Effect of Curcumin Enema Dosage Regimes on the Reduction of Giardia Cyst among Calves

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(received 19-02-2023, revised 12-09-2023, accepted 02-10-2023)

ABSTRAK

Ural K, Erdoğan H, Ural DA, Paşa S, Erdoğan S, Ayan A, Gültekin M, Balikçi C. 2023. Pengaruh dosis curcumin Enema terhadap pengurangan giardia cyst pada sapi pedet. JITV 28(4):237-243. DOI:http://dx.doi.org/10.14334/jitv.v28i4.3276.

Giardiasis, mungkin salah satu serangan parasit yang paling signifikan dan terabaikan pada anak sapi, yang menyebabkan infeksi usus. Agen protozoa ini memperoleh kepentingan diagnostik dan terapeutik karena potensi zoonosisnya sehingga intervensi terapeutik alternatif/pengganti baru menjadi cukup penting. Hal ini karena obat antiparasit yang biasa digunakan untuk pengendalian dan pemberantasan untuk melawan infeksi ini, biasanya menimbulkan efek samping. Oleh karena itu, proyek ini mengevaluasi aktivitas anti-protozoa kurkumin, pada dosis berbeda yang digunakan melalui rute rektal, konstituen utama kunyit. Kelompok enema rektal [Kelompok I: 2 gr/kg, Kelompok II: 80 mg/kg dan Kelompok III: 10 mg/kg] dan pengobatan oral Kelompok IV: 2 gr/betis terdiri dari pengobatan kurkumin dengan dosis yang sesuai ditunjukkan di sini sebagai kontrol kelompok V tidak diobati. Semua kelompok perlakuan menunjukkan penurunan jumlah kista yang signifikan secara statistik pada hari ke 10, dibandingkan dengan nilai hari ke 10 pada kelompok kontrol ($p<0,005$). Di antara kelompok perlakuan, pengurangan kista tertinggi terlihat pada 99,86% pada kurkumin 10 mg/kg enema rektal yang diberikan pada anak sapi (Kelompok III), yang menerima dosis penelitian terendah. Kurkumin pada semua dosis, namun terutama digunakan melalui rektal dengan rejimen dosis terendah 10 mg/kg, dapat mempercepat kemanjuran klinis dan parasitologis melalui kemungkinan penghambatan proliferasi dan adhesi Giardia, seperti yang dijelaskan sebelumnya. Modalitas pengobatan melalui rute rektal ini mampu mengubah keadaan, menyoroti protokol pengobatan baru.

Kata Kunci: Kurkumin, Giardia, Oral, Rektal, Perlakuan

ABSTRACT

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Giardiasis is one of the most significant and neglected parasitic infestations among calves, resulting in intestinal infections. This protozoan agent gained diagnostic and therapeutical importance because of its zoonotic potential, in which novel alternative/substitute therapeutical interventions are important, and this is because commonly used antiparasitic drugs for control and eradication for battling this infection commonly presented side effects. Therefore, the present project evaluated the anti-protozoan activity of curcumin at different dose regimes used via the rectal route, the main constituent of turmeric. Rectal enema groups [Group I: 2 gr/kg, Group II: 80 mg/kg, and Group III: 10 mg/kg] and oral treatment Group IV: 2 gr/calf consisted of curcumin treatment at appropriate dosages showed herein in which the control group V were untreated. All treatment groups showed a statistically significant decrease in cyst counts on day 10, compared to day 10 values of the control group ($p<0.005$). Among the treatment groups, the highest cyst reduction was evident at 99.86% in curcumin 10 mg/kg rectal enema-administered calves (Group III), which received the lowest dose of the study. Curcumin at all dosages, primarily used in the rectal route at the lowest dosage regimen of 10 mg/kg, could have hastened clinical and parasitological efficacy via probable inhibition of Giardia proliferation and adhesion, as described previously. This treatment modality via the rectal route can change the game, highlighting novel treatment protocols.

