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Research Article

Pharmacokinetic Evaluation and Studies on the Clinical Efficacy of Guar Gum-Based Oral Drug Delivery Systems of Albendazole and Albendazole-β-Cyclodextrin for Colon-Targeting in Human Volunteers

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The present investigation assessed the in vivo properties of the guar gum–based colon-targeted matrix tablets of albendazole-β-cyclodextrin (KA), albendazole matrix tablet (GA), and an immediate-release albendazole tablet (CA) in humans. A single oral dose was administered in healthy human volunteers and a completely randomized, two-way, three-period crossover design was adopted. The data were statistically analyzed and a value of $P<0.05$ was considered statistically significant. In healthy human volunteers, guar gum colon-targeted tablets showed delayed t_{max} and absorption time, decreased absorption rate constant, and unaltered $t_{1/2}$ indicating that albendazole is not released in stomach and small intestine, but is delivered to the colon, resulting in a slow absorption of the drug and making the drug available for local action in the colon. The increase in C_{max} and $AUC_{0-\infty}$ of KA shows that the bioavailability of albendazole in humans could be definitely improved by complexing the drug with β-cyclodextrin. Furthermore, an open, parallel, single-blind clinical study was conducted in patient volunteers who had helminthiasis. Clinical studies showed that the guar gum colon-targeted tablets could reduce eggs per gram faster than conventional tablets could, resulting in improved clinical symptoms, Hb content, and decreased total count as compared to conventional tablets of albendazole. The investigation shows that albendazole, when complexed with β-cyclodextrin, improves in vivo bioavailability of the drug and colon targeting using guar gum ensures drug delivery in the colonic fluids, and therefore improves clinical efficacy in human beings with helminthiasis. Drug Dev. Res. 67:154–165, 2006.

Key words: albendazole; β-cyclodextrin; bioavailability; clinical study; humans

Abbreviations: ABZ, albendazole; β-CD, β-cyclodextrin; CA, conventional albendazole tablet; GA, guar gum colon-targeted matrix tablet containing albendazole alone; KA, guar gum colon-targeted matrix tablet containing albendazole β-cyclodextrin complex; K_a, absorption rate constant; t_a, absorption time; $t_{1/2}$, half-life; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; epg, eggs per gram; HBS, hepetitis B surface.

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INTRODUCTION

Enteric nematodes are among the most common and widely distributed parasites residing in the human gastrointestinal tract (GIT) and well tolerated by the human host. More than 25% of the world population is affected with helminthiasis, of which nearly 286 million humans are affected in India alone [de Silva et al., 2003; Brooker et al., 2004]. Albendazole is a benzimidazole derivative developed more than 25 years ago; it acts by binding to parasite β-tubulin, inhibiting its polymerization and impairing glucose uptake. Albendazole is used in the treatment of intestinal helminthiasis and is also a useful drug to treat a number of other parasitic diseases including cystic echinococcosis, ascariasis, enterobiasis, and cestode parasitosis [Njorge et al., 2005; Albonico et al., 1994; Horton, 2000]. Albendazole is presently considered the drug of choice for the treatment of human intestinal capillariasis because it is effective against eggs, larvae, and adult worms [Cross, 1992; Andersib et al., 1984]. The side effects of albendazole are few and tend to be mild gastrointestinal upset, dizziness, rash, and alopecia, but do not require discontinuation of the drug [Venkatesan, 1998]. Patients who have helminthiasis show significant increase in total count and marked decrease in hemoglobin and also presence of eggs/cysts of parasites in stool samples. Albendazole has to reach its specific receptor inside the parasite cell to exert its action; concentrations achieved inside the intracellular space are critical to assure clinical efficacy. Because the solubility of albendazole is low, the plasma concentrations do not reach required therapeutic concentrations and are often erratic and variable. The therapeutic response is unpredictable because of its poor bioavailability [Dennis et al., 2002]. Several techniques of improving the bioavailability of albendazole were reported previously in animal models and in humans. In animals, the bioavailability of albendazole was enhanced by co-administering with co-solvents [Torrado et al., 1997], or with surfactants/liposomes [Wen et al., 1996] or as solid dispersions [Torrado et al., 1996; Kohri et al., 1999] or by inclusion complexation with HP-Cyd [Evrard et al., 2002]. In humans, albendazole was given along with a fatty meal [Lange et al., 1988] or with cimetidine (to inhibit the degradation of the active drug) [Schipper et al., 2000; Nagy et al., 2002], or with grapefruit juice [Nagy et al., 2002] and also, as an emulsion of soyabean oil [Mingjie et al., 2002]. Previously clinical trials that were conducted on albendazole conventional-release tablets in various diseases showed that albendazole immediate release is effective in the treatment of neurocysticercosis in children [Singhi et al., 2003], microfilaria either alone or in combination with diethyl carbamazine citrate [Pani et al., 2002], and cystic echinococcosis [Dennis et al., 2002]. A conventional tablet of albendazole disintegrates in the stomach and releases the drug in the stomach and small intestine and only a small fraction reaches the colon. This erratic and unpredictable absorption of albendazole in the GIT might result in less clinical efficacy; hence, sometimes long-term therapy of albendazole may be required in treating helminthiasis. Because conventional albendazole tablets release the drug throughout the GIT and the absorption is erratic and variable from various parts of GIT, a colon-targeted albendazole would be a suitable alternative in focusing the maximum amount of drug in the colon. However, the drug is poorly soluble, so there is a need to improve the solubility of albendazole and thereby its bioavailability could be enhanced. Such targeting of albendazole in the colon for local action would reduce unwanted systemic side effects, and even a lower dose of albendazole may be sufficient to treat helminthiasis. In light of this information, it was planned to study the pharmacokinetics and clinical effectiveness and to assess the actual benefit of colon-targeted guar gum matrix tablets of albendazole and albendazole-β-cyclodextrin in humans.

