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Toxicity, Anthelmintic Efficacy and Proteolytic Activity of Chitosan-Encapsulated Bromelain within the Gastrointestinal Tract of Small East African Goats

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ABSTRACT

The development of resistance to anthelmintic drugs has prompted researches into alternative methods for controlling intestinal nematodes in ruminants. This study aimed to evaluate the anthelmintic efficacy, proteolytic activity, and toxicity of bromelain encapsulated in chitosan within the gastrointestinal tract (GIT) of Small East African goats in Kenya. Twelve healthy indigenous male goats were divided into four groups contained three goats in each groups. Treatment groups included: G1, chitosan-encapsulated bromelain (90 mg/kg); G2, chitosan-encapsulated bromelain (270 mg/kg); G3, positive control (albendazole 7.5 mg/kg); and G4, negative control. The animals were orally treated with the drugs in a single dose. The hematological and serum biochemical parameters were determined using standard methods. The strongyle fecal egg count was evaluated weekly using a modified McMaster technique. To determine the proteolytic activity of nanoencapsulated bromelain within the GIT, another set of twelve goats was used and administered 270 mg/kg of encapsulated bromelain. Every four hours, three goats were sacrificed and the proteolytic activity of the drug was determined in the different organs of the GIT. Significant differences were observed between the mean PCV of goats treated with 270 mg/kg encapsulated bromelain and non-treated goats on days 21 and 28 post-treatment. The mean aspartate aminotransferase, urea, and creatinine levels of treated and control goats did not significantly differ during the experiment period. Also, no significant difference was observed between the mean alanine aminotransferase level of treated and untreated goats 28 days post-treatment. The administration of encapsulated bromelain was not associated with any clinical sign and mortality. The reduction in fecal egg count in G1 and G2 at 28 days post-treatment was 9.5% and 22.6%, respectively. The encapsulated bromelain remained proteolytically active along the goat GIT but its protease activity varied according to the type of GIT organ and time elapsed since administration. In conclusion, chitosan-encapsulated bromelain is safe, but have low efficacy against GIT strongyle nematodes when given as a single dose. Future studies should evaluate higher and repeated doses of encapsulated bromelain for controlling GIT nematodes.

Key words: Bromelain, Chitosan, Efficacy, Goats, Nanoencapsulation, Proteolytic activity.

INTRODUCTION

Livestock parasites are associated with major economic losses worldwide and have a considerable impact on farm profitability (Sackett et al., 2006; Roeber et al., 2013; Rashid et al, 2019). For instance, in Kenya, economic losses associated with haemonchosis alone in sheep and goats are estimated at US$26 million, while returns could be enhanced as much as 470% by controlling haemonchosis (Mukhebi et al., 1985; Kareru et al., 2008). A 15-year retrospective study in central Kenya revealed that 32% of sheep mortalities were due to parasitic diseases, of which 63% were associated with helminthiasis (Kagira and Kanyari, 2001). In addition to mortality, helminthiasis reduces growth and reproductive performance in goats (Waller, 2006; Lashari and Tasawar, 2011).

For control of helminthiasis, farmers mainly rely on anthelmintic treatments (Vercruysse et al., 2018). Unfortunately, the currently available commercial anthelmintics are associated with problems such as loss of efficacy as a result of the emergence of resistance (Wanyangu et al., 1996; Waruiru et al., 1998; Gatongi et al., 2003). Despite the high rate of resistance development in gastrointestinal tract (GIT) nematodes (Waruiru et al., 2003; Wanyangu et al. 1996; Nalule et al., 2011; Woodgate et al, 2017), the discovery of new anthelmintics is very slow, the main reason being the lack of investment and lengthy process in drug discovery (Behnke et al., 2008). Given the resistance to the anthelmintics, high cost of drugs, and lack of newer anthelmintics, there is a need to find alternatives or complementary methods for nematode control. The use of plant extracts has been considered a possible efficacious and environmentally acceptable method to control GIT nematodes (Newman et al., 2012; Ribeiro et al., 2014).

