AM-SLNP.

tion of nanoparticles will reduce intestinal membrane mediated metabolism of α-mangostin due to its smaller particle size and greater solubility compared to pure extract and further increase bioavailability of the formulation.

Conclusion

From the start, the research had significant difficulty because of α-mangostin's low bioavailability. It is a crucial and effective medicine in the anti-inflammatory, anti-cancer, and anti-oxidant categories. Using hot melt homogenization followed by ultrasonication, we created AM-SLNP's. The most advanced and extremely dependable equipment, LC-MS, was employed for the estimation of α-mangostin, which was a critical stage in the entire study procedure. The LC-MS findings were very reproducible, and the procedure had been well tested before being used for analysis of samples.

In-vitro experiments were crucial since they confirmed the theory underlying the research and encouraged the research to pursue more advanced and dependable technology such as Caco-2 cell monolayers and *in-vivo* animal tests. In the presence of the solid lipid and emulsifiers in the formulation, transport tests in caco-2 cell lines revealed an increase in the cellular absorption of α-mangostin. Nano particulate drug delivery system developed for α-mangostin is effective for transportation across P-gp inhibitors. The use of cell monolayer also aids in the determination of the optimal ratios for PK investigations.

In rat's plasma, pharmacokinetic tests revealed a substantial rise in the concentration of α-mangostin in the presence of additives in the formulation compared to pure extract. The fact that α-mangostin is the P-substrate gp's restricts its oral absorption is widely established. Because the developed formulation is nanoparticle, oral absorption of α-mangostin has been enhanced. Furthermore, it has been observed that α-mangostin's bioavailability is influenced by hepatic and intestinal first-pass metabolism. Developed technology of MSLNP showed some inhibitory actions against the Cyp-450 enzyme mediated metabolism and intestinal P-gp inhibition. As a result, we formulated SLNP's improved α-mangostin bioavailability even further. Because both cell monolayer and animal studies demonstrate the same pattern of drug intake mechanism for SLNP's and as they are almost identical, nanotechnology can be utilized in avoiding hepatic metabolism and improving bioavailability.

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Authors contributions

Concept –GSN., VNB.; Design – GSN., VNB., GKB.; Supervision – GSN., VNB., MJS.; Resources – ARS., MRK., CHN.; Materials – MJS., MRK., CHN.; Data Collection and/or Processing – GSN, VNB., ARS., RRB.; Analysis and/or Interpretation – VNB., GSN., GKB; Literature Search – VNB., RRB., GSN.; Writing – VNB., GSN., CHN.; Critical Reviews - GSN., VNB., GKB., ARS., MJS., RRB., MRK., CHN.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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