Colon-targeted drug delivery systems for albendazole were developed using guar gum as a carrier and in order to improve bioavailability of albendazole, it was complexed with β-cyclodextrin (β-CD). Albendazole–β-cyclodextrin complex equivalent to albendazole 200 mg was used in the study. The in vitro release kinetics have been reported [Somashekar et al., 2005]. Guar gum matrix tablets containing various proportions of guar gum were prepared and subjected to in vitro drug release studies. Albendazole matrix tablets containing 20% of guar gum (SAC-20) were found not suitable for colon targeting because they disintegrated in the simulated physiological environment of the stomach. Also, matrix tablets containing 40% of guar gum (SAC-40) were considered unsuitable for colon targeting because they released only 43.44% of albendazole even after 24 h of dissolution study. Matrix tablets containing 30% of guar gum (SAC-30) (KA) were intact and swollen in simulated stomach fluid but degraded in simulated colonic fluids releasing 67.7% of drug in rat cecal contents at the end of 24 h of dissolution study. Thus, the matrix formulation containing 30% guar gum (SAC-30) is most likely to target albendazole to colon without being released significantly in stomach and small intestine. Also, the matrix tablets of SAC-30 were found to be stable at 40°C/75% R.H. for 6 months [Somashekar et al., 2005]. Therefore, in the present study, it was planned to evaluate pharmacokinetic and clinical effectiveness of SAC-30

(KA) colon-targeted matrix tablets in humans, in comparison with a matrix tablet of albendazole alone and a conventional immediate release albendazole tablets.

The objective of the present investigation was therefore to evaluate the pharmacokinetic variables of three formulations, namely, a reference (a conventional tablet) and two test formulations of albendazole (a guar gum matrix tablet of albendazole alone, and a guar gum matrix tablet of albendazole–β-CD) tablets. The formulations were conveniently coded as **CA**, **GA**, and **KA**, respectively. After a single oral dose (200 mg) in six healthy male human volunteers in a completely randomized, two-way, three-period complete crossover design with a washout period of 7 days between the two treatment sessions, the plasma samples were analyzed for albendazole using ultraviolet–high-pressure liquid chromatography (UV-HPLC). And also to study the clinical effectiveness of CA, GA, and KA on patient volunteers who had helminthiasis. Because the dose of the conventional albendazole tablet is 400 mg, hence for the purpose of clinical study a uniform dose of 400 mg of albendazole or its equivalent was used in clinical evaluation of guar gum–based oral drug delivery systems containing either albendazole alone or albendazole–β-CD complex, respectively.

MATERIALS AND METHODS

Materials

Albendazole (98.5–102% purity) was a gift sample from M/s. Indechemie Laboratories Ltd., Mumbai, India. Mebendazole (98.6–101.4% purity) was a gift sample from M/s Cipla Ltd., Bangalore, India. Methanol (HPLC grade) was obtained from M/s Qualigens Fine Chemicals, Mumbai, India. Water (HPLC grade) was collected from Milli-Q RO system. Other materials used in the study such as orthophosphoric acid, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, and sodium hydroxide were of analytical grade and were obtained from M/s S.D. Fine Chemicals, Mumbai, India.

Products for Evaluation

Three products were used in this study: matrix tablets of albendazole–cyclodextrin (KA), a matrix tablet containing albendazole alone (GA), and a commercially available conventional albendazole tablet were used for pharmacokinetic studies.

Selection of Volunteers and Drug Assignment

All of the volunteers were in the age group 22–26 years (weight 55–60 kg) and each subject was assigned a subject number. They were nonalcoholics and nonsmokers. The required biochemical tests were carried out to ensure that the volunteers were free from either liver or kidney dysfunction. None of the volunteers was on drug treatment 10 days prior to participation in the study. The volunteers were divided into three groups (I, II, and III), and in each group for a particular period two volunteers were used. Each subject was randomly assigned to one of the following dosing sequences/groups as given in Table 1.

TABLE 1. Dosing Sequences of Different Albendazole Tablets in Healthy Humans (n = 6)

Group	Period I	Period II	Period III
Group I: 2 subjects	GA	CA	KA
Group II: 2 subjects	CA	KA	GA
Group III: 2 subjects	KA	GA	CA

Volunteers received all of the study formulations according to their respective code number with 240 mL of water. A standard breakfast was served 2 h after the commencement of the study. Lunch and dinner in standard quantity were served at regular time intervals. After a washout period of 10 days, i.e., on day 11, volunteers received any of the albendazole (200 mg) formulation as described above and in Table 1. An informed written consent was obtained from each healthy human volunteer, and they were free to withdraw from the study at any time during the study period.

HPLC Analysis of Albendazole in Matrix and Conventional Tablets of Human Plasma

The quantification of drug/metabolites concentrations in human plasma after a colon-targeted or conventional anthelmintic treatment orally is required to correlate information on host pharmacokinetics with the pattern of drug uptake by the target parasite. Therefore, a simple robust HPLC method was developed to quantitate albendazole from human plasma. A gradient high-pressure liquid chromatograph (Shimadzu, Class VP 6.01) with two LC-10 AT-VP solvent delivery system pumps, a SPD M-10AVP photo diode array detector, a Rheodyne 7725*i* injector (loop volume 20 μL), Lichrocrat C_{18}; 250 × 4.6 mm (i.d., 5 μm) analytical columns, and a solid phase extraction cartridge (Waters; Sep Pak C_{18}, 1 g cartridges) was used. The HPLC system was equipped with the software "Class-VP 6.01 data station." The method was validated for linearity, accuracy, and precision.