Bromelain, a cysteine proteinase, is one of the plant-derived products which possess anthelmintic properties (Hunduza, 2018). Its use as anthelmintic has faced some constraints including the requirement of multiple dosing and

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rapid movements in the ruminant GIT; it requires only 20 minutes to pass through the small intestine (Stepek et al., 2005; Buttle et al., 2011). Shiew et al. (2010) also found that the activity of bromelain is lowered by the low pH found in the abomasum of ruminants; the drug is further degraded by rumen microbiota, resulting in an ineffective contact between parasite and drug. A study by Hunduza (2018) indicated that the encapsulation of bromelain in chitosan enhanced the *in vitro* activity against all the stages of *Haemonchus contortus* isolated from goats. Moreover, it revealed that encapsulated bromelain compared to pure bromelain had a higher inhibitory activity on egg hatch. It is, therefore, necessary to conduct *in vivo* studies on the efficacy and safety of this compound.

To the authors' knowledge, the proteolytic activity of cysteine proteinases has not yet been examined in different parts of the GIT of goats but attempts have been made in several *in vitro* studies to treat the abomasal nematode, *H. contortus*, with these enzymes (Hunduza, 2018). Therefore, there is a need to assess the stability of the proteolytic activity of bromelain in various parts of the GIT of goats where a number of nematodes reside. Hence, this study was designed to evaluate toxicity, anthelmintic efficacy, and proteolytic activity of chitosan-encapsulated bromelain within the GIT of Small East African goats.

MATERIALS AND METHODS

Ethical approval

Approval for animal experiments was obtained from the Animal Ethics Committee of Jomo Kenyatta University of Agriculture and Technology (JKUAT, REF: JKU/2/4/896B). The protocols were approved by the Institutional Animal Care and Use Committee of JKUAT.

Animals

In total, 24 Small East African healthy male goats, aged between 8 and 30 months and weighing between 13-21 kg, were used in this study. Twelve of them were used for the assessment of the toxicity and anthelmintic efficacy of chitosan-encapsulated bromelain and another set of twelve goats was used to determine the proteolytic activity of nanoencapsulated bromelain within the GIT. They were ear-tagged and kept in a goat house where they were acclimatized to the diet within 14 days before the start of the study. Animals were group-housed in pens (3 goats in each) located within the JKUAT, in Kiambu County, Kenya. Prior to the start of the experiment, each animal was screened for the presence of strongyle eggs by the Fecal Egg Count (FEC) examination. The goats were fed on 1.5 kg of concentrate feed and 1 kg of wheat hay twice per day (9 a.m. and 3 p.m.). The concentrate feed comprised beet liquid molasses, maize germ, and soybean meal (Aroma Feed Suppliers, Kenya). Feed blocks (Aroma Feed Suppliers, Kenya) were used to supplement essential minerals.

Bromelain extraction and encapsulation in chitosan nanoparticles

Bromelain extraction was performed as described by Kahiro et al. (2018). The extracted bromelain was purified using a 10 kDa dialysis membrane. Thereafter the ionic gelation method was used to encapsulate bromelain into chitosan (Fan et al., 2012; Hunduza, 2018). Briefly, after mixing equal volume (30 ml) of extracted bromelain (4 mg/ml) with 1% sodium tripolyphosphate (STPP), 12 ml of the bromelain-STPP mixture was added to 20 ml of 1% chitosan under vigorous and continuous stirring. Following the centrifugation at 15000 rpm for 45 minutes, the obtained pellet was washed with distilled water prior to freeze-drying at -60 °C using a freeze dryer (MRC Ltd., Israel). The Fourier transform infrared spectrophotometer analysis was used to confirm the successful conjugation of bromelain to the chitosan nanoparticles.

Treatments

Treatment groups were formed after randomization based on the number of eggs per gram (EPG) of feces, such that the mean EPG of the animals in each group was more than 500 (Coles et al., 2006). Each group had three animals. The treatment was done in a single oral dose. Group 1 received 90 mg/kg of encapsulated bromelain and group 2 received 270 mg/kg of encapsulated bromelain. Group 3 was the negative control (infected, non-treated). Group 4 (albendazole 7.5 mg/kg body weight) only served as a positive control in the anthelmintic efficacy assessment. Goats were fasted overnight prior to dosing. Following the period of fasting, the animals were weighed and then the encapsulated bromelain (90 and 270 mg/kg) was administered orally using drenching guns. The goats were monitored for 28 days.

