Development of Colon Targeted Oral Guar Gum Matrix Tablets of Albendazole for the Treatment of Helminthiasis

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The objective of the present study is to develop colon targeted drug delivery systems for albendazole using guar gum as a carrier. Matrix tablets containing various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder. The tablets were evaluated for hardness and drug content, and were subjected to in vitro drug release studies. The amount of albendazole released from the matrix tablets at different time intervals was estimated by HPLC method. Guar gum matrix tablets released 4 to 17% of albendazole in the physiological environment of stomach and small intestine depending on the proportion of guar gum used in the formulation. When the dissolution study was continued in simulated colonic fluids (rat caecal content medium) the matrix tablets containing 10% of guar gum released another 83% of albendazole after degradation into 2-3 pieces at the end of 24 h of the study. The matrix tablets containing 20% of guar gum also released about 44% of albendazole in simulated colonic fluids at the end of 24 h of the study indicating the susceptibility of the guar gum formulations to the rat caecal contents. The results of the study show that matrix tablets containing either 10% or 20% of guar gum are most likely to provide targeting of albendazole for local action in the colon. The guar gum matrix tablets of albendazole showed no change either in physical appearance, drug content or in dissolution pattern after storage at 40°/75 % RH for 6 mo. Differential scanning calorimetry indicated no possibility of interaction between albendazole and guar gum.

Enteric nematodes are among the most common and widely distributed animal parasites of humans1. The most common intestinal nematodes causing helminthiasis are those transmitted through contact with the soil for e.g., Ascaris lumbricoides, Trichuris trichiura, the hookworms, and Strongyloides stercoralis1. In Stoll's estimate, these worms, with Enterobius vermicularis, accounted for three-quarters of all helminthic infections2. The heavy load of worms may irritate the intestinal mucosa, causing inflammation and ulceration. Some produce toxic substances. The larger worms may become entangled and block the intestinal tract. Larval worms that migrate through the tissue to complete their life cycle may lose their way, end up in the wrong organ, and cause severe disease3. Nutritional problems occasionally are associated with the intestinal parasitosis, and persons with deficient diets often suffer from polyparasitism.

Albendazole is the drug of choice for treating helminthiasis1 including trichuriasis (whipworm infections), ancylostomiasis (hookworm infections) and ascariasis (roundworm infections)1-2. The conventional albendazole tablets release the drug along the GI tract and may cause the unwanted systemic effects1. Targeting of albendazole for action only in the colon may be beneficial in avoiding the systemic side effects, and even a lower dose of albendazole may be sufficient to treat helminthiasis. Several polysaccharides are being investigated as carriers for colon-specific drug delivery. The polysaccharides that are under active investigation for colon-specific drug delivery include

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pectin and its salts\textsuperscript{19}, chondroitin sulfate\textsuperscript{10,11} amylose\textsuperscript{12} and inulinHP\textsuperscript{13}. In this context, it has been reported from our laboratory that guar gum is a potential carrier for colon-specific drug delivery\textsuperscript{14-18}. Colon-specific drug delivery systems for 5-amino salicylic acid\textsuperscript{17} have been developed using guar gum as carrier. In the light of this information, it is planned to develop guar gum matrix tablets of albendazole for colon targeting to provide an effective safe therapy of helminthiasis.

Guar gum is a polysaccharide derived from the seeds of \textit{Cyamopsis tetragonolobus}, family Leguminosae\textsuperscript{16}. It consists of linear chains of (1-4)-\(\beta\)-D-mannopyranosyl units with \(\alpha\)-D-galactopyranosyl units attached by (1-6) linkages\textsuperscript{16}. In pharmaceutical formulations, guar gum is used as a binder, disintegrant, suspending agent, thickening agent and stabilizing agent. The present paper describes the development and evaluation of colon targeted drug delivery systems for albendazole using guar gum as a carrier.