Procedure

Methanol and 50 mM phosphate buffer of pH 7.4 in a ratio of 70:30 were used as mobile phase at a flow rate of 0.8 mL/min and at a temperature of 40°C. Run

time was set at 8 min with a spike volume of 20 μL and the detector sensitivity was set at 0.0001 absorbance units full scale (a.u.f.s.). Plasma samples were injected with 1 μg of mebendazole as internal standard and 2 mL of phosphate buffer (pH 7.4), after mixing transferred to a preconditioned Sep-pak cartridges (C_{18}, Waters USA). The cartridges were preconditioned with 5 mL of methanol and 5 mL of pH 7.4 phosphate buffer. The cartridges were washed with 20 mL of phosphate buffer. The methanolic extract was dried under a gentle stream of nitrogen at 40°C. The residue was reconstituted with 1 mL of mobile phase. After filtering through a 0.2-μ membrane filter, 20 μL of this solution was injected into an HPLC column. The ratio of peak area of albendazole to that of internal standard (mebendazole) was calculated, and the regression of the peak area ratio against the plasma concentration of albendazole was calculated using the least squares method of analysis.

Screening tests for human subjects

The healthy human volunteers were subjected to the following biochemical tests to ensure that the volunteers do not have liver or kidney dysfunction. All these volunteers were nonsmokers and nonalcoholics.

a. Complete hemogram
b. Liver function tests
c. Renal function tests
d. Blood sugar
e. Lipid profile
f. Routine urine examination
g. Chest radiograph
h. Electrocardiogram
i. Virological tests—human immunodeficiency virus antibody, hepatitis B surface (HBS) antigen

Blood Collection

Volunteers were given code numbers and were allocated to the treatment either with CA or GA or KA in accordance with the randomization code. Their pulse rate and blood pressure was recorded and an indwelling intravenous catheter was introduced with strict aseptic precautions for blood collection. They received either of the study formulations according to their code number with 240 mL of water. Blood samples were collected, at 0 h (before drug administration) 0.5, 1.0, 2.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0, and 48.0 h. Through an I.V. cannula, blood samples (5 mL) were collected via disposable syringes in precitrated centrifugal tubes. The withdrawn samples were centrifuged at 3500 rpm for 10 min to separate plasma. They were transferred into airtight containers and stored in a deep freezer at −40°C. None of the volunteers was on drug treatment 10 days prior to participation in the study.

Study Population

The study was conducted in a 350-bedded, Navodaya Medical College Hospital and Research Centre, at Raichur, India. Twenty-seven patient volunteers of either sex between 21 and 38 years (mean = 27.66 y) and between 40 kg and 58 kg of body weight (mean = 46.81 kg), from in and around the rural places of Raichur, India, with signs and symptoms of gastrointestinal disturbances were screened initially for the symptoms of helminthiasis. The patients were examined for clinical symptoms, slight intermittent fever, chills, sweat, nausea, vomiting, weakness, cramping abdominal pain, discomfort, and diarrhea. These patients were also subjected to microscopic examination of the freshly obtained stool samples and blood samples. It was revealed that only nine patient volunteers (6 M and 3 F) were confirmed to be suffering with helminthiasis. All of these nine patient volunteers with helminthiasis participated in the study. The study was "blind" to the extent that patients, physicians, nursing staff, and laboratory staff were unaware of the individual therapy schedules. The dosage forms were coded and repacked in separate, look-alike containers by a senior physician who was not a part of the investigation. The purpose of the study was fully explained to the patient volunteers including the possible adverse effects. An informed written consent was obtained from every patient volunteer. They were free to withdraw from the study at any point of time without assigning any reason. The physicians and the nursing staff of the hospital conducted the study. The base clinical status (day "0" clinical status) of all these nine patient volunteers was recorded in their respective case sheets.

Inclusion Criteria

Patients reported positive to clinical symptoms for helminthiasis, hematological tests, and also were reported positive to coproparasitoscopic tests (eggs or cysts of *Ascaris lumbricoides*, or *Strongyloides stercoralis*, or *Trichuris trichiura*) which were included in the study.

Exclusion Criteria

Patients excluded from this study were those who were receiving or having received antiparasitic drugs 15 days prior to commencement of study, pregnant women, breast-feeding mothers, minor patients (below the age of 18 y), alcoholics, and patients with known hypersensitivity or contraindication to albendazole.

Study Design

An open parallel, single-blind study design was followed. All of the nine patient volunteers, confirmed as having helminthiasis, were divided into three equal groups. Group I (n = 3) patient volunteers received conventional commercial albendazole tablets, Group II (n = 3) patient volunteers received colon-targeted matrix tablets of albendazole and the last Group III received colon-targeted albendazole–β-CD complex tablets. Each patient volunteer of any group received 400 mg or its equivalent of albendazole once daily, and the treatment schedule was planned for 3 days. From all the three groups of patient volunteers, the stool samples and blood samples were collected early in the morning on each day, i.e., day 0, day 1, day 2, and day 3, and were immediately examined. All the patient volunteers, in all the groups, were questioned and clinically examined on days 0, 1, 2, and 3 by the medical team at 8-hourly intervals for fever, diarrhea, abdominal pain, weakness, flatulence, nausea, and vomiting, and were recorded in their respective case sheets. "0th" day values of hemoglobin (Hb), total count, and eggs per gram (epg) along with the symptoms of the individual patient volunteers, were considered as "base clinical" values for the purpose of the study. Standard diet was given, and no other supplement medications were administered to any patients of all the three groups during the study period.

