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Original Article

FORMULATION OF ATOVAQUONE TABLET USING BIOSURFACTANT IN A TERNARY SOLID DISPERSION SYSTEM: *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT

Objective: The goal of the present investigation was to improve the solubility and bioavailability of atovaquone tablet, using in-house biosynthesized biosurfactant in the ternary system of solid dispersion containing hydrophilic polymers with varying concentrations of biosurfactant. Atovaquone is an anti-malarial agent and belongs to biopharmaceutical classification system class IV.

Methods: The solid dispersion of binary and ternary mixture was prepared using hydroxyl propyl methyl cellulose (HPMC) and biosurfactant respectively by a solvent evaporation method. All the atovaquone tablet formulations were prepared by incorporation of physical mixture, binary and ternary solid dispersed products with excipients by direct compression method. Pre-compression and post-compression parameters of atovaquone tablets were evaluated. *In vivo* bioavailability study was performed using female albino rabbits.

Results: *In vitro* dissolution profile of binary and ternary system of solid dispersion products showed 8.65% and 34.64% respectively. Precompression and post-compression values of all atovaquone tablets formulations were within the specified limits. *In vitro* dissolution efficiency of F2 and F5 were 1.44 fold and 6.62 fold respectively, in accordance to the F1. *In vivo* study revealed that bioavailability of optimized formulation F5 was increased by 2.5 times and time to reach peak concentration was reduced to 1.4 h, in accordance to pure atovaquone suspension.

Conclusion: Potential application of biosurfactant in the solid dosage form of atovaquone tablet was proved for enhanced dissolution rate and bioavailability of atovaquone for malaria treatment.

Keywords: Atovaquone, Solid dispersion, Physical mixture, Ternary system, Biosurfactant, Bioavailability

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INTRODUCTION

Atovaquone is unique naphthoquinone with a broad-spectrum antiprotozoal agent, belongs to the biopharmaceutical classification system (BCS) class IV and exhibits low and variable oral bioavailability (46%) due to its poor aqueous solubility (less than 0.25µg/ml) [1]. Enhancement of dissolution and thereby its bioavailability is a primary requisite of an oral drug delivery of atovaquone. Numerous technologies have been developed for enhanced solubility of poorly water-soluble drug such as micronization, salt-formation, solid dispersion, complexation etc. Solid dispersion using a water-soluble carrier system is the most commercially viable technology applicable in recent years. The ternary system of solid dispersion is the addition of surfactants in a binary system and also plays essential processes of modification for maximum solubility of the drug in the aqueous fluid [2].

Nonionic surfactants were used for enhancement of solubility of poorly water-soluble drug in the range of 5%w/w to 20%w/w. Synthetic surfactants are non-biodegradable. In recent years novel biotechnological products are produced from various micro-organisms through fermentation. Biosurfactants are produced from specific microorganisms with economic substrates such as waste fried oils, molasses etc, in a suitable media and environmental conditions. Major advantages of biosurfactants over the synthetic surfactants are, at low concentration maximum solubility of poorly soluble drugs, stable in all pH range, very less toxicity and biodegradability. This is due to its unique chemical composition with fatty acid, monosaccharide and amino acids [3, 4].

From the literature survey, it was revealed that the potential application of biosurfactant has not been explored as a solubility modulator and permeability catalyst for in-soluble and less permeable drugs. In this study, atovaquone drug was selected as model drug for its enhancement of solubility and permeability. The aim of our present study was to formulate atovaquone tablet of the physical mixture, binary and ternary solid dispersion system using water-soluble carrier hydroxyl propyl methyl cellulose (HPMC) with biosurfactant and compare its dissolution rate and bioavailability with poloaxmer 407 a nonionic surfactant.

