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Research Article

FORMULATION AND EVALUATION OF SUBMICRON EMULSION CONTAINING ENTRAPPED FLUOROQUINOLONE FOR OCULAR DELIVERY

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ABSTRACT

Objective: The objective of the present work was to develop and characterize the submicron emulsion bearing antimicrobial drug sparfloxacin (SF) for improvement of ocular activity by improved retention in eyes. The developed delivery system was results with prolonged drug release as compared to the conventional dosage form.

Methods: Submicron emulsion (SE) prepared by high energy emulsification and sonication to obtain uniform globule size.

Results: Average internal droplets size of the optimized formulation was 0.278±0.6 µm, pH of the optimized formulation was 6.9±0.6 (average of three determinations), and viscosity 2.9±0.5 cps suitable for ocular use. Entrapment of SF was 63±3.4%. Prepared formulation was found to be stable under accelerated and long term storage at 4 and 37°C. No major changes reported on pH and viscosity of optimized formulations. *In vitro,* drug release pattern showed sustain release of SF, a cumulative percent release of SF was found 87.8±1.7% within 24 h. Transmission electron microscopy showed spherical shape and size within 1 µm.

Conclusion: Designed formulation can be a good candidate for ocular drug delivery for severe ocular infections where frequent dosing required for conditions such as endophthalmitis, corneal ulcer, and penetrating trauma.

Keywords: Ocular, Submicron emulsion, Antimicrobial, Prolonged drug release.

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INTRODUCTION

Delivery of drugs to the eye remains a challenge for physicians as well as pharmaceutical scientists. The conventional formulations available commercially for ocular drug delivery are associated with various problems such as loss of drug from drainage, induced lacrimation, normal tear turn over, enzymes present in precorneal area, and low conjunctival absorption [1,2]. Anterior segment of the eye diseases treated with eye drop solutions, unfortunately, these are rapidly drained from the ocular surface and absorption is about <5%. Nanoemulsions with average droplet size are between 100 and 500 nm, stabilized suitable surfactants, and cosurfactants improve penetration of drugs in deep ocular infections [3]. The nanoemulsion offers a sustained release effect and high penetration of the drug through the cornea. This would, therefore, provide the advantages of conventional eye drops (ease of application and high patient compliance) and eliminate their disadvantages (low bioavailability and frequent administration) [4-6].

The term submicron emulsion (SE) is applied to emulsions that possess a dispersed phase mean droplet diameter under 1 µm and also referred to as miniemulsion, ultrafine emulsions, nanoemulsions [7]. Phase behavior studies have shown that size of the droplets governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition [8]. SE can be given by a variety of route such as parenteral, topical, ophthalmic, and nasal delivery and as a vehicle in cosmetics. As compared to conventional ocular dosage form, SE gives better retention time into the eye. Long duration of action reduces the dosage frequency and patient compliance [9]. Fluoroquinoles are very effective for the treatment of severe ocular infection, Sparfloxacin (SF) is bacterial DNA gyrase (topoisomerase IV) inhibitor, and it inhibits DNA replication and transcription [10].

The aim of this study was to load drug, sparfloxacin in submicron emulsion for prolonged drug release and more retention in precorneal area. Designed formulation reduces the side effects of conventional dosage form due to more targeting in ocular globe. Novelty in work was SE, composed of lecithin and poloxamer for selected drug not available in market.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade. SF received as a gift sample from Orbit Pharmaceutical, and poloxamer 188, Soya lecithin, and Soya oil purchased from Loba Chemie.

Methods

Preformulation studies

Solubility of drug determined by equilibrium solubility method and drug identification done by Fourier- transform infrared spectroscopy (FTIR). The infrared spectral assignment of SF was obtained in KBr using FT-IR prestige spectrophotometer.

pH determined by digital pH meter (mkow optics).

λ_{max} determined by ultraviolet (UV)-visible spectrophotometer [Fig. 1] (UV-1700 Shimadzu Corporation, Japan).

Drug and excipient compatibility studies

FTIR spectra analysis: Levels of investigations:

- SF Fig. 2.
- SF+soya lecithin Fig. 3.
- SF+poloxamer Fig. 4.

SE preparation [11-13].

