Research Paper

Investigation of Different Formulation Approaches to Enhance Oral Bioavailability of Paromomycin

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Paromomycin, an aminoglycoside antibiotic, is used to treat visceral leishmaniasis as an intramuscular injection daily for 21 days since it has limited oral bioavailability. The objective of this investigation is to improve the oral bioavailability through formulation development for increased patient compliance and convenience. Comparative pharmacokinetic studies of intravenous and oral administration of paromomycin in mice to evaluate different formulation approaches to increase oral bioavailability were carried out. Following intravenous injection of 15 mg/kg to mice, paromomycin exhibited low plasma clearance (8 ml/min/kg) with elimination half-life of 2.6 h. Following oral administration of 500 mg/kg of a suspension formulation using carboxymethylcellulose to mice, referred to as formulation 1, paromomycin showed very low or negligible oral bioavailability (%F=0.3). Different formulation approaches were made by employing a variety of FDA-approved novel excipients belonging to glycols, fatty acids, alcohols and surfactants alone and in various combinations. *In vivo* pharmacokinetic studies with these formulations demonstrated improved oral bioavailability of paromomycin in mice. Formulation approaches tested in the present research project were successful in improving the oral bioavailability of paromomycin from 0.3% to a maximum of 9%. Overall, the increase in paromomycin bioavailability ranged 1-30 fold with different formulations compared to carboxymethylcellulose formulation. Formulation with 10% Gelucire 44/14, 10% Solutol HS 15, 30% propylene glycol and 50% normal saline showed higher bioavailability compared to formulation 1. This research provided evidence that improved bioavailability of paromomycin can be achieved through formulation development, which eventually could result in making the most tedious and painful intramuscular therapy redundant.

Key words: Paromomycin, visceral leishmaniasis, pharmacokinetics, formulation approach, permeability, metabolism, P-gp substrate

Visceral leishmaniasis (VL) is the most common vector borne parasitic disease caused by sand-fly. Out of 5 00 000 cases detected in the world, most were from Asia, Sudan and Brazil[1]. Treatment options for VL are limited to antimonials, paromomycin, amphotericin B, and miltefosine. The endemic region where antimonials failed with high drug resistance was recorded in India[2].

Paromomycin was first developed in 1950s for local infections and was approved by Government of India in 2006 for the treatment of VL[3,4]. Although it was proved to be effective against VL way back in 1960s, only in 2006 it was approved by Government of India for the treatment of VL[1]. The major drawback with paromomycin is its negligible intestinal permeability with faecal excretion of >99%[5,6]. Due to its limitation of no oral formulation availability due to poor bioavailability, the treatment is being done by intramuscular (IM) route of administration[7]. The treatment regime involves 21 d IM injection of paromomycin at the dose of 20 mg/kg and more than 95% cure rate can be obtained with 6 mo of treatment[7]. An additional challenge for the treatment in the rural areas like Bihar and other endemic regions has poor hospital infrastructure[8]. Therefore, an oral dosage form of paromomycin is necessary for patient amenability as an advantage over the available regime of IM injection.

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Not many research efforts are made pharmaceutical companies to improve paromomycin bioavailability, and as a result there is scarcity of data on paromomycin formulation and pharmacokinetics[9]. Hence, an attempt has been made in the current investigation to develop better oral formulations of paromomycin with the aim to improve oral bioavailability. Paromomycin is a substrate of efflux transporter, P-glycoprotein (P-gp) leading to its low oral bioavailability. Paromomycin is highly water soluble with a large molecular weight[10]. The oral bioavailability in human is almost zero or negligible following oral administration[5]. In view of these limitations, authors proposed that oral exposure of paromomycin could be enhanced using different formulation approaches either by increasing the membrane fluidity or by inhibiting efflux transporters. No studies have thus far reported oral pharmacokinetics of paromomycin in rodents. This would be the first preclinical report on the comparative pharmacokinetics of paromomycin using various formulations that are either permeability enhancers or P-gp inhibitors or their combinations to improve the oral bioavailability.

