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TRANSFEROSOMES: A NEW PARADIGM IN THE PERMEATION ENHANCEMENT OF VESICULAR DRUG DELIVERY SYSTEM.

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ABSTRACT

Back Ground: Transdermal drug delivery is proving to be superior to the conventional oral delivery of drugs due to its potential benefits like avoidance of first pass metabolism, Gastro intestinal disturbances, toxic effects. Despite of advantages it has got its own limitations like inability to transport large molecules such as vaccines, proteins, steroids and to overcome the barrier properties of stratum corneum. The cost of discovering new drugs are amplified in with increasing efficacy and less side effects of the drugs.

Main body: Transferosomes also called ultra-deformable liposomes, are an emergent lipoidal vesicular novel drug delivery system to prevail over these barriers. Transferosomes possesses numerous advantages over conventional liposomes due to their elastic nature, increased permeation, reduced dosing frequency and undesirable side effects. Lipophilic drugs can be achieved >90% of bioavailability through transferosomes. Transferosome are composed of phospholipids, edge activators, solvents, and buffering agents. Phospholipids are membrane component of stratum corneum and edge-activators like tweens, spans, sodium cholate, dipotassium glycyrrhizinate, sodium deoxycholate are responsible for their curvature and their ultra-deformation allows to pass through narrow constriction less than their own diameter.

Conclusion: Transferosomes are promising approach for delivery of herbal drugs, high molecular weight compounds, drugs undergo extensive first pass metabolism. This review presents the mechanism of action, advantages, disadvantages, composition, methods of preparation, characterization and applications of transferosomes.

KEYWORDS: Transferosomes, ultra-deformable liposomes, permeation enhancers, edge activators, lipophilic drugs.

BACKGROUND

Skin acts as a barrier between body and external environment. It consists of 3 layers namely epidermis, dermis, subcutaneous tissue. Outer layer of epidermis, stratum corneum plays a crucial role in permeation of foreign substances into the body.[1] Stratum corneum composes of cholesterol, free fatty acids, ceramides, and corneocytes. Corneocytes consists of monolayer of nonpolar lipids which forms brick and mortar layer. Lipophilic drugs are soluble in these lipids and can pass through the skin.[2] Conventional liposomes cannot modify their shape and penetrate the skin. Transferosomes are advantageous over other nanoparticulate drug delivery systems like liposomes, niosomes in the administration of drugs across the skin as their composition contains a greater amount of safe and biocompatible excipients. Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intercellular sealing lipid of the stratum corneum.[3]

The word transferosome is derived from the Latin word "transferre" means "to carry across" and the greek word "soma" means "body". The technology was first described in 1991 by Cevc and Blume.[4] Transferosomes acts as carrier for the delivery of various drugs which has serious side effects when given through oral and systemic route.[5] The difference between conventional vesicle and transferosomes are shown in Figure 1. Now a days transferosomes are gaining much importance due to its exceptional properties. They inherent potential advantages are highly utilized in transdermal immunization, peripheral drug targeting, delivery of high molecular weight compounds and herbal based products.[6] Like other nano vesicular systems, transferosomes also possesses hydrophilic core and lipophilic layer. They can incorporate both lipophilic and hydrophilic drugs.