Blood samples were examined quantitatively for white blood cells and hemoglobin. The blood samples from patient volunteers was stored at 20°C until examination. Each sample was separately examined for Hb content and total count.

Stool samples were examined for presence or absence of eggs and cysts, of *Ascaris lumbricoides* or *Trichuris trichiura* or *Strongyloides stercoralis* by microscopic method, as described below. The fecal epg were counted on the fresh feces of all these patients.

Examination of Feces

Fresh fecal samples were collected in sterile containers every day in the morning. Stoppered, labeled, and immediately examined in a microscope at 100 × magnification and eggs were identified and counted. Fresh feces sample was thoroughly mixed. The feces was picked up with a sterile spatula and 2 g of accurately weighed quantity was diluted with 10 mL of clean tap water. Then it was further diluted to 50 mL with tap water and was mixed well. Then the suspension was centrifuged at 2,000 rpm for 2 min. One hundred microliters of the supernatant was transferred to a clean and dry glass slide with Lugol's solution, and covered with a glass coverslip so as to spread out the liquid sample into a thin, fairly uniform and transparent layer [Williamson et al., 1998]. The prepared stained smear slides of the feces were examined first under 100 × power magnification of the microscope. These were examined for the presence of eggs and cysts of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Strongyloides stercoralis*. The eggs were counted and observation was tabulated.

Ethical Considerations

The Institutional ethical committee approved the protocol of the study involving the conventional and colon-targeted drug delivery systems of albendazole. All the volunteers had the full details of the possible side effects of the drug formulation explained to them and were given freedom to withdraw from the study as and when they felt such a need. An informed written consent was obtained from every volunteer who had participated in the study.

Pharmacokinetic Analysis

The peak plasma albendazole concentration (C_{max}) and the time to reach peak levels (t_{max}) were obtained from the time versus plasma concentration of albendazole. Other pharmacokinetic parameters such as absorption rate constant (k_a), elimination half-life ($t_{1/2}$), absorption time (t_a), and $AUC_{0-\infty}$ were also calculated [Gibaldi and Perrier, 1990].

Statistical Analysis

Because the same group of volunteers received all the three tablet formulations of albendazole, either the t-test for unequal variances (where the equality of variance rejected) or paired t-test (where the equality of variance accepted) was used to test the significance of difference in pharmacokinetic parameters. A value of $P < 0.05$ was considered statistically significant (n = 6) [Riegelman and Hirsch, 1989; Zar and Jerrold, 1996]. Mean Hb, white blood corpuscles (WBC) count, and epg of patients were compared with Student's t-test and a value of $P < 0.05$ was considered statistically significant (n = 3).

RESULTS AND DISCUSSION

The mean age of human volunteers who participated in the pharmacokinetic study was 23 ± 2.68 years. Their height and weight were 168 ± 3.346 cm and 56.5 ± 4.324 kg, respectively. Measurable drug-blood levels were noticed in all the subjects up to 48 h after administration of the conventional tablet and colon-targeted tablets. Plasma concentration of albendazole was measured by HPLC with UV detection at 291 nm. Also, when the peak area ratio was subjected to regression analysis by least-squares method, a high correlation coefficient was observed (r = 0.998) in the

range of 10–2,000 ng/mL. The lower limit of quantification of albendazole in plasma was found to be 5 ng/mL (signal-to-noise ratio 10:1). The interday and intraday coefficient of variation (precision) was found to be less than 4%. About 98.18% of albendazole was recovered (accuracy) from the preanalyzed samples. The results showed that the HPLC method used for the estimation of albendazole in human plasma is highly reproducible and accurate.

Albendazole was administered at a dose of 200 mg in immediate-release tablet and in both colon-targeted tablets. The HPLC chromatograms did not show any other peaks interfering with those of drug and internal standard (mebendazole). This shows that either the metabolites or other plasma components did not interfere in the estimation of albendazole by HPLC method used in the present study. The mean plasma levels of albendazole after oral administration of immediate-release tablets (CA), and colon-targeted tablet formulations GA and KA containing albendazole (dose 200 mg each) are given in Table 2 and shown in Figures 1, 2, and 3, respectively. Albendazole appeared almost immediately within 0.5 h of oral administration of immediate-release tablet. However, it took about 6 h for albendazole to appear in plasma from both the colon-targeted matrix tablets of albendazole alone (GA) and colon targeted matrix tablets containing albendazole-β-cyclodextrin complex (KA) when administered orally.

The immediate-release tablets of albendazole (CA) might have disintegrated very fast in GIT and absorbed quickly from stomach and small intestine, thereby producing peak plasma concentration (C_{max} of 585.08 ± 20.3 ng/mL) within 5.0 ± 0.04 h (T_{max}). It appears from the results (Table 3) that guar gum–based albendazole matrix tablets (GA) did not release significant amount of drug up to 6 h (average colonic arrival time is 5 h) until the formulation reached the colon, because there was no albendazole present in the plasma up to 6 h. Similarly, guar gum–based albendazole–β-CD (equivalent to albendazole 200 mg) did not release a significant amount of drug up to 6 h (average colonic arrival time is 5 h) until the formulation reached the colon because there was no albendazole present in the plasma up to 6 h.

It appears that both of the swollen guar gum matrix tablets might have released some amount of drug, especially present on the surface of the tablet, thereby resulting in undetectable plasma levels of the drug by 6 h. However, after reaching the colonic environment, the degradation of swollen guar gum–based tablets of albendazole might have been started by colonic bacteria, thereby releasing the drug contained in the formulation.