In the present investigation, various oral formulations were prepared using lipids, surfactants and co-surfactants excipients. *In vivo* experiments were performed in mice to determine the oral bioavailability of paromomycin from these formulations. Mouse was selected as a preclinical species for the pharmacokinetic studies since most of the *in vivo* efficacy models for VL are in mouse and the promising formulations can be tested orally to demonstrate pharmacokinetic and pharmacodynamics (PKPD) correlations.

MATERIALS AND METHODS

Paromomycin sulphate was purchased from Sigma, Germany. Labrasol was purchased from Gattefose, France. Menthol was purchased from Merck, India. Pluronic F-127 purchased from Sigma, USA. Sodium cholate was purchased from Sigma, New Zealand. Gelucire 44/14, Transcutol, Labrafil and Maisine were purchased from Gattefosse, France. Capmul MCM C8, Captex and Acconon MC 82 were obtained from Abitec Corp, Janesville, WI, USA. Cremophor EL, Tween 80 and propylene glycol were procured from Sigma Aldrich, St. Louis, MO, USA. Normal saline was procured from Baxter, India.

Preparation of dose formulations:

Formulation for intravenous (IV) dosing was prepared in 100% normal saline. Oral dosing formulations were prepared either in carboxymethyl cellulose (CMC, 0.5 % w/v in RO water) or with different excipients. All formulations were prepared freshly on the day of dosing. Different categories of excipients were used including alcohols and glycols, surfactants, terpenes and acids and fatty alcohols. The excipients of other categories like d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) were also used. Various excipients used in the present research project along with the proportions used in formulations were tabulated in Tables 1 and 2.

Experimental animals:

Animal studies were approved by the Institutional Animal Ethics Committee (IAEC) as per approval number FB-15-067 of Sai Life Sciences, Pune, India (an AAALAC accredited facility) and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy male BALB/c mice (10-12 w old) weighing between 25 to 35 g were procured from Bharat Serum, Mumbai, India. Three mice were housed in each cage. Temperature and humidity were maintained at 22±3° and 40-70%, respectively with 12 h light and 12 h dark cycle. Temperature and humidity were recorded by auto-controlled data logger system. All the animals were provided laboratory rodent diet (Vetcare India Pvt. Ltd, Bengaluru, India). Reverse osmosis water treated with ultraviolet light was provided *ad libitum* to all the animals.

Pharmacokinetic studies:

Studies were performed in male BALB/c mice

TABLE 1: LIST OF PERMEATION ENHANCERS

Permeation enhancers	
Alcohols and glycols	Ethanol, propylene glycol, polyoxyethylene glycols, mannitol, Transcutol and Phospholipon H
Surfactants	Tween 80, sodium lauryl sulphate, Pluronic F-127, Solutol HS-15, Cremophore EL and polysorbate 20 and 60
Essential oils and terpenes	Menthol, oleic acid and lauricidine
Sulfoxides and analogues	Dimethyl sulfoxide, dimethyl acetamide and dimethyl formamide
Acids and fatty alcohols	Caprilic acid, Capmul MCM C8, Maisine, Captex, Gelucire, Acconon and Labrafil
Others	Vitamin E-TPGS

(25-35 g). Paromomycin was administered IV at 15 mg/kg and at 500 mg/kg by oral gavage. There were total 12 groups and each group consisted of 9 mice. IV dose group (normal saline group) was received a single bolus dose administration through tail vein and other 11 groups were coded as oral formulation dose group from formulation 1-11 (Table 2). Mice were fasted 4 h prior to dose administration and food was provided 4 h post dose administration and water *ad libitum* during the entire period of experiments. Blood samples were collected by sparse sampling design (n=3 at each time point) at 0.08 (IV), 0.25, 0.05, 1, 2, 4, 8 and 24 h (K2EDTA anticoagulant, 10 µl/kg). Plasma was immediately harvested from the blood by centrifugation at 4000 rpm for 10 min at 4±2°and stored below –70±10° until bioanalysis. The dosing volumes were 5 ml/kg (IV) and 10 ml/kg (PO). The area under the concentration time curve (AUC), terminal half-life (T1/2), CLi.v, Vss was estimated by non-compartmental analysis module in Phoenix WinNonlin® (Version 6.3).