MATERIALS AND METHODS

Materials

Gift sample of atovaquone was procured from Matrix Lab Ltd Hyderabad. Poloaxmer 407 from venous ethoxy ether Pvt Ltd, Goa. Hydroxypropyl methyl cellulose (HPMC) from Colorcon industry, Goa. Lactose, polyvinyl pyrrolidone (PVP), magnesium stearate and talc from Balji chemicals, Mumbai. All other chemicals were from SD fine chem India (AR grade).

Methods

In-house biosynthesis of biosurfactant

Biosurfactant was produced from the biotechnology processes using Flavobacterium Sp 2495(Chandigarh) in a modified mineral salt media. Molasses 5%w/v, waste fried oil 5%w/v and 1%w/v peptone, 0.5%w/v ammonium chloride were used as carbon and nitrogen source respectively. Trace elements were ferric chloride and vitamin B6. Fermentation conditions were maintained at pH at 7.4, temperature 32±1 °C, aeration 40% and rpm 150. Duration of fermentation was 72 h (sorotus lite fermentor). The fermented fluid was separated by solvent extraction using a methanol-water mixture (1:2). Purification was carried out using 5N hydrochloric acid (HCl) by the precipitation method. The product was dried at 40 °C for 3 h and passed through 60 mesh and stored in an airtight container at room temperature. An oral toxicity study was performed on albumin rats as per organization for economic co-operation and development (OECD) guidelines. It was found that 2000 mg per kg body weight of albumin rats was safe without mortality and toxicity. The yield of biosurfactant was 2.5g per liter [5].

Preformulation studies

Compatibility studies

Compatibility study of drug and excipients was carried out through fourier transmission infrared spectrophotometer (FTIR) (Shimadzu Japan) using potassium bromide (KBr) probe technique, scanned from 4000-400 cm-1. Graphs were recorded and analyzed for comparatively for compatibility studies.

The solid dispersion of atovaquone with HPMC and bio surfactant

Solvent evaporation technique was adopted for the preparation of the solid dispersion product. Atovaquone was dissolved in ethanol. Hydroxyl propyl methyl cellulose was dissolved in water in the ratio 1:2 (drug: polymer) using a magnetic stirrer. The drug solution was added to the aqueous hydroxyl propyl methyl cellulose solution with constant stirring. The ternary system was prepared by adding various concentrations of surfactants to the drug-polymer solution as shown in the table 1. The mixture was evaporated on 8 cm diameter china dish for 48 h at room temperature. The solid mass obtained was dried at 40 °C in the oven for 4 h, pulverized, sieved through 60 mesh and stored in an airtight container until use [6, 7].

Table 1: Solid dispersion of atovaquone with hpmc and surfactants

Ingredients	Formulation code							
	Unit	S0	S1	S2	S3	S4	S5	S6	S7
Atovaquone	mg	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
HPMC	mg	----	125	125	125	125	125	125	125
Biosurfactant	mg	----	--	2	4	6	-	-	---
Poloxamer407	mg	----	--	--	-	-	10	20	30
Ethanol	ml	----	30	30	30	30	30	30	30

Preparation of atovaquone tablet using the solid dispersed product

Compressed tablets containing physical mixture and solid dispersed product with and without surfactants were prepared separately by direct compression method. Diluent lactose, dry binder polyvinyl pyrrolidone, and lubricants talc, magnesium stearate were used as excipients. All ingredients were sieved through 40 mesh and blended with lubricants and compressed in 10 stations rotary machine (M/s Karnavati) at 6 kg/cm2 hardness using 9 mm flat punches. All the formulation batches were shown in the table 2.