**MATERIALS AND METHODS**

Albendazole (98.5-102\% purity) and mebendazole (98.6 to 101.4\% purity) were gift samples from M/s. Indechemie Laboratories Ltd., Mumbai and M/s. Cipla Ltd., Bangalore respectively. The pharmacopeial grade of guar gum (USNF, viscosity of 1\% aqueous dispersion is 125 cps; particle size <75 \(\mu\)m) was obtained from M/s. Dabur Research Foundation, New Delhi. Acetonitrile, water (HPLC grade) and glacial acetic were obtained from M/s. Qualigens Fine Chemicals, Mumbai. Other materials used in the study such as microcrystalline cellulose (Avicel, FMC Type pH-105), starch, magnesium stearate and talc were of pharmacopeial quality (USNF).

**Preparation of albendazole matrix tablets:**

Matrix tablets of albendazole were prepared by wet granulation method. Microcrystalline cellulose (MCC) was used as diluent and a mixture of talc and magnesium stearate (2:1 ratio) was used as lubricant. Guar gum was included in the formulations in various proportions. The composition of different matrix formulations used in the study containing 200 mg of albendazole in each case is given in Table 1. In all the formulations, guar gum was sieved (<250 \(\mu\)m) separately and mixed with albendazole (<150 \(\mu\)m) and MCC (<250 \(\mu\)m). The powders were blended and granulated with 10\% starch paste. The wet mass was passed through a mesh (1600 \(\mu\)m) and the granules were dried at 50\°C for 2 h. The dried granules were passed through a mesh (1190 \(\mu\)m) and these granules were lubricated with a mixture of talc and magnesium stearate (2:1). The lubricated granules were compressed at a compression force of 4500-5500 kg using 11 mm round, flat and plain punches on a single station tabletting machine (M/s Cadmach Machinery Co. Pvt. Ltd., Ahmedabad). Matrix tablets (n=100) of each composition were compressed and tested for their hardness, drug content and drug release characteristics with a suitable number of tablets for each test. The hardness of the matrix tablets was determined by using Monsanto Hardness Tester (M/s Magumps Ltd., Mumbai).

**HPLC analysis of albendazole in matrix tablets and dissolution fluids:**

The quantitative determination of albendazole was performed by High Performance Liquid Chromatography (HPLC). A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wave length programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP Column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (250 mm, 4.6 mm I.D., particle size 5 \(\mu\)m) was used. The HPLC system was equipped with the software Class-VP series version 5.03 (Shimadzu).

The mobile phase used was acetonitrile water containing 0.4\% of triethylamine (pH adjusted to 3.6 with 5\% orthophosphoric acid) in the ratio of 46:54. The filtered mobile phase was pumped at a flow rate of 1.2 ml/min. The column temperature was maintained at 40\°C. The eluent was

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity per each matrix tablet (mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ALV-10</td>
</tr>
<tr>
<td>Albendazole</td>
<td>200</td>
</tr>
<tr>
<td>Guar gum</td>
<td>45</td>
</tr>
<tr>
<td>MCC</td>
<td>146.5</td>
</tr>
<tr>
<td>Starch</td>
<td>45</td>
</tr>
<tr>
<td>Talc</td>
<td>9</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>4.5</td>
</tr>
<tr>
<td>Total (mg)</td>
<td>450</td>
</tr>
</tbody>
</table>

Composition of albendazole matrix tablets containing 10\% (ALV-10), 20\% (ALV-20) and 30\% (ALV-30) of guar gum.
detected using UV detector at 254 nm and the data were acquired, stored and analyzed with the software Class-VP series version 5.03 (Shimadzu). A standard curve was constructed for albendazole in the range of 1 to 40 μg/ml using mebendazole as the internal standard. A good linear relationship was observed between the concentration of albendazole and the ratio of the peak area of albendazole to that of mebendazole (internal standard) with a high correlation coefficient (r=0.9999). The required studies were carried out to estimate the precision and accuracy of this HPLC method of analysis of albendazole. The present HPLC method was found to be precise (CV< 3.5 %) and accurate (99.97 to 100.15 %). The standard curve constructed as described above was used for estimating albendazole either in the matrix tablets or in dissolution fluids.