Key Words: Curcumin, Giardia, Oral, Rectal, Treatment

INTRODUCTION

Giardia duodenalis, a well-recognized protozoal parasite, is frequently detected worldwide among production animals. Subclinical infection in cattle is common, whereas giardiasis prone calves to diarrhea and diminished weight gain (Geurden et al. 2009). The emergence and spread of giardiasis among calves population are increasing problems in our country (Ural et al. 2022; Ural et al. 2022). For the treatment of

giardiasis, several drugs are preferred. Table 1 presented anti-giardia treatment options (Pérez-Arriaga et al. 2006), with the vast majority related to side effects. Unexpected, however, well-recognized side effects and therapeutical failures in treatment are known (Darlan et al. 2020). Besides, the parasite might be resistant to drugs, indicating the requirement for novel substitute therapy approaches, natural in origin if possible. Given the significance of natural plants as new antiparasitic compounds, several might be promising.

A famous plant, turmeric, best known as Curcuma longa, has traditional and scientifically therapeutical usage. The latter Asian tropical plant (Shahiduzzaman & Daugschies 2011) constituently involves yellow bioactive material obtained from the rhizomes of Curcuma are curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Dyab et al. 2016). Curcumin is the most significant bioactive ingredient related to the medicinal activity of Curcuma (Holt et al. 2005). It has anti-inflammatory, antitumoral, antioxidant, and antimicrobial efficacy, dedicated to its promising natural compound that might be beneficial against bacterial, fungal, and viral agents (Kumar et al. 2011). Writing down the state of the art regarding curcumin usage within literature data should be wise at the bottom lines.

Given the usage of curcumin, as was the subject of the present study, the antiparasitic efficacy of curcumin received arousing interest. For a better understanding of audiences, several researches were focused on *in vitro* usage of curcumin against giardiasis. Curcumin exhibited activity against several parasites (Pérez-Arriaga et al. 2006; Das et al. 2008; Shahiduzzaman et al. 2009; Pinlaor et al. 2010; Shahiduzzaman & Daugschies 2011). Given curcumin's cytotoxic efficacy on Giardia lamblia trophozoites (Pérez-Arriaga et al. 2006), this data must, at least herein, be an in-depth analyte. *In vitro*, research published in 2017 interpreted cytotoxic influence and morphological alterations of 50,000/mL Giardia parasites subjected to curcumin intervention. In that study, it was hypothesized that curcumin selectively caused inhibition of trophozoite adhesion/proliferation/growth. Abrupted morphology was dedicated to deranged membranes, dorsal/ventral surfaces, flagella, and ventral disk. It was also concluded that curcumin could bind to and interact thoroughly within the most profused protein of the giardia cytoskeleton, denoted as tubulins, establishing microtubules within the parasite. Consequently, microtubules were defragmented (Gutiérrez-Gutiérrez et al. 2017). Another exciting article denoted an 85% mortality rate for Giardia cysts after treatment with curcumin extract at a 50 mg/mL concentration after 60 min (Dyab et al. 2016). Furthermore, curcumin prescription at 20 mg/kg/day diminished cyst excretion and trophozoites (84.7%) within the fecal samples of treated ones (Dyab et al. 2016). Apart from those mentioned above *in vivo* studies, one previous field investigation searched the efficacy of 2 g/calf/day curcumin via rectal route for 10 days) compared to the control group of calves. There was 58.17% (P=0.125), 99.68% (P=0.001), and 100% (P=0.001) reduction on days 3, 7, and 10, respectively, among calves treated with curcumin enema against giardiasis and diarrhea (Ural et al. 2022). Apart from the last studies, the difference between the present research and other relevant ones was based on curcumin usage from a clinical perspective, as this was an *in vivo* model. As a novelty, different dose regimes were used in this study. With the purpose and wishes of searching for promising anti-giardial natural compounds, in the present work, *C. longa* (curcumin) was selected to evaluate the activity of clinical and parasitological efficacy against giardiasis among calves via rectal or oral route.