Table 2: Formulation of atovaquone tablets

Ingredients (mg)	Formulation code					
	F1	F2	F3	F4	F5	F6
Atovaquone	62.5	62.5	62.5	--	--	--
Solid dispersion system	---	---	---	S1	S3	S7
Biosurfactant	--	4	--	--	--	--
Poloxamer407	---	--	30	--	--	--
Lactose	159.5	155.5	129.5	34.5	30.5	4.5
PVP	5	5	5	5	5	5
Mg stearate	1	1	1	1	1	1
Talc	2	2	2	2	2	2
Total weight	230	230	230	230	230	230

S1, S3, S7. Solid dispersed products

Evaluation of atovaquone solid dispersed products

***In vitro* dissolution**

All solid dispersion products and pure drug were subjected for dissolution profile using USP dissolution apparatus (Electro lab India TD 80) at 50 rpm in 1.2 pH buffer media for 1 h. Sample equivalent to 62.5 mg of atovaquone was placed in the tea bag and tied to the paddle. Aliquot samples were withdrawn from 900 ml at specified intervals and analyzed using uv-vis spectrophotometer (UV 1900 Shimadzu Japan) at λmax 220 nm under sink condition [8].

Evaluation of atovaquone tablets

Pre-compression

All the formulation of atovaquone lubricated powder blends was subjected for micromeritics properties using conventional methods. Bulk density and tapped density was determined by using measuring cylinder method. Hausner's ratios, Carr's index, were determined using the bulk density and tapped density data. An angle of repose was determined by conventional funnel method [9].

Post-compression

All batches of atovaquone tablets were subjected to non-pharmacopieal and pharmacopieal tests. Tablet thickness, hardness, was determined using a vernier caliper and Monsanto hardness tester respectively. Friability, disintegration test was performed as per Indian pharmacopeia (IP 2014) [10].

***In vitro* dissolution rate study**

The dissolution rate of atovaquone tablets prepared was studied in 900 ml of acidic buffer of pH 1.2 using USP dissolution apparatus type I at 50 rpm and the 37±1 °C temperature was maintained throughout the study. Aliquot samples of dissolution media (5 ml) were withdrawn through a filter (0.45µ) syringe at different intervals of time up 60 min. All the samples were suitably diluted and assayed at λmax 220 nm and sink condition was maintained [11, 12].

Dissolution efficiency

The concept of dissolution efficiency (DE) was proposed by Khan and Rodes in 1975 is defined by

$$DEt\% = \frac{\int_0^t Y.dt}{Q100t} X100$$

Where Y is the percentage of drug dissolved at any time t, Q100 is the100 percentage of dissolved product, DE is the area under the dissolution curve between time points 0 and t expressed as a percentage of the curve at maximum dissolution, Q100, over the same time period [13].

***In vivo* bioavailability study**

Experiments were conducted in compliance with the committee for the purpose of control and supervision of experiments on animals (CPCSEA) and experimental protocol approval from institutional animal's ethics of our institution (Registered No221). Female albino rabbits (1.2 to 1.5 kg) were used in the study. Animals were divided into two groups. Each group containing three rabbits was housed in polypropylene cages in a room with the controlled temperature of 25±3 °C and relative humidity (RH) 60±5% in 12 h light/12 h night cycle. The animals were fed with a standard pelleted diet and purified water ad libitum throughout the experiment. Group I and group II received atovaquone (25 mg/kg) oral suspension and optimized atovaquone tablet formulation (F5) respectively, through oral silicon tube after 12 h fasting. Blood samples were collected from the marginal vein of the ear of rabbits at an interval

0.5 h, 1 h, 3 h, 6 h, 12 h in a heparinized tube. Plasma samples were obtained by centrifuged at 5000Xg for 10 min and stored at-20 °C until analyzed. All the plasma samples were analyzed for drug content through high-performance liquid chromatography (HPLC SPD-M20A Shimadzu) with the following conditions. Chromatographic separation was developed for the quantitative serum level of atovaquone using column C18. Mobile phase consists of 0.1%v/v formic acid and methanol in the ratio of 20:80. The aqueous phase was eluted at a flow rate of 1 ml/min, volume injected was 20 µl and retention time was 7 to 8 min with the 15 min run time. The effluent was monitored at UV 254 nm. Standard calibration was constructed by spike method using a different concentration of drug in serum. All other chromatographic separation conditions were kept constant [14].