On oral administration of colon-targeted tablets, GA and KA, albendazole started appearing in the plasma at about 6 h and reached the peak concentration (C_{max} of 528.85 ± 79.72 ng/mL and 916.49 ± 73.3 ng/mL, respectively) at 12 ± 0.72 h (T_{max}) and 12 ± 0.91 h (T_{max}) for GA and KA, respectively. The colon-targeted formulations were formulated so as to release a minimal amount of albendazole in the stomach and small intestine, yet to release most of the drug in the colon. This was evident from the in vitro drug release studies. Thus, the guar gum matrix tablets of albendazole might have disintegrated very quickly once the swollen guar gum layer was degraded by colonic bacteria and produced peak concentration of the drug by 12 ± 0.72 h (T_{max}). There is no significant difference in the C_{max} of colon-targeted GA formulation and that of conventional-release tablet CA. It should be noted here that although the T_{max} for both GA and KA was same 12.0 h, the C_{max} of KA was greater than that of GA, which could be attributed to improved solubility of albendazole-β-CD complex in in-vivo colonic fluids.

The absorption rate constant of the immediate-release (CA) dosage form (0.358 ± 0.14 h^{-1}) was found to be higher than that of either of the colon-targeted tablets, GA (0.156 ± 0.108 h^{-1}) and KA (0.181 ± 0.087 h^{-1}), indicating that the drug from either of the colon-targeted matrix tablets was absorbed slowly on reaching the less absorptive colon. However, the absorption rate constant of KA seems to be higher than that of GA (0.181 ± 0.087 h^{-1}), owing to the improved solubility of

TABLE 2. Mean ± SD Plasma Concentration of Albendazole After Oral Administration (Dose 200 mg) of Immediate-Release Tablet or Guar Gum–Based Colon Targeted Tablets of Albendazole Alone or Albendazole-β-CD in Human Volunteers (n = 6)

	Mean (±SD) plasma concentration (ng/mL) of albendazole in human volunteers administered with		
Time (h)	Conventional tablet CA	Colon-targeted tablet GA	Colon-targeted tablet KA
0	0	0	0
0.5	3.33 ± 1.67	0	0
1	18.12 ± 5.29	0	0
2	256.32 ± 84.03	0	0
5	585.08 ± 20.43	0	0
6	497.46 ± 91.66	426.54 ± 49.53	448.75 ± 108.6
8	455.33 ± 43.12	498.98 ± 65.01	896.82 ± 70.75
10	383.98 ± 40.81	513.95 ± 66.91	906.65 ± 68.54
12	312.62 ± 79.43	528.85 ± 80.49	916.49 ± 81.99
24	69.34 ± 15.54	279.23 ± 24.97	375.03 ± 49.3
36	3.8 ± 1.89	37.26 ± 13.81	63.11 ± 5.05
48	0	0	0

$Y = -0.000389 + 0.000937\ X$ ($r = 0.998$).

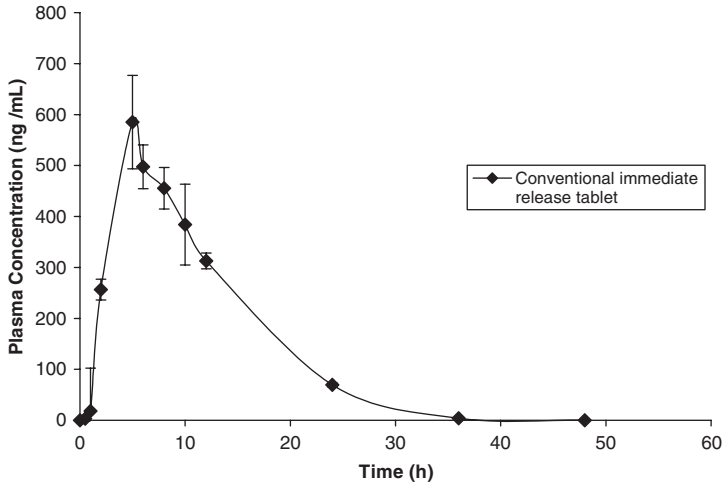

Fig. 1. Mean ± SD plasma concentration of conventional tablet containing albendazole (CA) in human volunteers (n = 6).

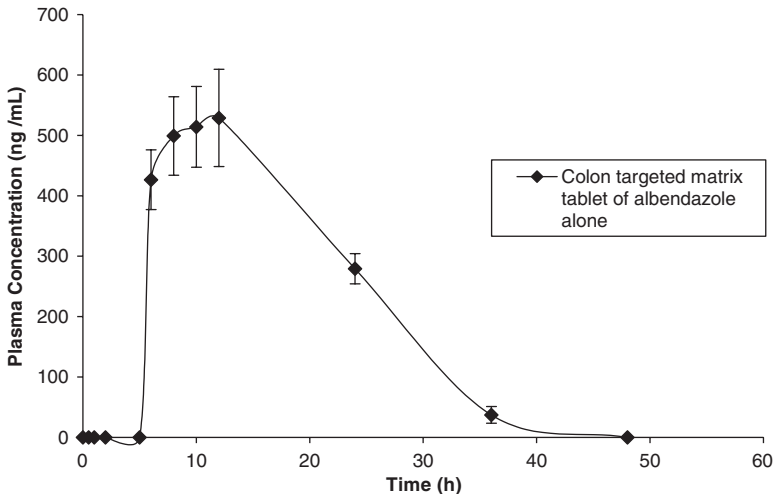

Fig. 2. Mean ± SD plasma concentration of colon-targeted matrix tablets of albendazole alone (GA) in human volunteers (n = 6).