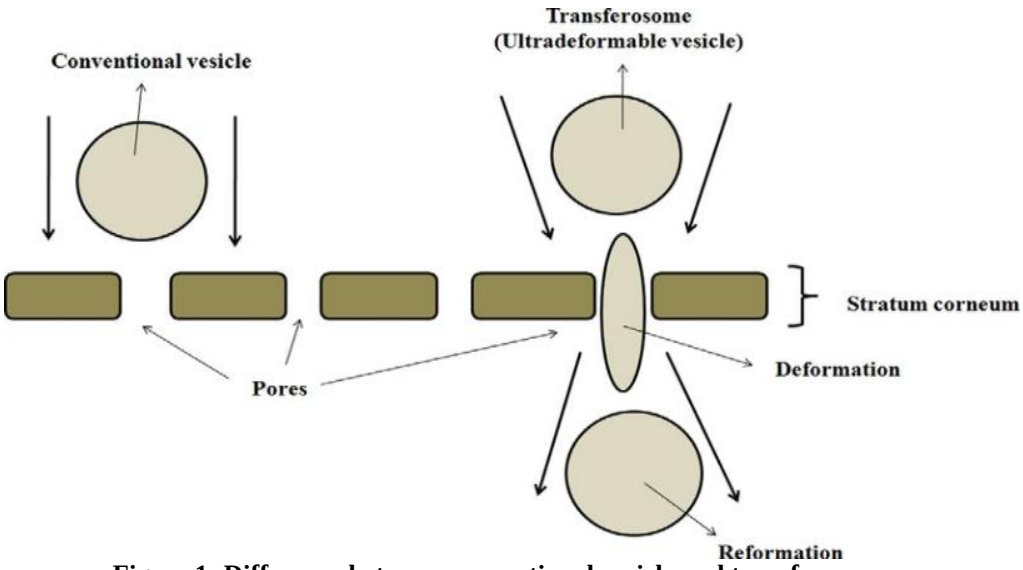

Figure 1: Difference between conventional vesicle and transferosomes.

Mechanism of transferosomes

The mechanism for penetration of the transferosomes is the generation of "osmotic gradient" due to evaporation of water while applying the lipid suspension (Transferosomes) on the skin surface.[4] Transferosomes when applied on an open biological surface, such as non-occluded skin, undergoes dehydration and tends to migrate into the water-rich deeper strata to secure its adequate hydration. The transport of these elastic vesicles is thus independent of concentration. They undergo deformation during penetration through the stratum corneum again undergo reformation once they reach deeper layers of skin.[7]

Advantages

- Avoidance of first pass metabolism
- Minimizing undesirable side effects
- Utility of short half-life drugs
- Transferosomes are a highly adaptable, stress responsive.
- They act as depot releasing their contents slowly and gradually, they can be used for both systemic as well as topical delivery of drug.[8]
- Transferosomes offers an advantage like high entrapment efficiency in case of lipophilic drug near to 90%
- They are biodegradable and biocompatible as they are made from natural phospholipids and high entrapment efficiency.[9]
- Transferosomes exhibit optimal zeta potential. Hence risk of aggregation is not seen and offers greater colloidal stability. Transferosomes had exhibited good colloidal stability up to three months both at 4^0C and 25^0C.

Disadvantages

- Transferosomes undergo oxidative degradation on long term storage.
- Microbial growth.

Main body

Composition of transferosomes

The main factors to develop optimal transferosomal formulations (high drug loading, and nanometric size) are the optimal ratio between the main components (phospholipids and edge activators) as well as the critical process parameters for the manufacture of transferosomes. Transferosome mainly composed of phospholipids, edge activators, solvents and buffering agents.[10] Each excipient has its own importance and the composition of transferosomes are shown in Table No. 1.

Table No 1: Composition of Transferosomes.

S. No	Excipients	Example	Use
1	Phospholipids	Natural lipids: soya phosphatidyl choline, egg phosphatidyl choline, dipalmitoyl phosphatidyl choline Cationic lipids: DOTAP, DOTMA	Membrane forming component
2	Edge activators	Non-ionic surfactants: span80, span60, tween80, tween60. Bile salts: sodium cholate, sodium deoxy cholate Terpenes: citrol and limonene.	Vesicle forming component
3	Polymers	Propylene glycol, polyethylene glycol	Permeation enhancers
4	Stabilizers	Surfactants: spans and poloxamers	Stabilizing agent

		Lipids: cholesterol Polymers: Polyvinyl pyrrolidine, HPMC	
5	Buffering agents	pH 5 phosphate buffer, pH 7 phosphate buffer	Hydrating medium
6	Solvents	Methanol, isopropyl alcohol, chloroform, ethanol etc.,	Core forming agent

Phospholipids forms the lipophilic bilayer of the vesicles. Phospholipids like soy lecithin, egg lecithin are widely used but soy lecithin contains a smaller number of unsaturated fatty acids, which is responsible for less microbial growth and shows good stability. Soya lecithin besides acting as a carrier, also nourishes the skin, that overall elasticity of skin increased 18.37% with novel cream and only 6% with base cream.[28] Vesicle size also affected by the nature of lipid. Least vesicle size with egg yolk phosphatidyl choline compared to soybean phosphatidylcholine was seen.[11] Phosphatidylcholine was used in the process; besides acting as a carrier, it also nourishes the skin, because it is an essential part of cell membrane. It showed better stability profile because chemical bonds are formed between phytoconstituent and excipients.[29] With the use of combination of lipids like cholesterol the stability of the vesicle also increases.