Pharmacokinetic analysis of data

The obtained data were expressed as mean±SEM. Student t-test and ANOVA were used to analyze and compare the results p< 0.05 was considered significant in all the tests. Systat 13 software was used in the study.

Maximum peak plasma concentration (Cmax) and time to reach maximum plasma concentration (t max) were obtained from the plasma concentration v/s time graph, area under the curve (AUC) calculated from the trapezoidal rule [15].

RESULTS AND DISCUSSION

Drug and excipients compatibility study

The FTIR graph interpretation of atovaquone and excipients showed the characteristics peaks of atovaquone at 3375 cm-1(C-O stretch),1658 cm-1(aromatic C=O stretch),729.09 cm-1(C-Cl stretch),2924(aromatic C-H stretch) were retained. No significant differences were observed, indicative of compatibility with drug and excipients. (fig. 1)

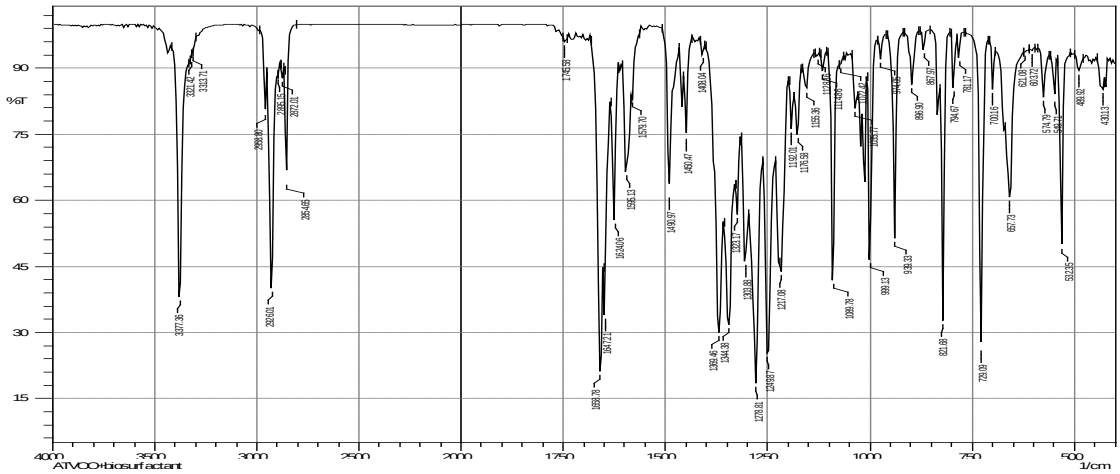

Fig. 1: FTIR of atovaquone and excipients

Solid dispersion of atovaquone

The dissolution medium in United State pharmacopeia (USP) for atovaquone drug is isopropyl alcohol 40% v/v with potassium hydrogen phosphate pH 8. In the present study 0.1N hydrochloric acid pH 1.2 dissolution medium was used to mimic the biological fluid. All the formulated solid dispersion batches, dissolution rate profiles were shown in the fig. 2. Batch S1 showed two fold increases in dissolution rate in accordance with batch S0. All the formulation batches of ternary systems of biosurfactant showed an average two-fold increase in the dissolution rate as compared to all batches of poloaxmer 407. Enhanced percentage of dissolution rate of atovaquone drug could be the effective penetration and wettability by biosurfactant, a similar study was reported by Swati S *et al.* [16]. Dissolution rates of batch S3 and S4 did not show significant differences in the percentage of drug release. Hence S3 formulation was chosen for further study. A comparative dissolution profile of binary and ternary systems of solid dispersion is shown in fig. 3.