Clinical observations

Observations were made and recorded systematically and continuously as per the guidelines (OECD, 2002). Each animal was observed during the first 30 minutes following the drug administration. Special attention was given during the first 4 hours and daily thereafter, for a total of 14 days to observe any changes in behavior and any clinical signs associated with toxicity. Temperature and body weight of goats were measured at 09:00 am using a digital thermometer

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(Kruuse, Denmark) and a 100 kg spring balance scale (Salter Model, Capital Scales, South Africa), respectively. This was done prior to treatment and weekly during the experiment period. Changes in the weight of individual goats were calculated and compared with that of the control animals. Changes were considered as adverse effects of drugs if the body weight loss observed was more than 10% of the initial recorded body weight (Nurul et al., 2018).

Blood Sample collection

Blood samples (3 ml) from each goat were taken from the jugular vein in ethylene-diamine-tetraacetic acid (EDTA) test tubes. This was done at 09:00 am weekly.

Packed cell volume

Packed Cell Volume **(**PCV) was determined using the microhematocrit method (Hansen and Perry, 1994; Githiori et al., 2004). Briefly, an aliquot of blood with anticoagulant from each goat was put in microcapillary tubes and then centrifuged at 14,000 rpm for 10 minutes. After centrifugation, samples were analyzed using a microcapillary reader (Hawksley, England).

Determination of serum biochemical parameters

The serum was prepared by allowing the whole blood to clot. Thereafter the clot was removed by centrifuging at 2,000 x g for 10 minutes. The resulting supernatant was used for biochemical tests. Aspartate aminotransferases (AST), alanine aminotransferases (ALT), urea, and creatinine were analyzed using standard diagnostic test kits on automated clinical biochemistry analyzer (Reflotron Plus System®, model: Cobas 4800 Detection Analyzer; India).

Assessment of the anthelmintic efficacy

FEC examination was performed before treatment and weekly during the experiment period. Fecal samples were weekly collected from the rectum of the goats using flesh gloves. Aliquots of the fecal sample from each goat were placed in a plastic bottle (Indosurgicals Pvt. Ltd., India). The fecal samples were analyzed using a modified McMaster technique (Zajac and Conboy, 2012) with a precision of 100 EPG using an Olympus B 201 microscope (Optical Element Corporation, Melville, USA) at 10× magnification. The percentage reduction in FEC was calculated using the formula described by Kochapakdee et al. (1995).

Assessment of proteolytic activity in gastrointestinal tract

Twelve goats were orally administered 270 mg/kg of encapsulated bromelain as a single dose. Every 4 hours, a group of 3 goats was sacrificed and the entire GIT was removed from each goat, from the rumen to the large intestine, then washed in a Petri dish of Phosphate-Buffered Saline (PBS), and split into six sections: the rumen, reticulum, abomasum, omasum, small intestine, and large intestine. These sections were opened longitudinally and the ingesta contents (100 grams) placed in a beaker containing PBS. Thereafter the contents were filtered using a sieve (250 µm size) into a beaker. The amount of enzyme present in each section over the 16 hours was measured by performing the casein enzymatic assay as described by Hunduza (2018).

Statistical analysis

All statistical analyses and graphical presentations were carried out using R (version 3.6.0) and GraphPad (Version 7.02), respectively. The Students t-test was used to compare the mean weight, temperature, AST, ALT, urea and creatinine levels of treated with those of non-treated goats. Differences in the proteolytic activity in the different organs of the GIT were tested using ANOVA. The significance was based on a p-value <0.05.

RESULTS

Toxicity assessment of encapsulated bromelain

Clinical observations

There were no mortality and clinical signs of toxicity observed in the goats after a single oral dose of 90 and 270 mg/kg of encapsulated bromelain. The mean body temperature of pre-treated goats ranged from 38.5 to 39.4 $^{\circ}$C. Following treatment, no significant differences were observed between the mean body temperature of goats treated with 90 mg/kg encapsulated bromelain and that of non-treated goats ($p>0.05$). The same observation was made between goats treated with 270 mg/kg encapsulated bromelain and non-treated goats. Prior to treatment, the mean body weight of treated goats was 16.8 kg and ranged from 16.1 to 17.3 kg while that for goats of the negative control group was 17.6 kg. Following treatment, there were no significant differences between the mean body weight of the non-treated goats and that of goats treated with 90 mg/kg encapsulated bromelain ($p>0.05$). Likewise, no significant differences were observed

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between the mean body weight of goats treated with 270 mg/kg encapsulated bromelain and that of non-treated goats ($p>0.05$).