Edge activators plays a key role as it provides a high radius of curvature that can destabilize the lipid bilayer increasing the deformability of the membrane. The amounts of edge activator up to 15 % are used. Increase in the concentration of edge activators lead to the disruption of lipid bilayer and releases the entrapped drug and also formation of mixed micelles which results in partial encapsulation within small micelles.[12] Sodium cholate, sodium deoxycholate, spans, tweens, dipotassium glycyrrhizinate are used as edge activators. Vesicle size increases with increase in hydrophilic and lipophilic balance (HLB)value of the non-ionic surfactants and size obtained in decreasing order for tween80>tween60>span60 >span80.[13] Manosroi *et al* work indicated that higher percentage of sodium deoxycholate led to lower particle size while large amounts of non-ionic surfactants resulted in an increase in particle size. Sodium cholate has amphiphilic chemical structure and steroid skeleton with OH groups provide a good hydrophilic binding through hydrogen bonds and it shows higher entrapment efficiency compared to non-ionic surfactants.[14]

Buffers act as a hydrating medium in preparation of transferosomes. Phosphate buffers like pH 5.5, pH 7.4, pH 7.5 were used. Solvents are responsible for the formation of aqueous core in transferosomes. Solvents like methanol, ethanol, chloroform are used individually as well as combinations. Stabilizers are used to stabilize the vesicle during the permeation of drug through skin. Cholesterol is commonly used as stabilizer. Permeation enhancers are used to further increase the skin permeation of drug.

Methods of preparation

1. Modified vortexing sonication method: A blend of lipids, edge activator and drug were dispersed in appropriate phosphate buffer and vortexed for required time to attain a milky suspension. the suspension formed was sonicated by LUC 410 power sonicator for 30 minutes followed by freezing at -20°C for 18hrs and thawing at room temperature for 6hrs for 3 times. The suspension is extruded through 0.2 mm sartorius membrane filter for 5 times at 50°C. The final product is stored in refrigerator.[15]
2. Reverse phase evaporation method: A blend of soy lecithin and cholesterol were taken in a beaker containing tween 80 as a surfactant. Organic solvents were added. This mixture was kept at room temperature for 24 hrs until thin film formed. Later drug was added and sonicated by probe sonicator at a frequency of 20kHz for 2 minutes. Sodium deoxycholate is added as edge activator and hydrated with pH 7.4 phosphate buffer and sonicated for 2 minutes.[16]
3. Lipid film hydration technique: Weighed amounts of drug, lecithin and edge activator were dissolved in round bottomed flask containing solvent mixture. Organic solvents were evaporated above lipid transition temperature (40°C) and fine traces of solvent were removed by vacuum. A thin lipid film was formed inside the flask. Prepared thin film was hydrated with appropriate buffer solution and stirred at speed of 60rpm for 1hr. The resultant vesicles are allowed to swell for 2hrs at room temperature.[17]
4. Ethanol injection method: Aqueous phase is prepared by dissolving drug into phosphate buffer and stirred at 400rpm. The organic phase containing lipids, edge activator, solvent is injected into aqueous phase under continuous stirring for 30minutes. Larger multilamellar vesicles formed are further sonicated for required time to form small uni-lamellar vesicles.[18]

APPLICATIONS

Transferosomes in herbal drug delivery

Green tea leaves contain epigallocatechin gallate (EGCG) which is responsible for antioxidant activity. due its high molecular weight and high hydrophilicity it is difficult to penetrate through the skin.[19] Hence, they were prepared as transferosomes by thin film hydration technique and formulated as cream. The *invitro* permeation studies were conducted and the amount of epigallocatechin gallate penetrated from transferosomal and non-transferosomal cream were 1003.6 and 400.09 µg/cm^2/hr respectively.[20] Permeation studies on caffeine

nanovesicles like transferosomes, phytosomes, niosomes were prepared by different penetration enhancers like oleic acid, eucalyptol and decyl polyglucoside as a non-ionic surfactant. Among that caffeine encapsulated as transferosomes showed higher permeation.[21] Capsaicin (obtained from capsicum) loaded transferosomes formulated for the treatment of rheumatoid arthritis. Arthritic activity study shows that transferosomal formulation possesses superior inhibitory activity than the marketed Thermagel formulation at the same dosage level.[23]