MATERIALS AND METHODS

Animals and housing

A sample size of 35 Holstein and Jersey calves aged 14 to 36 days was purchased from a commercial vendor in Aydin City, Turkey. Given that the incubation period of giardiasis usually takes 7 to 10 days before investigation, meaning before the treatment modality was applied, all calves were physically examined and determined to have varying degrees of diarrhea. During the allocation period (10 days), all calves were monitored daily at 12-hour intervals to confirm the presence/absence of G. duodenalis cysts within the fecal samples withdrawn. All calves were monitored for 6 months after therapy apart from the study period. No prior drug treatments were available, which was an exclusion criterion. During the trial, the housing prevented cross-contamination. Ethical guidelines were considered for enrollment into the groups, randomly performed by a coin toss.

This study was summarized partially from a national project funded by Adnan Menderes University, Research Projects Funding Unit, with Project number VTF-22027/2022. Along with the written owner consent of farm managers, ethical guidelines were followed: Aydın Adnan Menderes University HADYEK (Local Ethics Committee) with no: 64583101/2020/129 at 17/12/2020

Sub-classification to study groups

As denoted above, random selection by a coin toss permitted us to classify and denote the treatment groups as described in Fig. 1 below. Each group consisted of 10 calves enrolled. Curcumin was purchased from a commercially available pharmaceutical company with an ingredient of 95% pure curcumin powder available.

Table 1. Anti-giardia treatment options with mechanism of action anti-giardiasis compounds related to side effects

Antigiardiosic drugs		Mechanism of action
Nitroimidazoles	Metronidazole	induce DNA fragmentation influencing host cells contraindicated during pregnancy (Hausen et al. 2006; Benchimol et al. 2023)
Benzimidazoles	Albendazole	Effects on parasite cytoskeleton causing parasite detachment through intestinal epithelium (Harder et al. 2001)
Nitrofuran	Furazolidone	effects parasite adhesion capacity but not its viability (Hoyne et al. 1989)
Nitazoxanide	Tiazolidic derivative	Presented the same efficacy as metronidazole (Ponce-Macotela et al. 2001; Mehmood et al. 2022)

Source: Perez-Arriaga et al. 2006

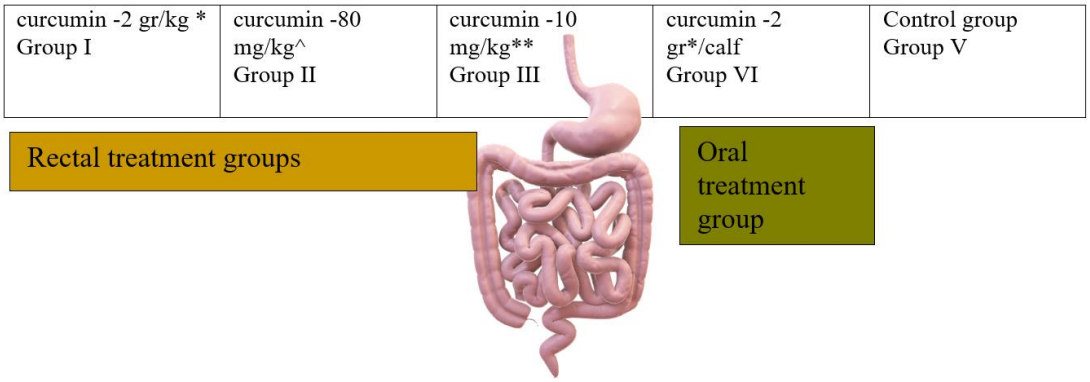

Figure 1. Classification of the groups and treatment decision tree methodology (*Oh et al. 2013; ^Hashemzadeh-Cigari et al. 2014; **Biswas et al. 2017). Rectal enema treatment groups (Groups I to III were administered curcumin per kg of calf body weight, whereas Group IV received 2 g per calf orally, and the control group received an equivalent volume of saline

Fecal flotation and microscopic examination of fecal samples

Pretreatment day 0 (d0) was designated as the start of the trial. Fecal samples from each calf were withdrawn in two sets by investigators in this study and were designated either d0 or day 10 (d10) (the last day of the treatment). Fecal samples (5-7 g) were withdrawn on d0 manually from the rectum of all calves that participated (Ural et al. 2014) and were submitted immediately to laboratory work for fecal flotation. A brief methodological explanation could be explained with previous work by some of the selected researcher groups (Ural et al. 2014) based on a prior description (Wilson & Hankenson 2010). Prepared slides were examined 40x power for the possible Giardia cysts. Microscopical fecal sample proof was based on (Escobedo et al. 2003).