Various formulations were prepared by changing excipient concentration, and drug concentration was equivalent to the marketed

formulation in all batches and prepared by standard procedure with minor modifications [14].

Step I - Preparation of oil phase: - Fixed amount of soya oil and egg lecithin taken and stirred properly in a magnetic stirrer at temperature 60°C, then finally drug added and stirred until it was mixed completely.

Step II - Preparation of aqueous phase: - Fixed amount of water mixed with poloxamer 188 with continuous stirring.

Step III - Oil phase was added to water phase dropwise with continuous stirring in high shear mixer until both the phases mixed completely. Then, this emulsion was subjected to sonication in probe sonicator for 10 min Table 1.

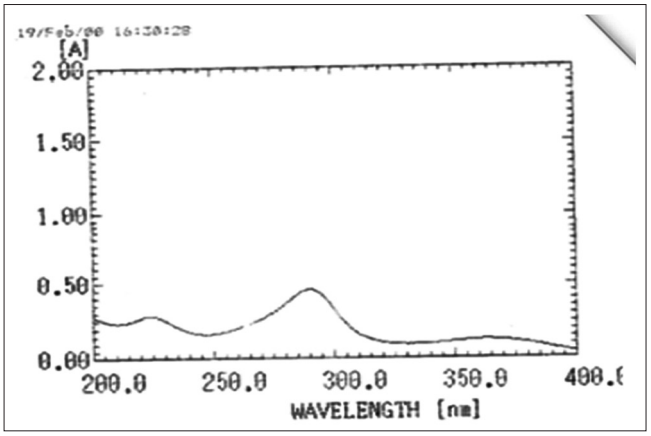


Fig. 1: Absorption maximum of sparfloxacin

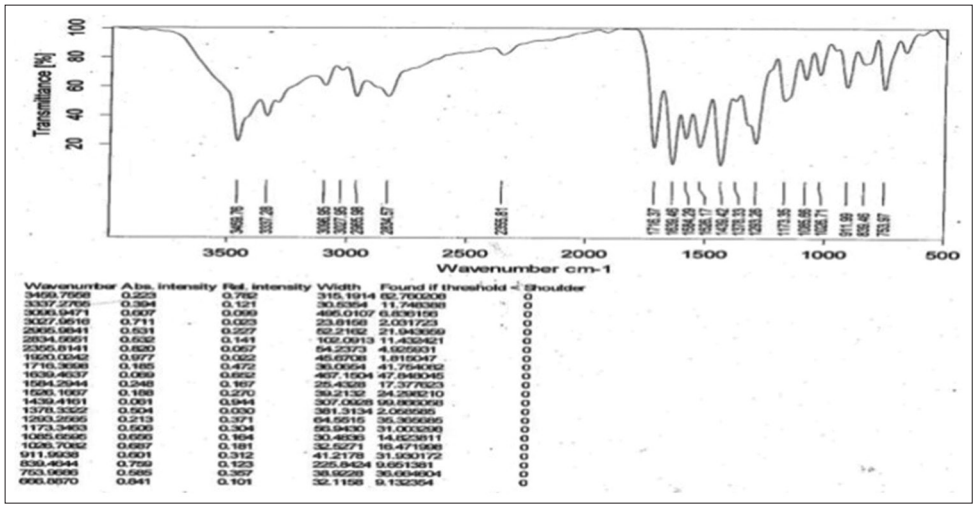
Evaluation of SE [15]

Various parameters were observed during studies like-particle size: Droplets size and size distribution of emulsion system were determined using Malvern Mastersizer 2000 laser diffraction particle analyzer (Malvern Instruments).

Transmission electron microscopy (TEM) was also performed using negative staining with sodium phosphotungstate solution (0.2% w/v). The formulation was dispersed in the staining solution for 30 min at room temperature, placed on a copper grid covered with nitrocellulose, dried under vacuum for at least 24 h and observed under TEM (TEM-1200 EX Japan) [Fig. 5] pH: Determined by digital pH meter. Viscosity: The viscosity of SE of SF was determined by brook field viscometer.