Effect of bromelain on the packed cell volume and serum biochemical parameters

The mean PCV of the untreated goats was 28.5% and 28.9% on day 21 and day 28 of the experiment, respectively. The pre-treatment mean PCV of goats treated with 270 mg/kg encapsulated bromelain was 33% (range: 32-34%). Significant differences were observed between the mean PCV of goats treated with 270 mg/kg encapsulated bromelain and that of non-treated goats on day 21 ($p=0.0460$) and day 28 post-treatment ($p=0.027$). However, no significant differences were observed between the mean PCV of goats treated with 90 mg/kg of encapsulated bromelain ($p>0.05$) and that of non-treated goats from day 7 to day 28 post-treatment (Figure 1). The mean ALT level of untreated goats was 15.74 U/L on day 0 of the experiment. Prior to treatment, the mean ALT level of goats treated with 90 mg/kg encapsulated bromelain was 15U/L while that of goats treated with 270 mg/kg encapsulated bromelain was 14.8 U/L. The mean ALT level of untreated goats on day 7 (15.7 U/L) was significantly higher ($p<0.05$) than that of goats treated with 90 mg/kg (14.8 U/L) and 270 mg/kg encapsulated bromelain (14.9 U/L) on day 7 post-treatment. On day 0, day 14, and day 28 post-treatment, no significant differences were observed between both groups treated with encapsulated bromelain and the non-treated goats. The mean AST level of untreated goats was 114.8 U/L (range: 112-117 U/L). Before treatment, the mean AST level of goats treated with 90 mg/kg encapsulated bromelain was 115.8 U/L while that of goats treated with 270 mg/kg encapsulated bromelain was 116.3 U/L. Following treatment, no significant differences ($p>0.05$) were observed between the mean AST level of treated goats (both 90 and 270 mg/kg encapsulated bromelain) and that of untreated goats (Figure 3). The mean urea level of untreated goats was 6.1 mmol/L (range: 5.5-6.5 mmol/L) on the day 0 experiment. The mean pre-treatment urea level of goats treated with 90 mg/kg encapsulated bromelain was 5.8 mmol/L while that of goats treated with 270 mg/kg was 6.2 mmol/L. Following treatment, no significant differences ($p>0.05$) were observed between the mean urea level of both treated groups and that of untreated group (Figure 4). The mean serum creatinine level of untreated goats was 56.9 µmol/L and ranged from 54 to 61.2 µmol/L. The mean pre-treatment creatinine level of goats treated with 90 mg/kg and 270 mg/kg were 57.5 and 60 U/L, respectively. After treatment, no significant differences ($p>0.05$) were observed between the creatinine levels of encapsulated bromelain treated goats (both 90 and 270 mg/kg) and the non-treated goats (Figure 5).

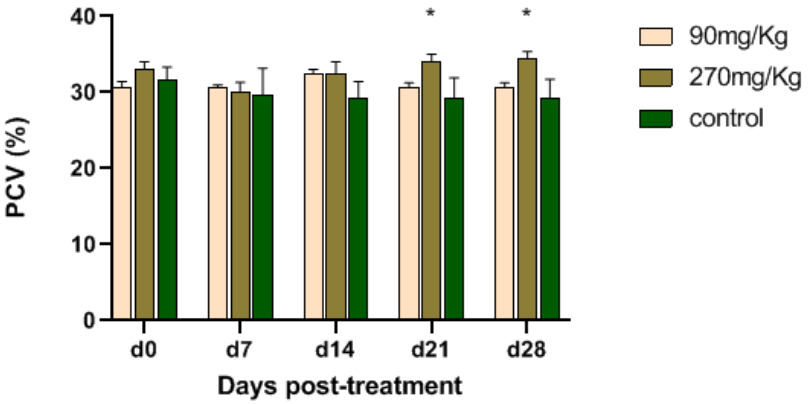

Figure 1. Mean packed cell volume of untreated and treated goats with single-dose of encapsulated bromelain. PCV: Packed Cell Volume; d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d21: day 21 post-treatment; d28: day 28 post-treatment. * Columns with an asterisk symbol are significantly different ($p<0.05$) from the control group on the same day. Each treatment (90 and 270 mg/Kg) was compared separately to the control group using the Students t-test.