Clinical observation of referring veterinarians

Data regarding clinical status (i.e., existence of diarrhea and several others) and fecal consistency were recorded before and after treatment. The fecal consistency was scored as previously described by Le Jambre et al. (2007) and closer to what has been described elsewhere (Geurden et al. 2011).

Assessment of efficacy of treatment

The therapeutical efficacy of curcumin, whether used by rectal route or orally in the present study, was assessed via microscopic examination of fecal samples collected on D0 and D10 (on the last day of treatment) and analysis based on the probable diminishment in cyst excretion for treated calves in comparison to those of control calves. Deduced cyst excretion was interpreted by use of the Henderson & Tilton's formula (Henderson & Tilton 1955), composing geometric mean cyst counts as described previously (Geurden et al. 2011):

$$100x[-\frac{Ta\ x\ Cb}{Tb\ x\ Ca}]$$

Ta and Tb represented the geometric mean cyst count in the secnidazole treatment group before and after treatment. In contrast, Ca and Cb were the geometric mean cyst count in the control animals before and after treatment (Presidente PJA. 1985).

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (version 13; SPSS), similar to what has been described previously (Ural et al. 2014).

RESULTS AND DISCUSSION

Statistical analysis along with cyst reduction to those of treatment (both rectal enema and oral) and control groups were shown in Table 2. All treatment groups showed a statistically significant decrease in cyst counts on day 10, compared to day 10 values of the control group ($P<0.005$). Among treatment groups, the highest cyst reduction was 99.86% in curcumin -10 mg/kg rectal enema-administered calves. Figure 3 shows groups I and III calves sampled. Given cyst counts, the geometric mean was shown in Figure. 4. There were no treatment side effects, and all calves accepted oral or rectal route usage of curcumin. Following 6 month period of monitorizatşon, no recurrence was observed.

At the initial planning of our study, we hypothesized that rectal enema curcumin usage would be beneficial for combatting giardiasis among calves in our country. As giardiasis is a common problem, reasonably priced, economical, and practical, thus natural, if possible, treatment is warranted. Given the antiparasitic efficacy of curcumin (Pérez-Arriaga et al. 2006; Das et al. 2008; Shahiduzzaman et al. 2009; Pinlaor et al. 2010; Shahiduzzaman & Daugschies 2011) and it is cytotoxic efficacy on Giardia lamblia trophozoites (Pérez-Arriaga et al. 2006), we herein at this study for the first time used different dose regimes of curcumin, comparatively by both oral and rectal enema routes, apart from the researches mentioned above. As a dissimilarity to *in vitro* studies of curcumin usage against giardiasis, we performed a clinical trial at this study.

In vitro, research published in 2017 interpreted cytotoxical influence and morphological alterations of 50,000/mL Giardia parasites subjected to curcumin intervention. In that study, it was hypothesized that curcumin selectively caused inhibition of trophozoite adhesion/proliferation/growth. Abrupted morphology was dedicated to deranged membranes, dorsal/ ventral surfaces, flagella, and ventral disk. It was also concluded that curcumin could bind to and interact thoroughly within the most profused protein of the giardia cytoskeleton, denoted as tubulins, establishing microtubules within the parasite. Consequently, microtubules were defragmented (Gutiérrez-Gutiérrez et al. 2017). Another exciting article denoted an 85% mortality rate for Giardia cysts after treatment with curcumin extract at a 50 mg/mL concentration after 60 min (Dyab et al. 2016).