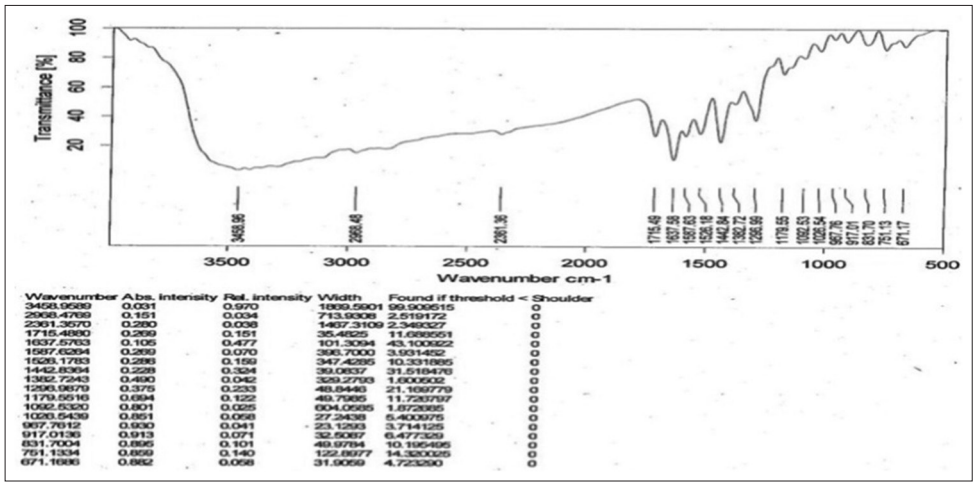
Drug entrapment efficiency: The SF loaded emulsions were centrifuged at 18,000 × g and 4°C for 15 min in ultracentrifuge to separate free drug from the entrapped drug. After centrifugation, the supernatant was collected and analyzed by UV visible spectrophotometer at 289 nm for the free drug or unentrapped drug (A1) concentration to determine the encapsulation efficiency from the total amount of drug (A2).

EE%=(A2-A1/A2) ×100



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold	Shoulder
3469.7559	0.253	0.782	315.1914	52.760208	0
3337.2705	0.394	0.121	30.63894	11.748368	0
3099.9471	0.607	0.069	495.0107	6.836196	0
3027.9616	0.711	0.023	23.8169	2.031723	0
2965.6941	0.531	0.227	52.21652	21.943959	0
2834.9951	0.532	0.141	102.0913	11.432421	0
2356.8141	0.820	0.057	54.2373	4.925931	0
1620.0342	0.977	0.022	46.67706	1.815047	0
1716.3098	0.185	0.472	30.0064	41.754002	0
1639.4637	0.069	0.652	467.1504	47.848345	0
1584.2944	0.248	0.197	25.4328	17.377823	0
1520.1097	0.188	0.270	39.2132	24.298210	0
1439.4161	0.001	0.944	307.0028	99.000000	0
1378.3322	0.504	0.030	381.3134	2.068585	0
1293.2595	0.213	0.371	64.5515	35.306986	0
1173.3403	0.509	0.304	56.9430	31.003508	0
1085.6995	0.656	0.164	30.4838	14.623911	0
1036.7062	0.697	0.181	32.5271	16.471936	0
911.9938	0.601	0.312	41.2178	31.930172	0
839.4944	0.759	0.123	225.8424	9.661381	0
753.9686	0.595	0.357	38.98288	36.004804	0
666.8870	0.841	0.101	32.1158	9.132364	0

Fig. 2: Fourier transform infrared spectra of sparfloxacin



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold	Shoulder
3458.9589	0.031	0.970	1869.5901	99.909515	0
2968.4769	0.151	0.034	713.9306	2.519172	0
2361.3670	0.280	0.038	1467.3109	2.349327	0
1716.4880	0.269	0.151	35.4825	11.686551	0
1637.5783	0.105	0.477	101.3094	43.100922	0
1587.6264	0.269	0.070	366.7000	3.931452	0
1526.1783	0.288	0.159	347.4235	10.331885	0
1442.8394	0.228	0.324	39.0837	31.516476	0
1382.7243	0.490	0.042	359.2788	1.600502	0
1290.9870	0.375	0.233	48.8448	21.109779	0
1179.5516	0.694	0.122	49.7585	11.729797	0
1092.5320	0.801	0.025	604.0585	1.872885	0
1030.5439	0.851	0.058	27.2438	5.400375	0
987.7612	0.930	0.041	23.1253	3.714125	0
917.0136	0.913	0.071	32.5087	6.477329	0
831.7004	0.896	0.101	49.9784	10.195495	0
751.1334	0.859	0.140	122.8677	14.320035	0
671.1886	0.882	0.058	31.9059	4.723290	0