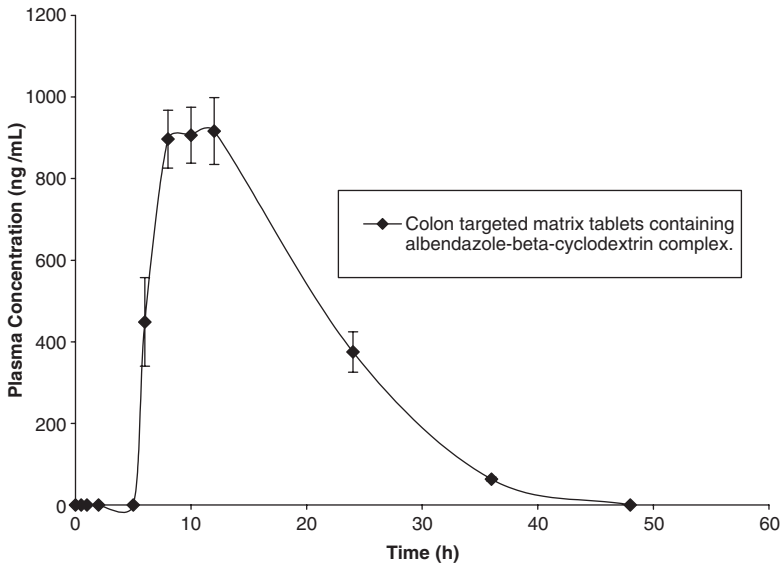

Fig. 3. Mean ± SD plasma concentration of colon-targeted tablets of albendazole-β-cyclodextrin complex (KA) in human volunteers (n = 6).

TABLE 3. Mean ± SD Pharmacokinetic Parameters of Albendazole in Human Volunteers (n = 6) After Oral Administration (Dose 200 mg) of Immediate-Release Tablet or Guar Gum–Based Colon-Targeted Tablet

	In human volunteers orally administered with			
Pharmacokinetic parameters	Conventional tablet CA	Colon targeted tablet GA	Colon targeted tablet KA	P <
$AUC_{0-\infty}$(ng/mL/h)	7,223.9 ± 203.2	10,253.21 ± 202.1	16,045.49 ± 137.26	NS
Terminal half-life (h) $t_{1/2}$	10.69 ± 1.45	5.79 ± 0.33	5.17 ± 1.9	0.05
Absorption time, (h), t_a	12.88 ± 0.9	29.55 ± 1.5	25.49 ± 0.9	0.01
Absorption rate constant (1/h) K_a	0.358 ± 0.14	0.156 ± 0.108	0.1808 ± 0.087	0.01
T_{max} (h)	5.0 ± 0.04	12 ± 0.72	12 ± 0.91	0.001
C_{max} (ng/mL)	585.08 ± 20.3	528.85 ± 79.72	916.49 ± 73.3	0.05

NS: not significant.

the complex and therefore faster absorption, but still less than that of CA.

The efficacy of the guar gum–based colon-targeted tablets of albendazole in targeting the drug locally in the colon is further evident from the pharmacokinetic evaluation of the plasma concentration versus time data. The t_{max} after administration of immediate-release tablets was 5.0 ± 0.04 h, which was significantly different ($P < 0.001$) from the t_{max} of GA and KA 12.0 ± 0.72 h and 12.0 ± 0.91 h, respectively, for guar gum–based colon-targeted tablets of albendazole.

The area under the plasma albendazole concentration versus time curves ($AUC_{0-\infty}$) for the immediate release (CA) and guar gum–based colon-targeted tablets of albendazole, GA, and KA were 7,223.9 ± 203.2, 10,253.21 ± 202.1, and 16,045.49 ± 137.27 ng/mL/h, respectively. The mean elimination half-life ($t_{1/2}$) for albendazole after oral ingestion of immediate release (CA) and colon-targeted tablets GA and KA were found to be 10.69 ± 1.45 h, 5.79 ± 0.33 h, and 5.17 ± 1.9 h, respectively, which were found to be decreasing. Thus, the $t_{1/2}$ of both of the colon-targeted matrix tablets of albendazole were not affected on administering the drug as a colon-targeted tablet, indicating that the extent of absorption and elimination was not affected. However, there was a significant decrease in the absorption rate constant ($P < 0.01$) of albendazole when administered as a colon-targeted matrix tablet of albendazole alone (GA). The increase in absorption rate constant of KA may be attributed to improved solubility of albendazole–β-CD complex in colonic fluids. It is also observed that there was a significant delay in the absorption time ($P < 0.01$) and T_{max} ($P < 0.001$) of albendazole on oral administration of both GA and KA colon-targeted tablets. Thus, it is clear from the pharmacokinetic evaluation of the colon-targeted tablets of albendazole that these targeted formulations did not release the drug in the stomach and small intestine yet released the drug in colon; from the colon the drug is absorbed slowly because of low permeability and less absorptive surface area.

There was good agreement of the in vitro drug release pattern [Somashekar et al., 2005] with that of the plasma concentration of albendazole when immediate-release and colon-targeted formulations were subjected to in vitro and in vivo evaluation. The immediate-release tablet released its drug content almost immediately within 2 h, wherein it resulted in early T_{max} of the drug in the volunteers. The colon-targeted tablet did not release any significant amount of drug up to 6 h, wherein it resulted in delayed T_{max} of the drug during in vivo studies in healthy volunteers. The fast release of the drug from the immediate-release tablet might have been available for immediate absorption from the stomach and small intestine, thereby resulting in early T_{max} and higher C_{max}. In contrast, guar gum–based colon-targeted matrix tablet containing albendazole alone (GA) did not release its drug content significantly up to 6 h, yet released the majority of its drug content in the physiological environment of the colon at about 12.0 h (Table 3), resulting in delayed T_{max} and absorption time, lower C_{max} and decreased absorption rate constant. Also, the colon-targeted tablets of albendazole-β-CD complex (KA) showed similar results, delayed T_{max}, reduced $t_{1/2}$, and absorption time but because of improved solubility, higher C_{max} and $AUC_{0-\alpha}$ resulted. This is according to our initial contemplation that albendazole-β-cyclodextrin in a ratio of 1**:** 0.25 would be sufficient to improve the bioavailability of albendazole in colonic fluids, which was based on our solubility experiments, in which an increase of 700% in solubility of albendazole was observed [Somashekar et al., 2005].