Transferosomes as Nutricosmetics:

Commonly used cosmetics have little percutaneous absorption and also side effects.[24] By incorporating biologically active phytoconstituents into transferosomes can improve both the aesthetics and performance of a cosmetic product, increased absorption, enhanced delivery to the tissues.[25] The alteration of retinoids levels in the skin cause different disorders in the maturation of epithelial skin cells.[26] Retinyl palmitate is formulated into transferosomes evaluated for penetration of the active ingredients and biodistribution by *in vitro* and *in ex-vivo* studies. Transferosomes showed a significant increase in the administration of retinyl palmitate to the epidermis by quantification of the active ingredients in the different layers of the skin, as well as by fluorescence microscopy of biopsies of pig-ear skin. These results suggest that transferosomes may be an efficient vehicle for the delivery of retinoids to inner layers of the skin, such as the epidermis.[27] **C**urcuma longa extract loaded transferosomal cream showed better absorption and stability compared to base cream. Saponification value of base cream was found to be higher than transferosomal cream and also possible microbial growth. Due to their poor lipid solubility and molecular size, studies on quercetin, naringin, simonenine, piperine, glycyrrhizin etc., demonstrated that by formulating into transferosomes has a capability to enhance their bioavailability.

Transferosomes in wound healing

Conventional topical burn formulations are required to be applied 3 to 4 times a day. By applying transferosomal cream of acriflavine patient compliance can be increased by reducing its dosage frequency.[30] They are also used in the treatment of vitiligo and in diabetic caused wounds and other skin diseases**.** Miconazole nitrate was formulated as transferosomes. Miconazole transferosomes were incorporated into a carbopol 934 gel base, the prepared transferosomal gel showed higher antifungal activity than miconazole cream.[31]

Transferosomes in targeted drug delivery

Transferosomes have the ability to localize activity of drug at the site or organ of action there by lowering its concentration at the other sites in body.[33] they protect the encapsulated drug from metabolic degradation. They act as a carrier for low and high molecular weight drugs. Size rages from 1 to 300nanometers(nm), hence move more freely in systemic circulation compared to bigger particles.[34] To overcome the difficulties of subcutaneous delivery of insulin, transferosomal gel was prepared by rotary evaporation sonication technique.[35] Lactoferrin has antiviral activity against Human papillomavirus (HPV). Transferosomes prepared by two methods including reverse phase evaporation and thin film hydration with different ratios of cholesterol: lecithin: DOTAP in the presence of Tween 80. The optimized transferosomes have 100nm size with good polydispersity index and encapsulation efficiency of 91% for lactoferrin. The viral inhibitory concentration (IC_{50}) of transferosomal lactoferrin has been significantly improved to nearly one tenth in comparison to free lactoferrin.[32]

Resveratrol is used to treat Alzheimer disease for brain targeting drug delivery due to its low bioavailability (<1) and solubility and extensive hepatic metabolism, resveratrol is formulated as transferosomes by reverse phase evaporation method.[36] Transferosomes displayed higher permeation of up to 81.29 ± 2.64%. Transferosomes significantly enhanced behavioural acquisition and spatial memory function in the amnesic rats compared with both the nano emulsion formulation and the pure drug. The developed transferosomes may be considered as a well-designed brain targeting system that might further be applied for targeting many drugs to be used in the treatment of central nervous system diseases.[37]

Transferosomes bearing loratadine were prepared by conventional thin film hydration method and optimized using sequential Quality-by-Design approach. The transferosomal gel proved superior to control, transferosome-free gel. Bioavailability of the transferosomal gel was comparable to Claritin® oral tablets.[38] Raloxifene used for breast cancer protection in HIV patients. Due to its poor bioavailability (2%) formulated as transferosomes using D alpha tocopheryl polyethylene glycol 1000 succinate which augments (Trans-Activator of transcription) TAT- HIV protein., inhibitory concentration IC_{50} results showed 1.42-fold improvement in cytotoxicity compared to raw raloxifene against MCF-7 cells.[39]