Fig. 3: Fourier transform infrared spectra of sparfloxacin+soya lecithin

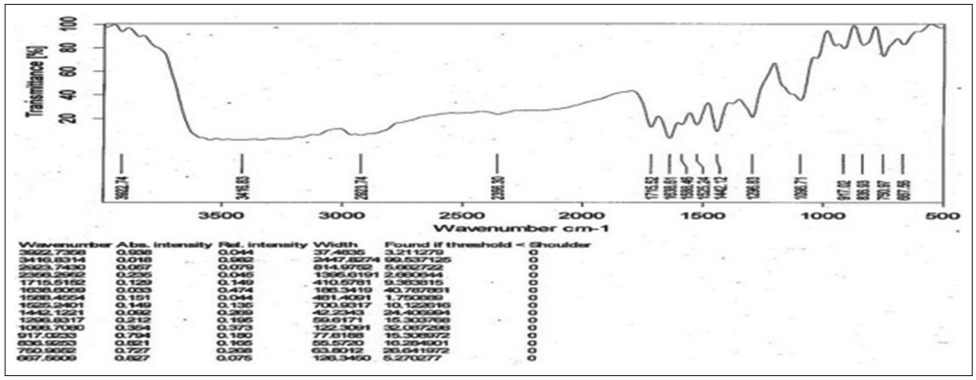


Fig. 4: Fourier transform infrared spectra of sparfloxacin+poloxamer 188

Table 1: Composition of optimized formulations (%w/w)

Excipients	SE	SE-SF
Soya oil	10	10
Poloxamer 188	2.5	2.5
Egg lecithin	1.25	1.25
SF	-	0.3
Water	100	100

SE: Submicron emulsion, SF: Sparfloxacin

Stability assessment: During stability, different parameters such as the drug content, pH, and droplet size distribution were monitored over periods of 90 days stored at 4°C, 23°C, and 37°C [16]. The creaming and the phase separation were assessed visually at different time intervals. To evaluate its mechanical and physical resistance, the emulsion was subjected to accelerated mechanical stress and its globule size distribution was measured before and after shaking at 100 strokes per min over 48 h at room temperature [17,18].

In vitro drug release: The *in vitro* release profile of SE was performed using dialysis bag technique [19]. The SE within dialysis bag immersed into 100 ml of phosphate buffer solution, pH 7.4 at 37°C, and magnetically stirred at 100 rpm. Aliquots were withdrawn from the release medium and replaced with the same amount of the phosphate buffer to maintain sink condition. The data obtained from *in vitro* drug release studies were fitted to various release models such as zero-order, first-order, Higuchi, and Korsmeyer's–Peppas model to understand the mechanism of drug release from the emulsion. The content of SF from the withdrawn sample was measured after dilution at UV visible spectrophotometer at 289 nm [20,21].

RESULTS

Solubility

The solubility of the drug was found in 0.1 N sodium hydroxide and dilute acetic acid. Melting point was found to be 262–265°C.

pH and viscosity of the SE

Observed pH was 6.9±0.6 (average of three determinations), and viscosity of SE was found 2.9±0.5 cps (average of three determinations).

Mean particle size

Mean particle size was found to be 0.278±0.6 µm.

Drug entrapment efficiency

The drug entrapment in the SE was found to be 63±3.4%.

Drug excipient compatibility

Drug-excipient compatibility studies done using FT-IR spectroscopy. The drug was compatible with excipient as found in FT-IR analysis.

SE was a plain formulation without drug and SE-SF contained SF in a concentration equivalent to the marketed formulation.