Bioanalysis:

Paromomycin and the internal standard (antipyrine) were analysed in *in vivo* samples using ultra-performance liquid chromatography (UPLC, Acquity, Waters, USA) mass spectrometry (AB Sciex, USA). Positive-ion electron spray ionization mode was used with MRM transitions of 455.30/308.60 for paromomycin and 189.4/104.0 for antipyrine. Chromatographic separation was obtained using Acquity, BEH, HILIC column (50×2.1 mm, 1.7 µm) maintained at 45° with an injection volume of 5 µl.

In vivo plasma samples were extracted by using protein precipitation technique. A 25 µl of plasma sample was added with 25 µl of internal standard prepared in water (antipyrine, 500 ng/ml) and vortexed. A 100 µl of perchloric acid (10% prepared in water) was added and vortexed for 5 min. Samples were centrifuged for 10 min at a speed of 4000 rpm at 4°. Following centrifugation, 100 µl of clear supernatant was transferred in 96 well plates and analysed using LC-MS/MS.

The lower limit of quantification (LLOQ) was 50.6 ng/ml and the calibration curve was linear over a 1000-fold concentration range. All the study samples were processed along with calibration and quality control samples. An acceptance criterion of ±15% for calibration curve and ±20% for quality control samples was used.

Statistical analysis:

All values are expressed as mean±standard error of mean (SEM). Data were analysed for statistically

TABLE 2: COMPOSITIONS OF DIFFERENT EXCIPIENTS USED IN ORAL FORMULATIONS OF PAROMOMYCIN USED IN MOUSE PK STUDIES

Excipient	Percent in normal saline										
	Formulation										
	1	2	3	4	5	6	7	8	9	10	11
Carboxy methylcellulose	0.5										
Menthol		5			5						
Pluronic F-127		10									
Sodium cholate		10									
Tween 80			20			10		20			
Sodium lauryl sulphate			10								
Phospholipon H			20								
Gelucire 44/14				5			10	20	15		
Capmul MCM C8				5	10						20
Cremophor EL					10						15
Captex						10				35	
Transcutol						5		20			20
Solutol HS-15							10			30	
Propylene glycol							30		30	20	
Acconon MC8									15		
Labrafil									20		
Maisine										15	15

significant differences using analysis of variance (ANOVA) followed by the two-sided unpaired Student's t-test. Differences were considered to be significant at a level of $P<0.05$.

RESULTS AND DISCUSSION

After IV dose administration, paromomycin showed low plasma clearance (8 ml/min/kg) in mice (CL less than 10% of normal hepatic blood flow) with elimination half-life of 2.6 h. Compound exhibited high volume of distribution steady state (>0.7 l/kg, Table 3; fig. 1). After a single oral dose administration of paromomycin with CMC formulation, it showed multiexponential disposition with a very low oral bioavailability (0.3%). Oral exposures (AUC) of different paromomycin formulations (formulation 2 to 8 and 11) showed statistically significant improvement compared to 0.5% CMC formulation of paromomycin (Table 3). Formulation 9 and 10 didn't show improvement in oral exposure of paromomycin. The maximum exposure observed was for formulation 7, which was 30-fold higher than formulation 1 (fig. 2A, Table 4). Similar trend of increase in C_{max} was also observed with different formulation approaches compared to CMC formulation. A maximum of 12.62 µg/ml of C_{max} was observed with formulation 7 compared to CMC formulation. The mean concentration-time profiles for various formulations were depicted in fig. 2.