Pharmacokinetic results discussed above were encouraging; therefore, further clinical investigation on patient volunteers was carried out to discover the possible clinical benefit of albendazole–β-CD complex over guar gum–based colon-targeted matrix tablets of

albendazole alone and also over conventional immediate-release tablets of albendazole in a limited number of patient volunteers of either sex with helminthiasis. The present study was focused on the ability of the albendazole formulations to cure helminthiasis only. All of the patient volunteers who participated in the study were found to have helminthiasis as indicated by slight intermittent fever, chills, sweating, nausea, vomiting, weakness, cramping abdominal pain, diarrhea, increase in WBC, and decreased Hb content. Furthermore, on microscopic examination of the freshly obtained stool samples showing the presence of eggs and cysts of *Ascaris lumbricoides*, *Trichuris trichiura*, or *Strongyloides stercoralis*, it was revealed that the nine patient volunteers participating in the study had helminthiasis.

An open parallel, single-blind study followed, wherein each group consisted of three patient volunteers. Group I (n = 3) was treated with conventional albendazole tablets (CA), Group II (n = 3) was treated with guar gum–based matrix tablets of albendazole (GA) and Group III (n = 3) was treated with guar gum–based matrix tablets of albendazole–β-CD complex (KA). Each patient volunteer received 400 mg of albendazole or its equivalent dose of tablet. The clinical symptoms of each patient volunteer of all the groups were examined every 8 h by a medical team. In all the treatment groups, the symptoms were found to be improving to normal. No supplement medications were administered to any patient volunteers during the study period.

With conventional immediate-release tablets (CA), raised body temperature was found to be gradually decreasing. Abdominal pain was reported to be persistent on all 3 days in all three patients. Diarrhea, nausea, and vomiting were observed only with one patient volunteer at the end of the third day. However, weakness was reported by all three patients on all 3 days. Hb content was found to be improving (Table 4) whereas decrease in total count (Table 5) was observed in all three patients of group I, although normal values were not achieved at the end of the third day. There was no significant difference in means at $P < 0.05$ was observed. Percentage reduction in epg from base clinical value was found to be increasing (Table 6), albeit few eggs were still present in the stool samples after 3 days of treatment in all three patient volunteers. There was no significant difference in means at $P < 0.05$ was observed. The helminthiatic infection in Group I patients was reducing; hence, epg and total count were found to be reducing. Although a few of the clinical symptoms were decreasing, there was no significant improvement in Hb content of patients, which may be because any supplement medications were not provided during the study period.

TABLE 4. Mean ± SD of Hb (g/dL) Values in Patient Volunteers Suffering (n = 3) of Different Groups with Helminthiasis on Various Days

Days	Group I	Group II	Group III
0	8.53 ± 1.23	7.47 ± 0.35	7.97 ± 1.36
1	8.53 ± 1.23	7.9 ± 0.8	8.9 ± 1.77
2	9.37 ± 1.02	8.7 ± 0.7	10.4 ± 1.45
3	9.67 ± 0.92	11 ± 1.8	12.97 ± 0.46

TABLE 5. Mean Values ± SD (× 10^3) (no./cm^3) of WBC in Patient Volunteers (n = 3) of Different Groups with Helminthiasis on Various Days

Days	Group I	Group II	Group III
0	15.63 ± 1.10	14.3 ± 3.39	14.37 ± 1.61
1	14.7 ± 7.21	13.6 ± 3.45	12.33 ± 9.6
2	13.6 ± 6.08	12.47 ± 2.67	10.67 ± 1.43
3	12.93 ± 3.21	11.1 ± 2.07	9.21 ± 1.16

TABLE 6. Percentage Reduction of Eggs/g from Base Clinical "0" Day in Patient Volunteers (n = 3) of Different Groups with Helminthiasis on Various Days

	Percent reduction in epg		
Days	Group I	Group II	Group III
1	29.4	29.67	48.275
2	52.94	62.96	86.2
3	76.47	88.89	100

When guar-gum–based colon-targeted oral drug delivery systems of albendazole alone (GA) were administered to patient volunteers, raised body temperature was found to be gradually decreasing. Abdominal pain and weakness were reported by two patients at the end of 3 days of treatment. None of the patients in group II reported diarrhea, nausea, and vomiting at the end of the third day. Hb content was found to be improving in all three patients (Table 4) and total count was found to be decreasing (Table 5), although values did not become normal at the end of the third day of the study. There was no significant difference in means at $P < 0.05$ was observed. Percentage reduction in epg was found to be increasing; however, no eggs were observed in only one patient volunteer at the end of the third day (Table 6). There was no significant difference in means at $P < 0.05$ was observed. Patients reported slight relief in few

of the clinical symptoms; epg and total count were found to have decreased more than that was observed with group I, but there was no significant improvement in Hb content of patients, which may be because no supplement medications were provided during the study period.