**Determination of drug content:**

The albendazole matrix tablets were analyzed for drug content. Ten tablets were finely powdered, and 100 mg of the powder was accurately weighed and transferred to 100-ml volumetric flask. Initially, about 50 ml of glacial acetic acid was added to the volumetric flask and allowed to stand for 6 h with intermittent shaking (Remi Equipments, Mumbai) to ensure complete solubility of the drug. Then the volume was made up to 100 ml with glacial acetic acid, the mixture was centrifuged, 1 ml of the supernatant liquid was suitably diluted, filtered and analyzed for albendazole content by reverse phase HPLC method as described above.

**In vitro Drug Release Studies:**

The ability of guar gum matrix tablets of albendazole to remain intact in the physiological environment of stomach and small intestine was assessed by conducting drug release studies under conditions mimicking mouth to colon transit. Drug release studies were carried out using USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37”) for 2 h in 0.1N HCl (900 ml). Then the dissolution medium was replaced with pH 7.4 Sorensen’s phosphate buffer (300 ml) and tested for drug release up to 3 h. At the end of the time periods, 1 ml of the samples were taken without a pre-filter. The dissolution samples were taken without a pre-filter to include drug particles that might erode from the outer layer of the swollen guar gum matrix tablets. One millilitre of glacial acetic acid was added to the dissolution sample along with 20 μg of mebendazole (internal standard), the volume made up to 10 ml with water, filtered through 0.2 μm membrane filter and analyzed for albendazole by HPLC as described previously.

The susceptibility of the matrix tablets to the enzymatic action of colonic bacteria was assessed by performing the drug release studies in medium containing rat caecal material as described previously. The drug release studies were carried out using USP dissolution rate test apparatus with slight modifications. A beaker (capacity 150-ml) containing 100 ml of rat caecal content medium was immersed in water maintained in the 1000-ml vessel, which, in turn, was kept in the water bath of the apparatus. The tablets were placed in the baskets of the apparatus and immersed in the rat caecal content medium. As the caecum is naturally anaerobic, the experiment was carried out with continuous CO₂ supply into the beakers.

At different time intervals, 1 ml of the sample was withdrawn without a pre-filter and replaced with 1 ml of fresh phosphate buffered saline (PBS) bubbled with CO₂ and the experiment was continued for 19 h as the usual colonic transit time is 20-30 h. One millilitre of glacial acetic acid was added to the dissolution sample along with 20 μg of mebendazole (internal standard), the volume made up to 10 ml with water, centrifuged, the supernatant liquid was filtered through 0.2 μm membrane filter and analyzed for albendazole by HPLC as described previously. Glacial acetic acid was added to the dissolution samples to ensure the complete dissolution of the water-insoluble albendazole particles that may be eroded out of the guar gum matrix tablets.

To assess the long-term stability (1-2 y), drug release studies in simulated gastric and intestinal fluids and in rat caecal content medium were also carried out on albendazole matrix tablets ALV-10 and ALV-20 after storage at 40°/ 75% RH for 6 months as described earlier.

**Differential scanning calorimetry (DSC):**

The possibility of any interaction between albendazole and guar gum during the tablet processing was assessed by carrying out the thermal analysis on guar gum, pure drug (albendazole), powdered sample of matrix tablets formulation ALV-20 (before storage) and powdered sample of matrix tablet formulation ALV-20 (after storage) using differential scanning calorimetry (DSC 220C, Seiko Instruments Inc., Japan). Samples (10 mg) were accurately weighed into aluminum pans and hermetically sealed with aluminum lids. The thermograms of the samples were obtained at a scanning rate of 10°/min conducted over a temperature range of 30° to 300°.