Paromomycin is a large molecule with good water solubility and limited oral bioavailability due to its absorption limitation and/or substrate to efflux transporter(s). In view of this, selection of excipients

TABLE 3: PHARMACOKINETIC PARAMETERS OF PAROMOMYCIN AFTER IV AND PO DOSE ADMINISTRATION

Formulation no.	Route	T_{max} (h)	C_0/C_{max} (µg/ml)	AUC_{last} (h×µg/ml)	$T_{1/2}$ (h)	CL (ml/min/kg)	V_{ss} (l/kg)	%F[b]
Normal saline	IV	-	16.27	30.63	2.60	8.16	1.84	-
1	PO	1.00	0.69	3.43	-	-	-	0.3
2	PO	0.5	5.23	26.85	-	-	-	2.63
3	PO	0.5	11.13	67.26	-	-	-	6.59
4	PO	0.5	6.24	47.03	-	-	-	4.61
5	PO	0.5	9.95	57.94	-	-	-	5.68
6	PO	1.0	5.02	31.24	-	-	-	3.06
7	PO	1.0	12.62	90.77	-	-	-	8.90
8	PO	0.5	4.62	31.64	-	-	-	3.10
9	PO	0.3	1.04	2.89	-	-	-	0.28
10	PO	0.5	2.05	8.92	-	-	-	0.87
11	PO	1.0	5.38	44.92	-	-	-	4.40

In male BALB/C mice (n=3). C_0; extrapolated concentration on y-axis for IV data

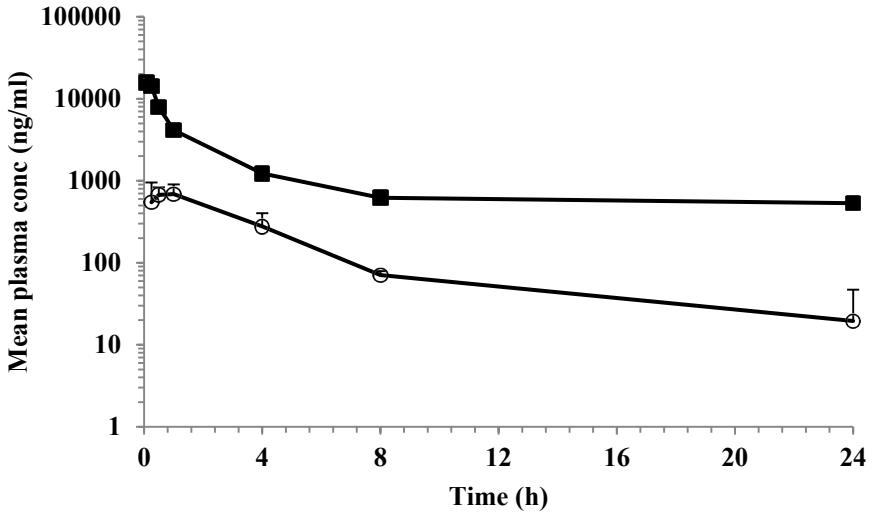

Fig. 1: Mean plasma concentration-time profiles of paromomycin
—■— IV, paromomycin (15 mg/kg) and PO dose (500 mg/kg), —○— formulation 1 (CMC)

was based on their effect on intestinal membrane or efflux transporter(s) or both. The excipient selection strategy covered maximum categories like alcohols and glycols: Phospholipon H and Transcutol; surfactants: Tween 80, Cremophore EL, Pluronic F-127, Solutol HS-15; terpenes: menthol; acids and fatty acids: Capmul, MCM C8, Maisine, Captex, Gelucire 44/14, Acconon and Labrafil.

The fatty alcohols used are medium chain fatty acids including capric acid (C10), lauric acid (C12) and long chain fatty acids, such as oleic acid fatty acids have been shown to increase the permeability of a series of hydrophilic drugs by dilating the tight junction and/or changing the cytoskeleton of the intestinal epithelial cells without prominent cytotoxicity. The main advantage of these excipients is the ease of integrating into the conventional oral dosage forms without the need for difficult or costly formulation technique, which is an advantage of providing cost effective formulation apart from improved bioavailability[11].

Terpenes are biosynthetically derived isoprene units, which increase the permeability of compounds by dilating the intestinal epithelium[12]. Glycols like phospholipon H is a lipoid characterized by natural and hydrogenated lecithin fractions and phospholipids that would enhance the absorption by changing the intestinal membrane fluidity[13]. The excipients like alcohols and glycols, fatty alcohols, surfactants and Solutol increase the permeability by increasing the membrane fluidity and/or disrupting the membrane structure[14-17].