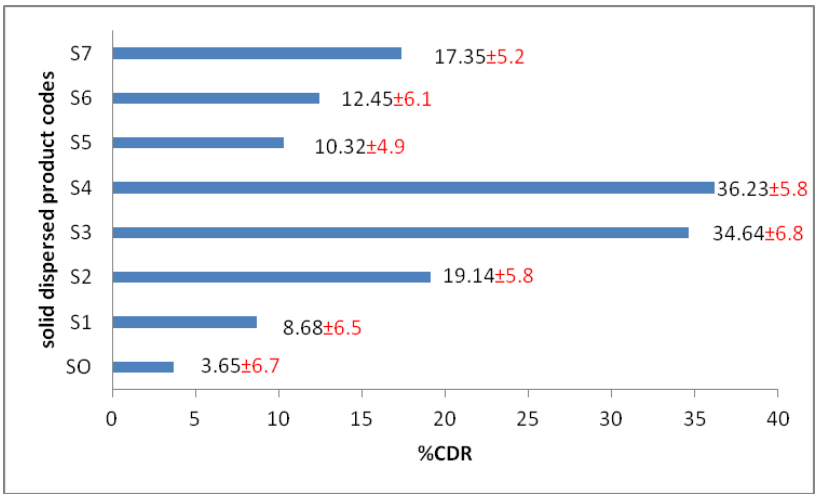

Fig. 2: Comparative dissolution profile of solid dispersed products from S0 to S7 for 1h. (n=3)(mean±SD)

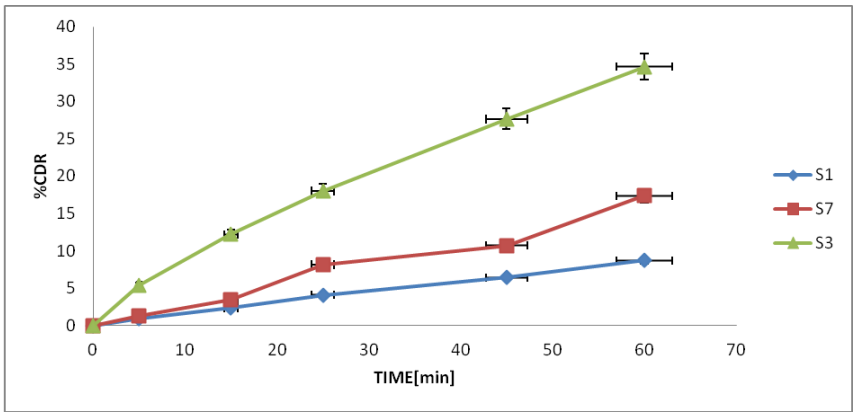

Fig. 3: Comparative dissolution profile of binary (S1) and ternary system of solid dispersion (S3, S7). (n=3) (mean±SD)

Atovaquone tablet evaluation

Atovaquone tablets formulations were prepared from the physical mixture (F1, F2, F3) and solid dispersed product blends of binary (F4) and ternary systems (F5, F6) using the dry granulation method. These two processing steps (physical mixture and solid dispersion) were adapted to atovaquone tablet preparation, to evaluate the appropriate method of addition of surfactants to achieve an enhanced dissolution rate of atovaquone.

Pre-compression

All the formulated atovaquone formulation powder blends were subjected for micromeritics properties. The compressibility index, Hausner's ratio, and angle of repose were within the specified limits of 10.8% to 12.19%, 1.11 to 1.27 and 25.60 to 28.30 respectively. From table 3 results indicates that all formulations were having a good free flowing and compressible properties.