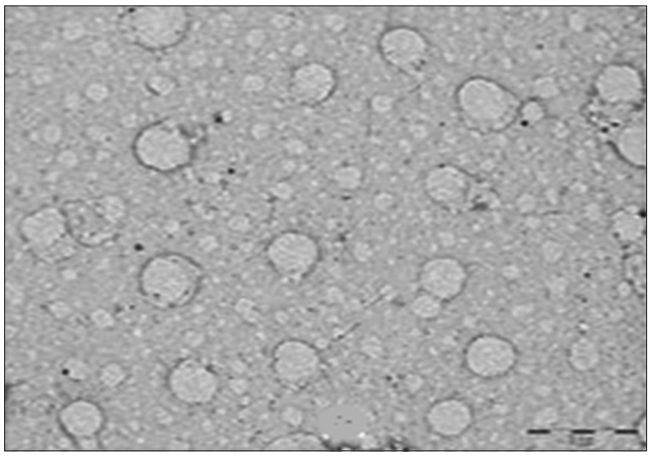


Fig. 5: Transmission electron microscopy of submicron emulsion-sparfloxacin Stability studies of optimized formulation in Table 2 showing stability study data of optimized formulation.

The storage stability of formulations was checked in terms of change in globules size, viscosity, and pH of emulsion sample stored at 4°C and 37°C over a period of 3 months, no major changes noticed. Values are expressed as means of three different batches.

DISCUSSION

SF available as eye drops for corneal and conjunctival infection but <1% bioavailability observed [22]. SE is more suitable than solutions, ointments, gels, and nanoparticles. Ointment improves retention but not comfortable due to the semisolid consistency of the preparations. Nanoparticles consisting of poly alkyl cyanoacrylate damaged the corneal epithelium by disrupting the cell membrane [23]. Blurred vision reported with gels as more viscous formulation than SE. SF entrapped successfully within oil phase of SE lecithin was used as surfactant and poloxamer 188 as cosurfactant. Various factors mentioned in Fig. 6 can be overcome by designed formulation. The appearance of SF was visually observed and complies with the standard limit. Melting point, UV and FT-IR spectroscopy of SF confirms that drug comply with standard specifications. The drug was practically insoluble in water so entrapped in the internal phase of oil in water emulsion. Cosurfactant is very essential for the stability of SE [24,25]. Poloxamer 188 concentration is critical for prolonged emulsion stability [26]. Formulations were prepared below 70°C as higher temperature degrades lecithin.

Optimization of batch was done by evaluation of their droplets size, viscosity, pH, and stability. The pH of the optimized formulation was satisfactory and suitable for ocular use. The average globule size in the dispersed phase of the formulation was in submicron range indicated its suitability for ocular use. As particle size requirement in such cases is <2 µm [27]. Sonication of formulations further reduces

the droplets size. Homogenization speed also plays an important role to achieve submicron size as speed more than 15000 results with a reduction in droplets size [28]. The drug content in the SE was found to be 63±3.4% represents sufficient entrapment of drug in the internal phase. Viscosity and pH of final formulation indicate its suitability for the ocular route. Viscosity for ocular use reported, 2–3 cps considered as adequate viscosity for ocular preparation [3]. The data obtained from *in vitro* release studies of SE were satisfactory and showed sustained release profile Fig. 7 thus more drug retention is possible in the precorneal area. Release mechanism of the drug from formulation was due to diffusion and partition from oily core to the aqueous phase. TEM confirms spherical shape and size within 1 µm which is suitable for the ocular application. TEM freeze-fracturing examination revealed that a mixed-emulsifier monolayer film was established at the o/w interface of the SE.

In stability studies, during the excessive shaking, no phase separation or creaming and change in mean droplet size observed because the interfacial film formed by lecithin and poloxamer was strong enough to prevent droplet coalescence on any physical and thermal condition.

CONCLUSION

From this study, it is concluded that the SE can be an effective dosage form for more retention in eyes. It has the convenience of drop and comfortable due to droplet size <1 µm and will improve the activity of SF as compare to another conventional ocular dosage form. The

Table 2: Long-term stability studies at 4°C and 37°C for 3 months

Days	pH	Viscosity cp	Particle size µm	pH	Viscosity cps	Particle size µm
	4°C			37°C		
0	6.9±0.6	2.9±0.5	0.278±0.6	6.9±0.6	2.9±0.5	0.278±0.6
15	6.9±0.4	2.2±0.6	0.302±0.7	6.9±0.3	2.8±0.2	0.279±0.8
30	6.7±0.2	2.3±0.6	0.305±0.6	6.9±0.3	2.7±0.4	0,274±0.6
60	6.5±0.4	2.4±0.3	0.311±0.8	6.7±0.3	2.5±0.5	0.300±0.2
90	6.3±0.3	2.4±0.6	0.320±0.9	6.4±0.3	2.1±0.2	0.320±0.3