After IV dose administration, paromomycin exhibited low plasma clearance (8 ml/min/kg) and moderate volume of distribution (1.84 l/kg) suggesting extravascular distribution. Paromomycin showed negligible oral bioavailability (0.3%) when administered in a CMC formulation. Our research data and study outcomes are in agreement with the study reported

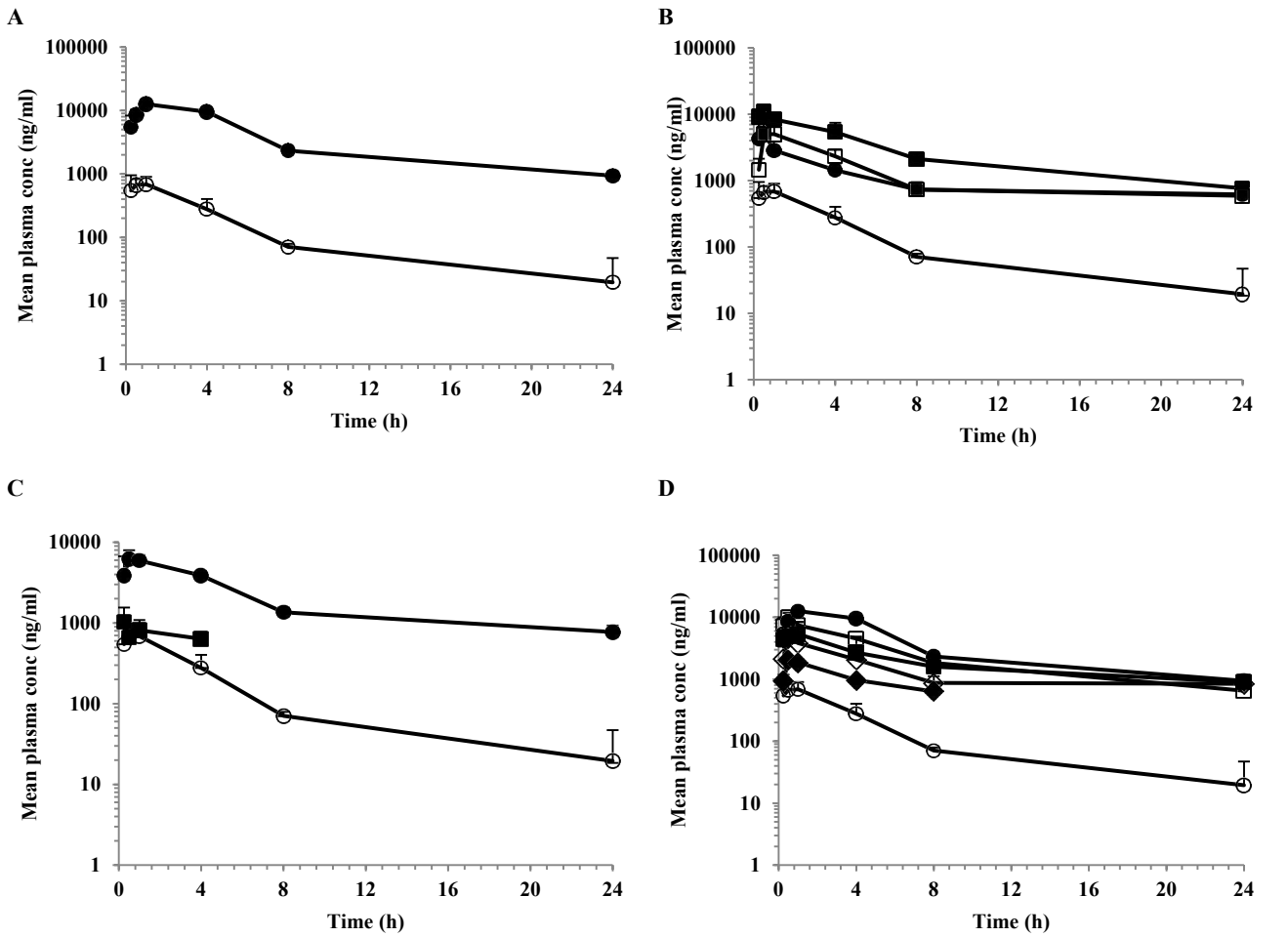

Fig. 2: Mean plasma concentration-time profiles of paromomycin after PO dose (500 mg/kg)
A: —○— formulation 1 (CMC), —●— formulation 7; B: —○— formulation 1 (CMC), —●— formulation 2, —■— formulation 3, —□— formulation 6; C: —○— formulation 1 (CMC), —●— formulation 4, —■— formulation 9; D: —○— formulation 1 (CMC), —□— formulation 5, —●— formulation 7, —◊— formulation 8, —♦— formulation 10, —■— formulation 11

by Bissuel *et al*., where the absence of paromomycin in plasma after oral dose was attributed to absorption limitation[5]. This could be due to permeability limitation as it is a large molecule with a molecular weight of 615 Da and a P-gp substrate. Considering large molecular weight, paromomycin could not be permeating through the intestinal tract and it being a P-gp substrate whatever permeated compound could be effluxed into the intestinal tract[18]. The present study was hypothesized that the absorption can be enhanced either by increasing the membrane fluidity and/or by inhibiting the efflux transporters. Hence to improve the oral bioavailability, the excipient that enhances the permeability and inhibit the P-gp efflux were employed in the formulation vehicle. The excipients used were belongs to different categories of permeation enhancers like glycols, surfactants, terpenes, fatty alcohols and others like TPGS. Glycols used were propylene glycol, Phospholipon H and transcutol and were reported to increase the permeability by changing the fluidity of intestinal membrane[19]. It was also reported that glycols increased the permeability by inhibiting the P-gp efflux transporter[19]. The use of 30% of propylene glycol in the present study showed much greater exposure than the reported value, which could be due to increasing the permeability