Table 3: Micrometric properties of lubricated powder of atovaquone

Formulation code	Bulk density* (g/cm^3)	Tapped density* (g/cm^3)	Compressibility index (%)*	Hausner's ratio*	Angle of repose*
F1	0.49±0.02	0.55±0.02	12.19±0.03	1.13±0.08	25.60±2.07
F2	0.48±0.01	0.54±0.04	10.83±0.04	1.11±0.07	26.71±0.57
F3	0.47±0.09	0.55±0.03	10.89±0.04	1.12±0.04	27.13±0.75
F4	0.48±0.08	0.53±0.02	13.01±0.01	1.13±0.04	26.40±0.43
F5	0.47±0.01	0.53±0.02	11.70±0.07	1.10±0.02	27.58±0.35
F6	0.48±0.03	0.53±0.04	10.93±0.09	1.13±0.09	28.30±0.01

*mean±SD (n=6)

Table 4: Post-compression parameters

Code	Weight variation * (mg)	Thickness (mm) *	Hardness (kg/cm^2)*	Friability (%)*	Drug Content (%)*	Disintegration time (min)*
F1	230±2.01	3.5±0.5	3.3±0.35	0.32±0.091	98.22±0.50	7.50±0.01
F2	230±3.04	3.5±0.6	3.5±0.50	0.39±0.015	96.70±1.06	5.5.±0.04
F3	230±3.25	3.5±0.5	3.3±0.35	0.36±0.028	99.05±0.43	6.60±0.25
F4	230±2.02	4.5±0.5	3.4±0.40	0.19±0.041	98.03±0.37	6.30±0.02
F5	230±3.05	4.25±0.5	3.3±0.35	0.21±0.028	97.45±0.43	4.50±0.05
F6	230±3.04	3.35±0.5	3.40±0.40	0.25±0.013	98.25±0.37	5.50±0.04

*mean±SD (n=6)

Post-compression

Pharmacopieal and non–pharmacopieal tests for atovaquone tablets results were shown in the table 4. Weight variation test, thickness, hardness, friability, and drug content results were within the specified limits. Results of disintegration test for batch F2 and F5 indicated that addition of biosurfactant in the ternary system can improve the disintegration time. Similar results were also reported by Shaji A *et al.* [17].

***In vitro* dissolution profile**

In vitro dissolution of a physical blend of atovaquone tablets F1, F2 and F3 showed 5.7%, 9.0% and 7.23% drug release respectively. Binary and ternary blend system atovaquone tablets, F4, F5 and F6 showed 10.95%, 37.65% and 18.64% respectively (fig. 4). The numbers of fold increase in dissolution efficiency for F2, F3, F4, F5, and F6 were 1.44, 1.28, 1.94, 6.62, and 3.51 respectively (table 5). *In vitro* dissolution profile revealed that physical mixture blend(F2) and the ternary system blend (F5) of biosurfactant showed 2 fold and 6.6 fold an increase in the percentage of dissolution rate respectively, calculated at DE45. From these results conclude that the most effective step for the addition of surfactant in the tablet formulation was in the ternary system. Mohamed FK *et al.* [18] reported that addition of surfactant in ternary solid dispersion system showed an increased in dissolution rate of less water-soluble drug.

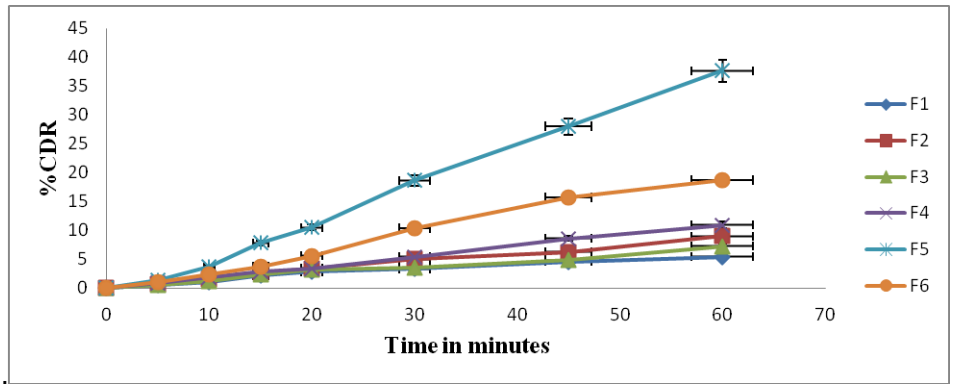

Fig. 4: *In vitro* dissolution of atovaquone tablet F1 to F6. (n=3) (mean±SD)