**Statistical analysis:**

The cumulative percent of albendazole released from
guar gum matrix tablets (n=3) in the dissolution medium at 15 h and 24 h with and without rat caecal contents was compared, and the statistical significance was tested using Student's t-test. A value of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The present study was aimed at developing oral colon targeted formulations for albendazole using guar gum in the form of a matrix tablet. It was reported earlier that guar gum could be used as a carrier for colon-specific drug delivery in the form of either a matrix tablet or as a compression coat over a drug core tablet[17]. Matrix tablets, though release a low percent of the drug in stomach and small intestine, are the choice for colon-targeting of drugs. With indomethacin, it has been reported earlier by us that guar gum matrix tablets release about 21% of the drug in the physiological environment of stomach and small intestine majority of its drug content delivered in the physiological environment of colon[16]. However, the release of even such a small percent of drug from the surface of the matrix tablets in the physiological environment of stomach and small intestine is a serious consideration for drugs (for e.g., anticancer drugs in the treatment of colon cancer) showing deleterious effects on stomach and small intestine[8]. In such a situation, it was suggested to apply guar gum as a compression coat over the drug core tablet[4]. In this direction, compression coated 5-aminosalicylic acid (5-ASA) tablets were developed for colon targeting. The compression coat containing even 125 mg of guar gum over 5-ASA core tablets failed to achieve colon targeting[7]. In the light of this information, it is clear that film coating of guar gum over plain tablets of albendazole (likely to contain not more than 45 mg of guar gum for a tablet of 450 mg weight), as an alternative for matrix tablets, would not tolerate the physiological conditions of stomach and small intestine. Hence, it is planned to target albendazole to the colon in the form of guar gum matrix tablets.

Since guar gum is found to have poor flow properties and is to be incorporated in the matrix tablets in a larger proportion, albendazole tablets were prepared by wet granulation technique using starch paste as a binder. Assaying three samples of the powder mix, drawn randomly from the lot, assessed the uniformity of mixing of albendazole, guar gum and MCC. The matrix tablets were prepared by applying maximum force of compression, and the hardness of the tablets was found to be in the range of 4.4 to 5.0 kg/cm² (Table 2). The albendazole tablets (ALV10, ALV20 and

<table>
<thead>
<tr>
<th>Matrix formulation</th>
<th>Hardness (kg/cm²)</th>
<th>Percent Drug content Before storage</th>
<th>Percent Drug content After storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALV-10</td>
<td>4.5±0.1</td>
<td>99±0.9</td>
<td>99</td>
</tr>
<tr>
<td>ALV-20</td>
<td>4.8±0.1</td>
<td>100±1.0</td>
<td>100±0.1</td>
</tr>
<tr>
<td>ALV-30</td>
<td>5.0±0.9</td>
<td>102±0.9</td>
<td>102±0.2</td>
</tr>
</tbody>
</table>

*Values shown in the Table indicate Mean±SD. Characteristics of albendazole tablets (n=3) containing 10% (ALV-10), 20% (ALV-20) and 30% (ALV-30) of guar gum.

ALV30) were subjected to drug content and in vitro drug release studies containing guar gum in the proportion of 10, 20 and 30% respectively. The matrix tablets were found to contain 99.4 to 102.3% of the labeled amount indicating uniformity of drug content in the formulation (Table 2).

The matrix tablets were subjected to in vitro drug release studies in varied dissolution media 0.1 N HCl (2 h), pH 7.4 Sorensen’s phosphate buffer (3 h) and simulated colonic fluids (rat caecal content medium at 4%w/v level after 7 d of enzyme induction). It was reported earlier from our laboratory[4] that rat caecal content medium at 4%w/v level after 7 d of enzyme induction provide the best conditions for assessing the susceptibility of guar gum to colonic bacterial degradation.

When albendazole matrix tablets containing 10% of guar gum were subjected to in vitro drug release studies, the matrix tablet degraded into 2-3 pieces at about 20 h of the dissolution study conducted without rat caecal contents in the medium (control). However, albendazole matrix tablets containing 20% (ALV-20) and 30% (ALV-30) of guar gum retained their physical integrity up to 24 h of the dissolution study conducted without rat caecal contents in the dissolution medium.

The percent of albendazole released from the matrix tablets containing 10% of guar gum (ALV-10) is shown in fig. 1. Matrix tablets containing 10% of guar gum (ALV-10) degraded into 2-3 pieces at the end of 10 h dissolution study in the presence of simulated colonic fluids (rat caecal contents in the dissolution medium). The percent of albendazole released from the ALV-10 matrix tablets at the end of 15 h and 24 h was found to be 93.7±2.8% and 99.5±0.1 whereas in control study (without rat caecal contents in the dissolu-
Fig. 1: Release profile of 10% guar gum albendazole matrix tablets

Mean (± SD) percent of albendazole released from matrix tablets (n=3) containing 10% of guar gum in dissolution study with (-●-) and without (-■-) rat caecal contents.