in GI tract. Surfactants like sodium lauryl sulphate, Tween 80 and Cremophor EL were used at ≥10% and were reported to inhibit P-gp and SLS by increasing the fluidity of intestinal membrane resulting in increase in the plasma exposure[20]. Cremophor EL was also reported to increase the permeability by disrupting the intestinal barrier[21]. Terpenes like menthol were shown to increase the permeability either by increasing the fluidity or by disruption of tight junctions of intestinal membrane[22]. Fatty alcohols like Gelucire 44/14, Capmul MCM C8, Captex, Acconon, Labrafil and Maisine were shown to increase the permeability by disrupting the intestinal barrier[22-24]. Solutol HS-15, a non-ionic surfactant was reported to increase the permeability by disrupting the membrane structure and/or increasing the membrane fluidity[25]. Peltier *et al.* also proved that Solutol HS-15 increased the plasma exposure of paclitaxel by inhibition of P-gp[26]. Paromomycin prepared with different combinations of excipients either P-gp inhibitors and membrane disruptors or both, showed increase in the oral bioavailability compared to CMC formulation. All the formulation tested showed an increase in 9 to 30 fold oral bioavailability compared to CMC formulation (0.3%) except the formulation 8 and 9. Amongst all the tested formulations, the formulation 7 showed highest bioavailability (8.9%) for paromomycin. Formulation 7 consisted of Gelucire 44/14, Solutol HS-15 and propylene glycol, which increased the intestinal membrane fluidity/disrupt the membrane or inhibit the P-gp transporter. Overall, the increase in bioavailability across the tested formulation ranged 1-30 folds higher than the CMC formulation (0.3%; formulation 1). The data was depicted in Table 4. Comparison of bioavailability with different formulations was depicted in fig. 3. In specific, as per the literature the increase in bioavailability could be due to increase in the intestinal membrane fluidity/ disrupt the membrane by Gelucire 44/14 and by both increase in intestinal membrane fluidity/disrupt the membrane or inhibition of the P-gp efflux transporter by Solutol and propylene glycol.