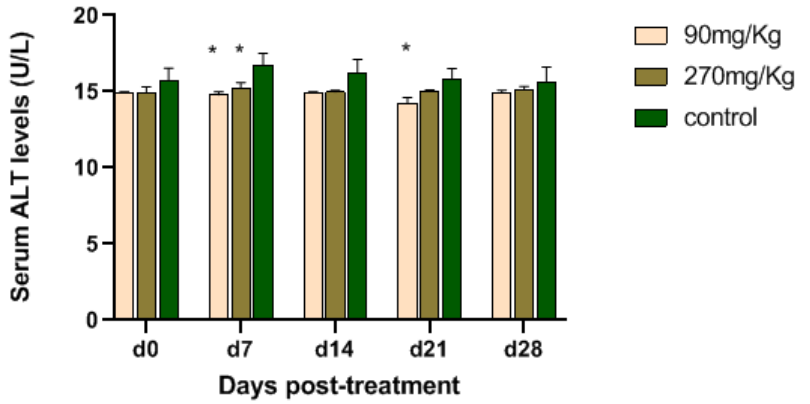

Figure 2. Mean concentration of alanine aminotransferase in untreated and treated goats with single-dose of encapsulated bromelain. ALT: Alanine aminotransferase; d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d21: day 21 post-treatment; d28: day 28 post-treatment. * Columns with an asterisk symbol are significantly different ($p<0.05$) from the control group on the same day. Each treatment (90 and 270 mg/Kg) was compared separately to the control group using the Students t-test.

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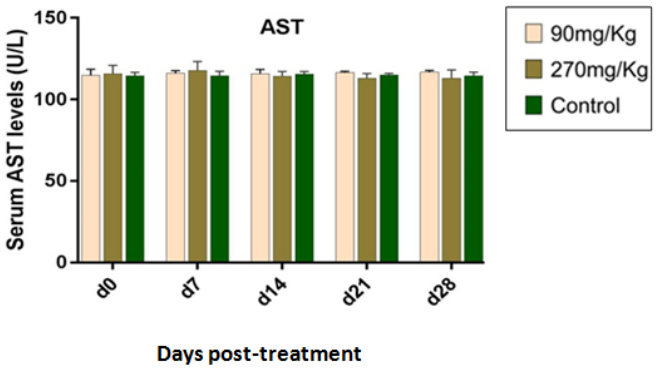

Figure 3. Mean concentration of aspartate aminotransferase in untreated and treated goats with single-dose of encapsulated bromelain. AST: Aspartate aminotransferase; d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d21: day 21 post-treatment; d28: day 28 post-treatment.

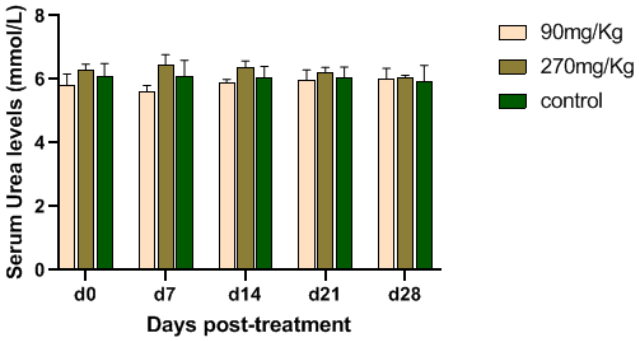

Figure 4. Mean urea level of untreated and treated goats with single-dose of encapsulated bromelain. d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d 21: day 21 post-treatment; d28: day 28 post-treatment.

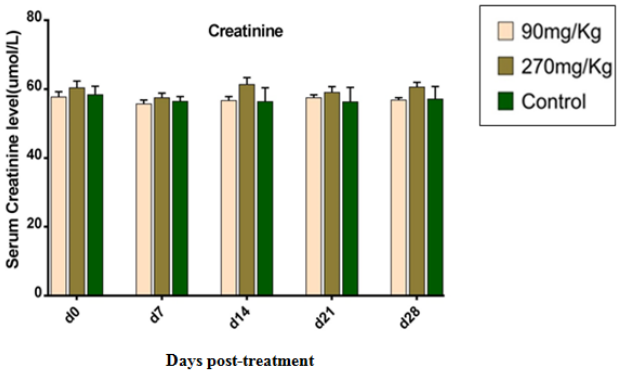

Figure 5. Mean serum creatinine level of untreated and treated goats with single-dose of encapsulated bromelain. d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d21: day 21 post-treatment; d28: day 28 post-treatment