Furthermore, curcumin prescription at 20 mg/kg/day diminished cyst excretion and trophozoites (84.7%) within the fecal samples of treated ones (Dyab et al. 2016). In the present study, as an *in vivo* model, the efficacy of both rectal enema and oral curcumin was detected among calves with giardiasis. Group I, II, and III of calves received rectal enema curcumin at different dosages, giving 99.84%, 99.68%, and 99.86% efficacy, respectively. The highest efficacy was received in group III of calves administered curcumin at the lowest dosage. A direct comparison among those studies is difficult as most were *In vitro* studies. However, comparatively, Ural et al. (2022) determined the efficacy of 2 g/calf/day curcumin via rectal route for 10 days compared to control ones. There was a 100% ($P=0.001$) reduction on day 10 among calves with giardiasis. In the present study, as mentioned above, we received 99.86% efficacy on giardia cyst count regression compared to the previous study. Also, in the present study, the lowest dose of curcumin exhibited the best efficacy.

Not surprisingly, but interestingly, giardia deranges homeostasis of the whole intestinal tract through competition with the commensal gut microbiota (for attachment sites and nutritional conditions). During the latter condition, giardia i) deranges commensal gut microbiota biofilms, ii) exhaust mucus, and iii) diminish the activity of the immune system. Furthermore, at upper-end growing conditions, parasites elevated intestinal epithelial permeability, epithelial apoptosis induction, bacterial translocation promotion, and pro-inflammatory cytokine production (Allain et al. 2017). Even if this was the condition in the present study (not a far relationship), curcumin, primarily used as a rectal enema, could have helped hasten clinical and parasitological recovery. A brief explanation, thus, should include 1) regulation of intestinal barrier functioning (Wang et al. 2017; Ghosh et al. 2018; Liu & Zhu 2022), 2) beneficial effects via gut microbiota [neutralizing diminished butyric acid-producing bacteria along with elevated richness and diversity] (Zhai et al. 2020), 3)anti-apoptotic activity on intestinal epithelial tissue (Loganes et al. 2017), 4) reduction of bacterial translocation and cytokine levels *(*Sözen et al. 2015) all by curcumin. It should not be unwise to draw a preliminary suggestion that curcumin interacts with giardia via the aforementioned probable mechanisms, which could also explain the efficacy of this study.

In a prior investigation (to those in which selected researchers of this manuscript were also involved), the anti-giardial therapeutical efficacy of citrus extract was analyzed. Goat kids, at the age of 12 to 68 days, with giardiasis were classified as the control group (n=8) [without any receipt], and the other relevant group (n=10) received oral bitter orange extract at a dosage of 10 ml/goat kids for seven days. Antigiardial combatting of bitter orange extract was detected by oocyst shedding on days 0 and 10, with 99.9% efficacy on day 10. Interestingly, it is a natural compound against giardiasis with significantly diminished oocyst shedding supporting the anti-giardial activity of bitter orange extract (Ural et al. 2021). In the present study, another natural compound was the choice. The efficacy of both rectal enema and oral curcumin was shown in this study among calves with giardiasis. Group I to III of calves

Table 2. *G. duodenalis* cyst excretion among curcumin-treated and control groups of calves enrolled at days 0 and 10

	Group I (-2 g/kg in rectal enema)	Group II (-80 mg/kg in rectal enema)	Group III (-10 mg/kg in rectal enema)	Group IV (-2 g/calf p.o.)	Control (saline p.o.)	P-value
Day 0	79709±70145	54923±63340	49014±65423	34798±68076	69133±79519	0.792
Day 10	129.9±566[a]	175±1253[a]	69.96±253.5[a]	75.58±563[a]	23846±16054[b]	0.005
Cysts Reduction (%)	% 99.84	% 99.68	%99.86	% 99.78	% 65.51	

[a,b]: Different letters in the same line are statistically different.

Figure 3. Randomized classification to rectal enema curcumin groups involved a) 2 g/kg, b) 10 mg/kg.