Interestingly, paromomycin being a substrate for efflux transporter and having a permeability limitation, bioavailability can be increased by adopting an alternative formulation approach, which was evident in present research. The compounds, which fall in negligible permeability and no oral bioavailability like paromomycin and other drugs can be used to increase the oral bioavailability by efficient utilization of excipients that can increase intestinal membrane fluidity, permeability and or P-gp inhibitor. Our hypothesis of increasing the permeability of paromomycin by incorporating the novel formulation excipients has proved to improve the oral bioavailability.

The present study provides first time detailed pharmacokinetic experiments that showed the improvement of oral exposure of paromomycin using different excipients (formulation based approaches) in mice. The formulation additives, i.e. the permeation enhancers, and/or P-gp inhibitors were well-tolerated

TABLE 4: INCREASE IN BIOAVAILABILITY WITH DIFFERENT FORMULATION APPROACH COMPARED TO CMC FORMULATION (FORMULATION 1)

Group no.	% F	Fold increase
Formulation 1	0.3	-
Formulation 2	2.63	9
Formulation 3	6.59	22
Formulation 4	4.61	15
Formulation 5	5.68	19
Formulation 6	3.06	10
Formulation 7	8.9	30
Formulation 8	3.1	10
Formulation 9	0.28	1
Formulation 10	0.87	3
Formulation 11	4.4	15

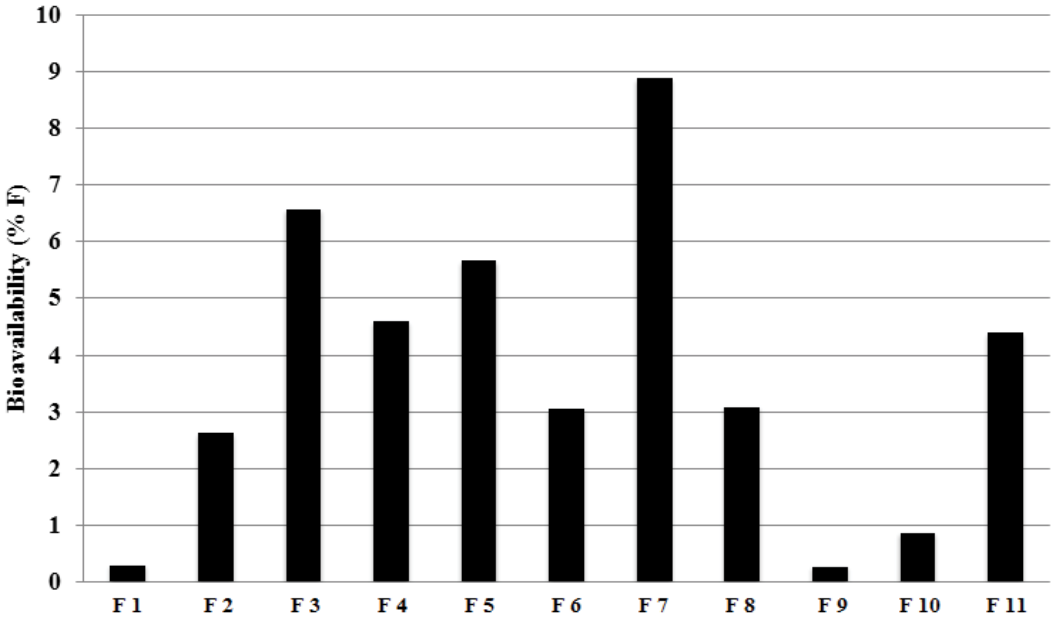

Fig. 3: Comparison of bioavailability of different oral formulations of paromomycin after PO dose (500 mg/kg)

in mice at the administered doses in single dose IV and oral pharmacokinetic studies. The highest oral relative bioavailability of paromomycin was observed in formulation 7, which was significantly high compared to CMC formulation 1. This evaluation provide evidence that paromomycin can be administered orally, which in turn help patient compliant. The outcome of this research is important for treatment compliance of paromomycin and further clinical studies required in future to establish our findings in clinic.

Conflict of interest:

The authors declare no conflict of interest.

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