Table 5: Dissolution efficiency of F1 to F6 (n=3) (mean±SD)

Formulation	F1	F2	F3	F4	F5	F6
DE45	5.02±5.3	7.2±6.8	6.45±7.2	9.75±5.8	33.25±4.8	17.65±5.6
No of fold increase in dissolution rate	---	1.44	1.28	1.94	6.62	3.51

***In vivo* study**

From *in vivo* data, model-independent pharmacokinetic parameters, Cmax, tmax, and AUC were summarized in the table 6. The mean AUC of F5 and oral suspension were 240.73 µg/ml/h and 88.71 µg/ml/h respectively. Formulation F5 exhibited a significant increase Cmax: 3 folds and tmax was shortened by 1.4h in accordance to oral suspension (fig. 5). Formulation strategy for BCS class IV drug has been met with moderate success by incorporation of biosurfactant in the atovaquone dosage form. The beneficial effect of biosurfactant could be enhanced solubility-dissolution kinetics is the probable driving force behind the improved pharmacokinetic properties and effective softening of tight junction of the intestinal cell lining. Improved bioavailability study was reported by Borhardev *et al.* [19].

Table 6: *In vivo* study of F5 and oral suspension

PK parameters	Oral suspension*	F5*
Cmax µg/ml	10.56±12.51	30.12±14.32
t max h	6.5±14.32	5.1±13.58
AUC µg/ml/h	88.71±23.17	240.73±24.56

*mean±SD (n=3)

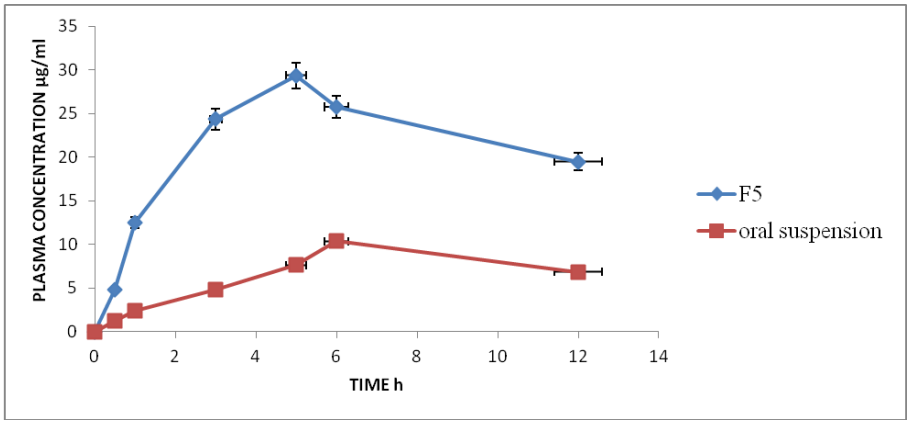

Fig. 5: *In vivo* study of F5 and atovaquone suspension. (n=3) (mean±SD)

CONCLUSION

The main objective of the present study was to evaluate the effect of biosurfactant on dissolution rate and bioavailability of BCS class IV drug. Atovaquone was selected as a model drug. Formulations of a solid dispersion in a binary and ternary system with biosurfactant and synthetic surfactants were prepared and compared. The ternary system was proved to be better than the binary system for enhancement of dissolution rate. Atovaquone tablets prepared from physical mixture blend and solid dispersed products. Evaluated results indicated that addition of biosurfactant in the ternary system better than the physical mixture. *In vivo* study indicated the biosurfactant can also act as bioenhancer for BCS class IV drugs. However, detailed toxicology and stability studies are needed for the biosurfactant to be approved from regulatory authorities as the surface active agent.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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