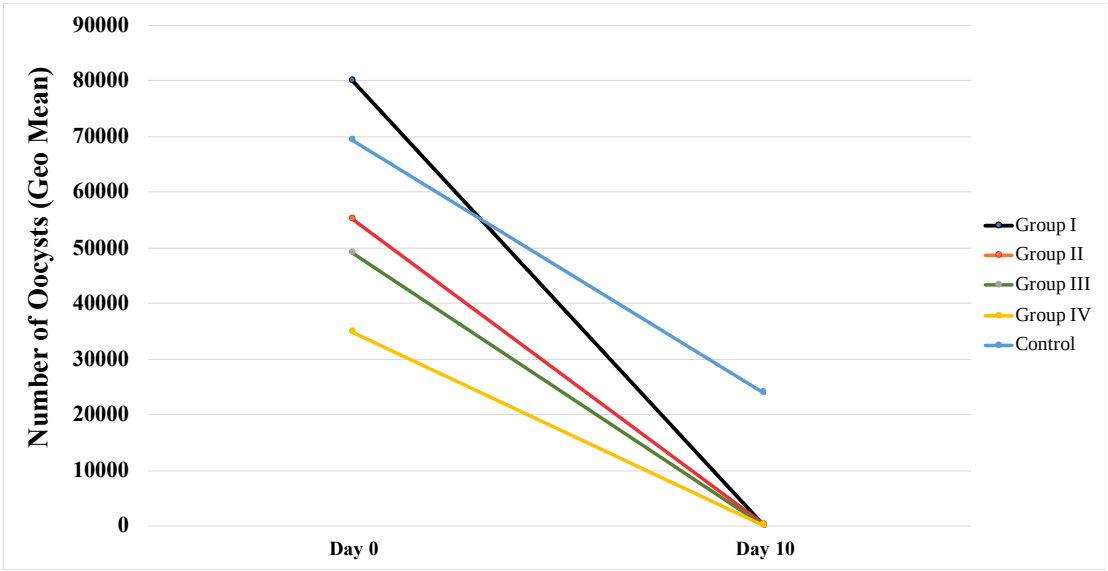

Figure 4. Geometric mean of cyst counts among curcumin-treated and control groups of calves enrolled at days 0 and 10

received rectal enema curcumin at different dosages (apart from the purpose of the present study), resulting in %99.68 to 99.86 efficacy, with the highest reflection in group III. Those calves in group III administered curcumin at a dosage of 10 mg/kg (the lowest among all treated calves) showed the best efficacy. The concept of low-dose curcumin may be beneficial for health promotion (DiSilvestro et al. 2012; Fança-Berthon et al. 2021), lower dose in comparison to higher doses (15 mg. versus 500 mg) showed delivery of higher concentrations, in which our efficacy with the lowest dose at the present study could be briefly explained.

Several frequently observed side effects of oral curcumin might include gastrointestinal complaints (Goulart et al. 2021), whereas, as was also the selected route for the present study with 3 different enema dose regimes, administering curcumin through rectal route (enema), were reported in humans with ulcerative colitis. This methodology could bypass first-pass metabolism within the liver, which is elevated for oral consumption of curcumin (Singla et al. 2014). Our interest was competitively growing in this subject and era of rectal enema curcuminoids, in which our subsequent study would meet these criteria.

CONCLUSION

Our study reveals that curcumin, when administered either orally or via rectal enema, effectively resolved diarrhea within 2 to 4 days in treated groups. Notably, the lowest dosage of curcumin delivered as an enema showed the highest efficacy. By day 10 of treatment, a significant reduction ($p<0.005$) in cyst excretion was observed across all treated groups compared to control calves. Given its cost-effectiveness and favorable tolerance profile, curcumin is a promising, natural therapeutic option, potentially surpassing conventional drugs. This research underscores the potential of curcumin as a therapeutic agent in managing giardiasis in calves, providing invaluable insights for future studies and field applications. The observed benefits of curcumin address immediate clinical outcomes and present broader implications for the long-term health and well-being of treated animals.

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