Transferosome as bioenhancer

Topical clindamycin phosphate has less bioavailability, to overcome these limitations an attempt has been made to prepare transferosomes and optimize it for enhanced delivery through the skin.[40] To increase the solubility of sertraline, the drug was formulated into transfersomal gel by using span 80, soya lecithin, and Carbopol. Enhance skin delivery of sertraline because of excellent drug release and permeation of the drug. Also, no skin irritation was observed when the gel formulation of transethosomes were applied for enhancing the transdermal delivery of olmesartan and medoxomil. *In vivo* evaluation studies showed higher permeation rates

compared to oral tablets.[41] Autoimmune disorders are distinct with over production and accumulation of free radicals due to its undisclosed genesis. The imbalance could only be combat by supplementing natural defensive antioxidant enzymes such as superoxide dismutase and catalase. The efficiency of these enzymes is enhanced by use of colloidal carriers which include cellular carriers, vesicular and particulate systems like transferosomes.[42] Felodipine[43], Risperidone[45] and Piroxicam[44] loaded transferosomal gels shows excellent drug release and better therapeutic effect. Permeation rate is improved by incorporation of different permeation enhancers.

Characterization of transferosomes

1. Vesicle morphology and size characterization

Vesicle morphology studies were conducted by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The mean diameter and the polydispersity index (PDI) of the transferosomes were determined by Laser particle size analyser.[45] PDI value of less than 0.1 was considered as a homogeneous distribution of vesicles, whereas a value of greater than 0.3 was of higher heterogeneity.[46]

2. Entrapment Efficiency

Transferosomes were centrifuged at high rpm (10,000 - 20,000 rpm) for 3h. The supernatant was collected, diluted with suitable buffer and analysed for the entrapment efficiency.[47]

3. Zeta potential

The magnitude of the zeta potential provides in sequence about particle stability. Zeta potential is an assessment of the efficient electric charge on the transferosomes surface, quantifying the charges. The higher the magnitude of zeta potential exhibit amplified electrostatic repulsion and therefore amplified stability. The zeta potential of the transferosomes was measured using a zetasizer. The zetasizer consists of capillary cell which was cleaned with 90% ethanol and distilled water before analyses.

4. Fourier Transform Infrared Spectroscopy (FTIR) studies

Fourier transform infrared spectroscopy studies are conducted to study the interaction between drug and excipients. The drug, lipids, edge activators and physical mixture of drug and lipid samples are prepared with potassium bromide (KBr). All the prepared samples were subjected to FTIR spectroscopic studies to determine drug-carrier interaction.[48]

5. Elasticity measurement

This study was carried out by extrusion method at 7.5 psi pressure through 0.2 µm polycarbonate filter membrane fixed to stainless steel pressure holder of 50 millilitre(mL) capacity barrel. 0.5mL of the suspension was diluted up to 10 mL with phosphate buffer saline(PBS) pH 7.4 and then extruded for 10 min through the filter medium.[49]

6. *In-vitro* diffusion studies

In vitro drug release studies are performed in open end tube in which one end of the tube is tied with cellophane membrane, acts as donor compartment the tube end which is tied with membrane is dipped into the receptor compartment which consists of pH 7.4 phosphate buffer. 2 mL of transferosomes are taken and placed into the donor compartment. The setup is placed on magnetic stirrer and agitated at speed of 1000rpm under optimized temperature. The samples were withdrawn at predetermined intervals and replaced with fresh buffer in receptor chamber.[50] Finally, the release was quantified by spectroscopy methods.

7. Ex-vivo Skin permeation Studies

The tested formulations contained equivalent amount of drug to those of the control. The skin was placed onto vertical Franz diffusion cells with the stratum corneum side up and dermal side down facing the dissolution medium (22 mL of phosphate buffer pH 7.4). The medium was maintained under stirring at 50 rpm and thermostated at 32 ± 0.5 C. 1mL Sample were withdrawn from the receptor compartment at predetermined time intervals and replaced with fresh dissolution medium to maintain sink conditions.[51]

8. Stability studies

Samples of the selected formula were kept in glass amber vials and stored in a dark place at three different temperatures (0°C, 4°C and 25°C) for three months. Samples were withdrawn every two weeks and observed for their appearance and growth of microorganisms and drug content.[52]

Table No: 2 List of drugs representing research work carried on transferosomes.