Patient volunteers treated with guar-gum–based colon-targeted oral drug delivery systems of albendazole–β-CD complex (KA) showed gradual decrease in raised body temperature and reached clinical status at the end of the third day. Abdominal pain, diarrhea, and weakness were reported by only one patient volunteer, whereas nausea and vomiting were not reported by any patients from the second day of treatment. Hb content improved faster (Table 4), and the number of WBC was found to be decreasing rapidly (Table 5) each day, and at the end of third day the Hb content and total count reached nearly normal clinical status. No significant difference in means at $P<0.05$ was observed. Percentage reduction in epg (Table 6) was also found to be decreasing from base clinical values (0th day), and no eggs or cysts were observed in the stool samples at the end of the third day of treatment in all of the three patients of group III. There was no significant difference in means at $P<0.05$. Few patients reported relief from few of the clinical symptoms; epg and WBC decreased more quickly than in either of the groups. Although no supplemental medications were provided in group III patients, significant improvement in Hb content of patients was observed during the study period. These results could be attributed to faster reduction in eggs or cysts of parasites, because of the availability of maximum concentration of albendazole and its improved solubility in the colonic fluids. Thus, albendazole diffuses more quickly and in increased concentrations into the intercellular space of the parasite cell, exerting its lethal action on parasitic cell at the specific target.

The results above indicate that albendazole is useful in the treatment of helminthiasis, but the dosage form and improvement in bioavailability of albendazole is what influences recovery rate in patients with helminthiasis. Conventional-release tablets of albendazole reduce the infection, but complete absence of clinical symptoms or epg would not be achieved. Therefore, a longer duration of therapy might be required with conventional-release tablets of albendazole. Similarly, guar-gum–based colon-targeted oral drug delivery systems of albendazole would deliver the drug into the colon, thereby improving clinical symptoms more quickly; however, complete absence of either the clinical symptoms or epg still cannot be achieved, whereas the reduction in number of eggs (epg) is faster in the guar-gum–based colon-targeted oral drug delivery systems of albendazole–β-CD complex (Fig. 4), than guar-gum matrix colon-targeted of albendazole alone or with conventional matrix tablets of albendazole. Also, patient volunteers in group III reported faster relief from clinical symptoms when treated with guar gum colon-targeted matrix tablets of albendazole-β-CD complex. This study indicates that the colon-targeting of albendazole–β-CD cures helminthiasis faster; thereby, the possible side effects with extended treatment may be avoided.

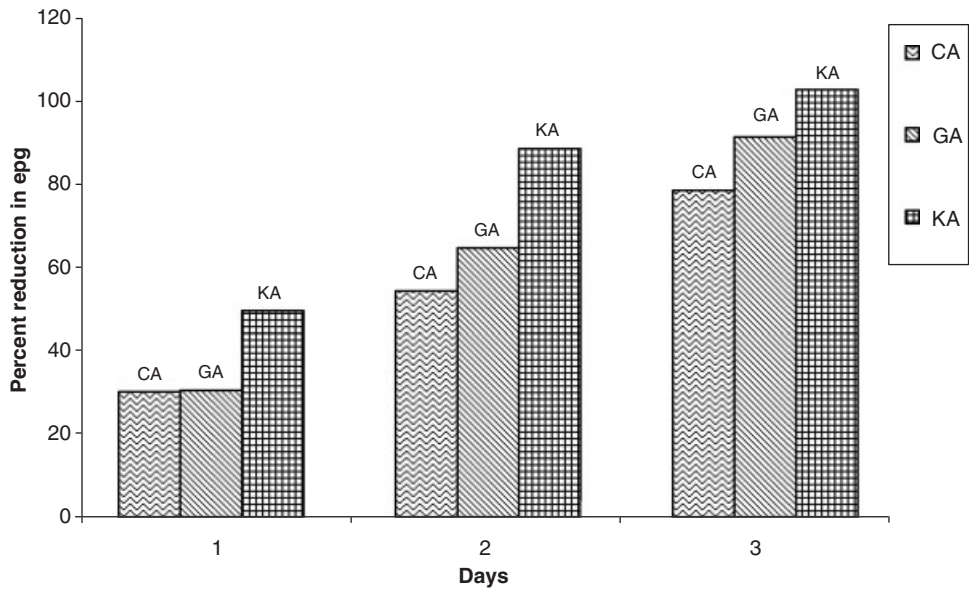

Fig. 4. Percent reduction in eggs per gram of feces from base clinical ''0'' day in patient volunteers with helminthiasis on various days.

However, the adverse effects of albendazole associated with the systemic absorption from conventional immediate-release tablets could not be addressed in this study because it requires controlled clinical study involving treatment with a placebo. However, the results of the present investigation definitely provide information on the possible benefit of the guar gum–based colon-targeted tablets of albendazole and albendazole-β-CD in providing an effective therapy over conventional immediate-release tablets.

CONCLUSIONS

The in vivo evaluation of guar gum–based colon-targeted albendazole tablets in healthy human volunteers showed delayed t_{max}, and delayed absorption time (t_a), decreased absorption rate constant, and unaltered $t_{1/2}$ indicate that the drug is not released in stomach and small intestine, but delivered to the colon, resulting in a slow absorption of the drug and making the drug available for local action in the colon. The increase in $AUC_{0-\infty}$ shows that the bioavailability of albendazole could be definitely improved by complexing the drug with β-CD. This clinical study revealed that albendazole is a useful drug in treatment of helminthiasis but might require longer duration of therapy when administered as a conventional immediate-release tablet. Guar-gum–based colon-targeted oral drug delivery systems of albendazole either alone or albendazole-β-CD reduce the infection faster and treat the disease quicker than conventional immediate-release tablets.

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