***In vivo* assessment of anthelmintic efficacy of encapsulated bromelain**

Before treatment, the mean strongyle FECs for treated goats with a single dose of 90 and 270 mg/kg of encapsulated bromelain were 1,050 and 1,550 EPG, respectively. Following treatment (on day 28 post-treatment), the percentage reduction in FEC was 9.5% and 22.6% for treated goats with 90 and 270 mg/kg of encapsulated bromelain, respectively. The percentage reduction in strongyle eggs of goats treated with 270 mg/kg was significantly higher ($p < 0.05$) than that of the group treated with 90 mg/kg encapsulated bromelain on all treatment days (day 7, 14, 21 and 28 post-treatment). In comparison, the mean percentage of strongyle FECs for the untreated group increased by 22% while a reduction of 100% was recorded for the goats treated with albendazole (7.5 mg/kg) on day 28 post-treatment (Figure 6).

Protease activity of encapsulated bromelain in the different organs of the gastrointestinal tract

The protease activity of chitosan-encapsulated bromelain in the different digestive organs varied with time as the encapsulated bromelain was passing through the GIT of goats. Four hours after the drug administration, the encapsulated bromelain activity was lowest in the reticulum (0.124 U/ml, range: 0.04-0.18 U/ml) while 8 hours after the drug administration, the activity was highest in the rumen (3.11 U/ml, range: 2.83-3.3 U/ml) and lowest still in the reticulum (0.117 U/ml, range: 0.02-0.18 U/ml). The overall encapsulated bromelain activity (sum of activities in the different organs) gradually increased over 12 hours and then decreased ($p=0.001$). The highest enzyme activity (3.142 U/ml, range: 2.83-3.3 U/ml) was recorded in the large intestine at 12 hours after drug administration (Table 1).

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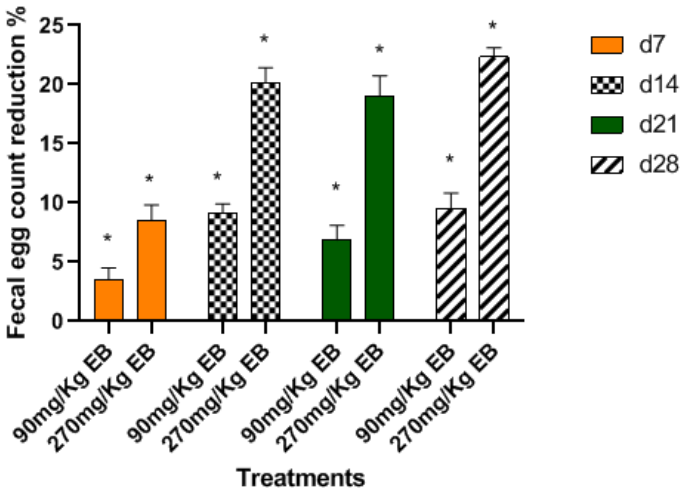

Figure 6. Mean percentage reduction in fecal egg cou nt of goats treated with a single dose (90 or 270 mg/kg) of chitosan-encapsulated bromelain. EB: Encapsulated bromelain; d0: day 0; d7: day 7 post-treatment; d14: day 14 post-treatment; d21: day 21 post-treatment; d28: day 28 post-treatment. * The asterisk symbol indicates a significant difference ($p<0.05$) between treatment groups on the same day.

Table 1. Mean enzyme activity of encapsulated bromelain in digestive organs of goat at different time points

Organ	Enzyme activity after drug administration (Units/ml)				p-value
	4 hr	8 hr	12 hr	16 hr	
Reticulum	0.124±0.062	0.117±0.073	0.106±0.058	0.034±0.016	0.408
Rumen	0.665±0.361	3.11±0.217	1.146±0.288	0.251±0.117	<0.0001
Omasum	0.603±0.141	2.128±0.257	1.808±0.451	0.397±0.186	0.001
Abomasum	0.191±0.018	0.398±0.151	1.446±0.294	0.345±0.161	<0.0001
Small intestine	0.002±0.0001	0.398±0.124	0.339±0.087	0.074±0.034	0.099
Large intestine	0.001±0	0.018±0.004	3.142±0.218	0.402±0.18	<0.0001
Overall activity	1.586±0.582	6.169±0.826	7.987±1.396	1.503±0.69	0.001
p-value	0.007	<0.0001	<0.0001	0.064	