The percent drug released from the matrix tablets containing 20% of guar gum is shown in fig. 2. Drug released from the ALV-20 matrix tablets at the end of 24 h was found to be 44.0±1.55%, whereas in the control study (without rat caecal contents in the dissolution medium) it was only 20.9±0.6%. Significant difference (P<0.001) was observed in the amount of albendazole released at 15 h and 24 h of the dissolution study with rat caecal content medium when compared to the dissolution study without rat caecal contents.

Fig. 2: Release profile of 20% guar gum albendazole matrix tablets

Mean±SD percent of albendazole released from matrix tablets (n=3) containing 20% of guar gum in dissolution study with (-●-) and without (-■-) rat caecal contents.

The study shows that the release of albendazole in the physiological environment of colon is due to the microbial degradation of guar gum matrix tablets in the presence of rat caecal contents. The dissolution study was carried out without rat caecal contents (control study) to ensure that the drug release is not due to mechanical erosion likely to occur because of the bowel movements in humans. On exposure to the dissolution fluids, the gum gets hydrated and forms a viscous gel layer that slows down, further seeping-in of dissolution fluids towards the core tablets. The hydration of guar gum seems to be unaffected by the pH of the dissolution medium.

On increasing the amount of guar gum in the matrix tablets, the release of albendazole decreased at the end of 24 h of dissolution study. The ALV-30 released 21.0±0.9% of albendazole in the presence of rat caecal contents, whereas in the control study, the formulation released only 12.3±1.8% of albendazole. Though the matrix formulation ALV-30 released only 21.0±0.9% of albendazole in simulated colonic fluids, a significant difference was observed (P<0.05) in the dissolution pattern at 15 h and 24 h of study.
when compared to the dissolution study without rat caecal contents (fig. 3). The results showed that ALV-30 formulation was degrading slowly in simulated colonic fluids indicating that 30% of guar gum in the matrix formulation is high enough for the colonic bacteria to act upon the formulation and degrade it.

The matrix formulation ALV-10 released almost the entire quantity of the drug at the end of 24 h dissolution study. The formulation ALV-20 also released about 44% of its drug content in the physiological environment of stomach, small intestine and colon. It appears from these results that ALV-10 could target albendazole to colon. The ALV-20 tablets are also considered as potential formulations for targeting of albendazole to colon because of the fact that the human caecal contents would be far more than what was used in the present study. Just on increasing the proportion of guar gum from 20% to 30% there was a sharp decline in the percent of drug release in simulated GI fluids, i.e., only 21% of the drug was released at the end of 24 h of dissolution study. The high gel strength of the swollen matrix formulation might have prevented the drug release from the formulation. Probably, the colonic bacterial action of the rat caecal medium is insufficient to degrade such a high strength gel barrier of the swollen ALV-30 matrix formulation. Unless the swollen guar gum barrier degrades, further swelling of the matrix formulation does not occur. Even in humans, despite higher caecal contents, the complete degradation of the ALV-30 may not be possible because of such a high quantity of guar gum in the formulation. However, the relative potential of the formulations ALV-20 and ALV-30 needs to be evaluated in human volunteers.

In view of the potential utility of ALV-10 and ALV-20 formulations for targeting albendazole to colon, stability studies were carried out at 40°C 75% RH for 6 months (climatic zone IV conditions for accelerated testing) to assess their long term (2 years) stability. The protocol of the stability studies was in conformation with the WHO recommendations for stability testing of products intended for global market. After storage, the formulations were analyzed for the drug content and the in vitro drug release pattern was determined. The powdered sample of ALV-20 formulation after storage was also subjected to DSC study. When the matrix tablets ALV-10 and ALV-20 were stored at 40°C 75% RH for 6 mo, no change appeared either in physical appearance or in drug content. When the dissolution study was conducted in the simulated physiological environment of stomach, small intestine and colon as described above, no significant difference (P>0.05) was observed in the cumulative percent of albendazole released from both ALV-10 and ALV-20 stored at 40°C 75% RH for 6 mo when compared to that released from the same formulations before storage (Tables 2 and 3). The insignificant change either in the physical appearance, drug content or in dissolution profile of ALV-10 and ALV-20 formulations after storage at 40°C 75% RH for 6 mo indicate that the formulations could provide a minimum shelf life of 2 years.