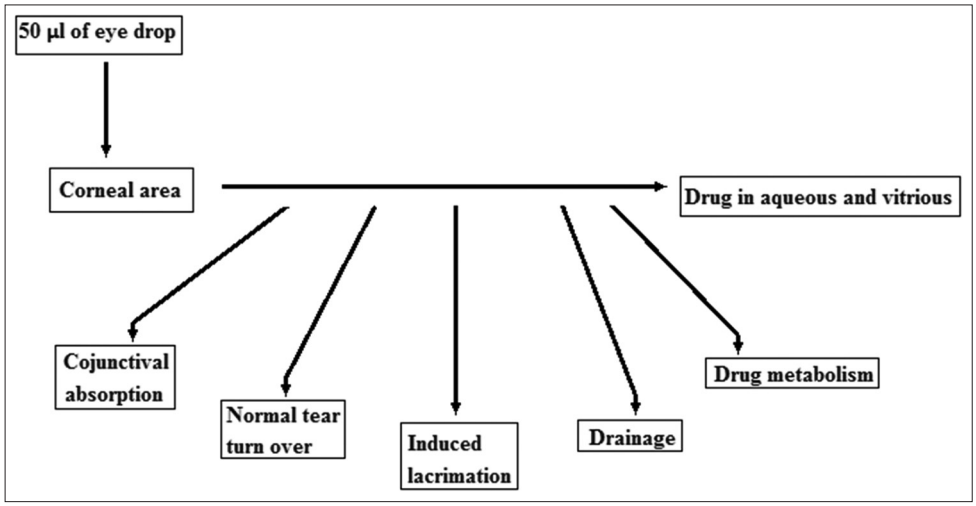


Fig. 6: Various factors competing corneal absorption [3]

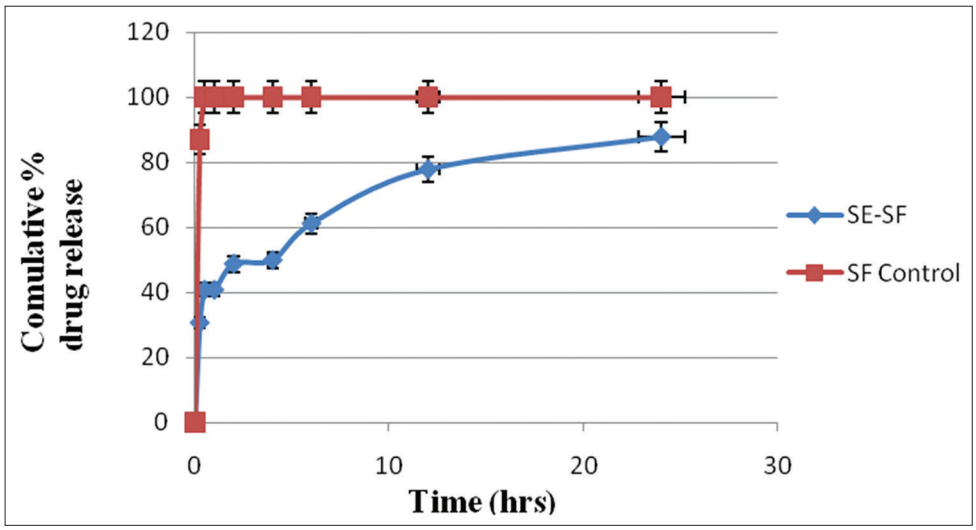


Fig. 7: *In-vitro* drug release behavior of sparfloxacin, prolonged release observed as compared to marketed control. 87.8±1.7% found in 24 h. Data represented as a mean average value of three determinations (n=3)

discussed work will be benefitted by researchers and formulators for preparation of safe, stable, and effective formulation.

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AUTHOR'S CONTRIBUTION

Design of the study, all experimental work and data compilation done by author Durga Pandey under the guidance of Dr. Deepti Jain.

CONFLICTS OF INTEREST

Declared none.

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