Name of the drug	Excipients	Remark	Ref
HBsAg	DOTMA, SDC Triton X-100	Cationic unilamellar transferosomes based topical genetic vaccine against hepatitis B elicited significantly higher anti-HBsAg antibody and cytokines level compared with naked DNA.	[53]
Bleomycin	Egg lecithin, sitosterol and tween 80	Bleomycin-incorporated transferosomes enhanced distribution of drug in epidermis and dermis of rat skin.	[54]
Lidocaine	Poloxamer 407	Lidocaine gel containing PANAM G3(permeation	[55]

	soy lecithin dipalmitoyl phosphatidyl choline chloroform	enhancer) showed 1.62 folds increased local anaesthetic efficiency. Gels showed pseudo thixotropic behaviour and 80% of drug released less than 6hrs.	
Famotidine	Polyvinyl alcohol propylene glycol PhospoliponG, Span80.	The % drug release of famotidine transferosome patch was found to be higher than plain famotidine patch. Sustained drug release over 24 hours. The particle size was found to be 215nm. Low poly dispersity index 0.31 indicates particle size uniformity.	[56]
Sildenafil	span80 tween80 Sodium hydroxide Phosphate buffer	Transferosomes encapsulating sildenafil shows 5-fold higher *in-vitro* permeation compared with drug suspension.	[57]
Insulin	Sodium cholate Triethanolamine-HCl buffer.	Ratio of lipids to surfactants 1:1 and ratio of lipids are 5:3{soy lecithin and cholesterol} were incorporated in transferosome gel for transdermal insulin delivery. *In vitro* diffusion studies show zero order kinetics and case 2 transport mechanism of insulin release.	[58]
Acriflavine	Cholesterol Cetosteryl alcohol Soft paraffin Glyceryl monostearate	Transferosomal cream of acriflavine obtained sustained drug release (65.18%) in 24hrs comparison with conventional cream (97.54%) in 8hrs.	[29]
Pentoxifylline	Soy lecithin span80	Optimization of elastic transferosomes formulations for transdermal delivery of pentoxifylline obeys zero order kinetics with fickian diffusion mechanism.	[46]
Cilnidipine	Soy lecithin span80	Cilnidipine loaded transferosomes improved bioavailability than the drug suspension. Gel formulation was safe, less irritant and well tolerable.	[59]
Raloxifene	Soy lecithin span80	Nano transferosomes of raloxifene HCl with sorbitan80 showed better results.	[60]

CONCLUSION

Delivery of various hydrophilic drugs into the skin can be modified by using transferosomes vesicular formulations. Various drugs which are toxic when given through oral route can be given as transferosomes. Transferosomes can be coated and used as carrier for both topical and systemic drug delivery. Hence transferosomes are promising approach for delivery of cosmetics, which improves the skin permeation of drug also used in the targeted drug delivery for various herbal drugs, vaccines and transfection of genetic material. BCS Class III and IV drugs whose bioavailability can be increased and also drugs which undergo extensive first pass metabolism.

Abbreviations

FTIR: Fourier transform infrared spectroscopy, DEE: drug entrapment efficiency, nm: nanometre, HCl: hydrochloric acid, span 80:polyoxyethylene sorbitan monooleate 80, HLB: hydrophilic and lipophilic balance, SEM: scanning electron microscopy, TEM: transmission electron microscopy, RPM: revolutions per minute, EGCG: epigallo catechin gallate, MIC: miconazole, DOTMA: Di-Oleoyloxomropyl-Trimethylammonium Chloride, DOTAP:1,2-dioleyl-trimethylammonium propane, IC_{50}: inhibitory concentration, CT: computed tomography, GNPs: gold nanoparticles, HIV: human immunodeficiency virus, TAT protein: Trans-Activator of transcription, MCF: Michigan Cancer Foundation, SD: standard deviation, KBr: Potassium bromide.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets generated during the current study are available in the Mendeley repository.

Competing interests

Not applicable

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Authors contributions

VM was a major contributor in writing the manuscript. MV analysed and interpretated the data and done corrections.

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