The occurrence of any drug-excipients interactions in the formulations was predicted by using DSC studies. A sharp endothermic peak corresponding to the melting point of crystalline albendazole was found at 222.1°C for the drug sample. The endothermic peak corresponding to the melting point of albendazole in the sample of matrix formulation (ALV-20) slightly shifted to 214.3°C, which may be, because of bound water present in guar gum. Even after storing at 40°C 75% RH for 6 mo, the thermogram of the powdered sample of ALV-20 formulation did not show any significant shift in the endothermic peak. Based on the thermograms of DSC, there appears to be no possibility of interaction.

Fig. 3: Release profile of 30 % guar gum albendazole matrix tablets
Mean (±SD) percent of albendazole released from matrix tablets (n=3) containing 30% of guar gum in dissolution study with (-•-) and without (-□-) rat caecal contents.
**TABLE 3: PERCENT OF ALBENDAZOLE RELEASED FROM MATRIX TABLETS.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Percent of albendazole released from ALV-10</th>
<th>Percent of albendazole released from ALV-20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before storage</td>
<td>After Storage</td>
</tr>
<tr>
<td>2</td>
<td>11.3±1.1</td>
<td>11.4±0.6</td>
</tr>
<tr>
<td>5</td>
<td>17.4±0.5</td>
<td>17.8±1.6</td>
</tr>
<tr>
<td>10</td>
<td>61.5±1.3</td>
<td>65.2±4.8</td>
</tr>
<tr>
<td>15</td>
<td>93.7±2.8</td>
<td>95.2±4.5</td>
</tr>
<tr>
<td>20</td>
<td>98.9±0.3</td>
<td>99.8±2.2</td>
</tr>
<tr>
<td>24</td>
<td>99.5±0.1</td>
<td>99.8±0.9</td>
</tr>
</tbody>
</table>

*Values shown in the Table indicate the mean±s.d. percent of albendazole released from ALV-10 and ALV-20 matrix formulations (n=3) before and after storage at 40°/75% RH for 6 mo.*

between albendazole and guar gum and other excipients used in the matrix tablets.

The conventional tablets usually containing 400 mg of albendazole, releases majority of the drug in stomach and small intestine and a very small proportion reaches colon. The novel albendazole tablet (containing, 200 mg) developed in the present study (ALV-10) released only 17% of the drug in the physiological environment of stomach and small intestine, but released 83% of the drug in the physiological environment of colon. Thus, the guar gum matrix tablets of albendazole developed in the present study might be useful in selectively targeting majority of the drug to the colon. This should be further confirmed by subjecting both the conventional and guar gum matrix tablets of albendazole to bioavailability studies in human volunteers.

The present study was carried out to develop colon-targeted delivery systems for albendazole using guar gum as a carrier. Matrix tablets containing various proportions of guar gum were prepared and subjected to in vitro drug release studies. Albendazole matrix tablets containing 30% of guar gum are considered unsuitable for colon targeting, as they released only 21% of albendazole even after 24 h of dissolution study. Though matrix tablets containing 10% of guar gum completely degraded in the presence of rat caecal contents releasing almost all albendazole, matrix tablets containing 20% of guar gum also degraded partially in simulated colonic fluids. In conclusion, the matrix formulations containing either 10% or 20% guar gum are most likely to target albendazole to colon without being released significantly in stomach and small intestine. The matrix formulations (ALV-10 and ALV-20) after storing at 40°/75% RH for 6 mo showed no significant change either in physical appearance, drug content or in dissolution pattern. The DSC thermograms indicate no possibility of interaction between albendazole and guar gum/other excipients used in the matrix tablets. Bioavailability studies are in progress to assess the relative usefulness of the ALV-10 and ALV-20 formulations in comparison with conventional albendazole tablets.

**ACKNOWLEDGEMENTS**

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