Data are expressed as mean ± standard deviation

DISCUSSION

The current study evaluated the anthelmintic efficacy, toxicity, and proteolytic activity of bromelain encapsulated in chitosan in the GIT of Small East African goats. In this study, no mortality and clinical signs were observed when encapsulated bromelain as a single dose was administered to goats. Therefore, the median lethal dose (LD_{50}) of encapsulated bromelain may be considered to be greater than 270 mg/kg in goats. This finding is in agreement with the results of Dutta and Bhattacharyya (2013) who similarly did not observe any toxicity after oral administration of acute and sub-acute doses of the aqueous extract of *Ananascomosus* (pineapple) crown leaf to rats. According to Taussig et al. (1975), bromelain has very low toxicity and its LD_{50} is greater than 10 g/kg in mice, rats, and rabbits. Similarly to this study, Pavan et al. (2012) also did not report any toxic effect associated with daily administration of bromelain to dogs in increasing levels up to 750 mg/kg after six months. The PCV observed in this study for healthy goats was lower compared to the values (32.5-43.7%) reported by Al-Bulushiet al. (2017) for Sahrawi goats. Earlier reports for Jabali goats showed PCV values in the same range (37.4-43.7%) (Al-Bulushi et al., 2017). Further, the obtained PCV values were within the normal range of the species whose values vary among breeds of goats (Radostitis et al., 2000). The observation that there were no significant differences between the PCV of treated and untreated goats indicates that the administration of encapsulated bromelain does not affect erythrocyte production and physiology and thus is safe for use in animals.

Following treatment, the serum creatinine, urea, ALT and AST levels of encapsulated bromelain treated goats were within the normal range of the species and did not show any significant difference across the groups. This indicates no liver and kidney damage up to 270 mg/kg of encapsulated bromelain administered as a single dose. The range of ALT level observed in this study is comparable with the finding by Tibbo et al. (2008) who reported the ALT level of indigenous Arsi-Bale, Central Highland, and Long-eared Somali goat breeds ranged from 14.0-20.2 U/L. The obtained values for urea were within the range reported for normal healthy goats described by other studies (Kaneko et al. 2008; Chikwanda and Muchenje, 2017). This study is consistent with previous results obtained by Buttle et al. (2011) who did not observe any sign of toxicity or gross lesions in the sheep administered single and repeated doses of papaya latex cysteine proteinase during the post-mortem examination.

In comparison to the anthelmintic efficacy obtained in this study (22.6%) when encapsulated bromelain (270 mg/kg) was administered to goats as a single dose, Domingues et al. (2013) reported a lower efficacy for plain bromelain

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(180 mg) in sheep (3.7%). This disparity can be attributed to the differences in the animal species and the administered dosages. Another possible explanation of the differences in anthelmintic efficacy can be the beneficial effects of nanoformulation as a drug delivery system (Bhatnagar et al., 2014). According to Dimitrov (2012), nanoparticles including chitosan improve the efficacy of drugs by preventing enzymatic degradation and enhancing the absorption of the intestinal epithelium. Similarly, George and Abraham (2006) reported that incorporation of proteins into a chitosan matrix protected these biomolecules. In the same trend, Mahlangu (2018) declared that the encapsulation of bromelain into chitosan enhanced its activity against bacteria isolates from mastitis infected goats. A critical fact to take into consideration regarding the use of proteins as anthelmintic is their possible degradation by proteases found in the GIT, including the rumen. Thus, the nanoformulation is a very important step in the development of protein drugs for oral delivery since it protects and promotes their availability in nematode habitat organs (Dos Santos Soares et al., 2019).

The anthelmintic efficacy reported in this study is consistent with the findings of Buttle et al. (2011) who found sheep receiving repeated treatment of papaya latex supernatant (100 µmol active cysteine proteinase), daily for 4 days, had fewer *H. contortus* worms compared to those that received the single treatment. The lower anthelmintic efficacy of a single dose treatment as compared to repeated doses can be explained by the fact that repeated administration of the drug extends contact period between cysteine proteinases and the parasite resulting in an increased efficacy (Buttle et al., 2011). Another possible reason is the rapid movement of cysteine proteinase in the GIT of ruminants (Stepek et al., 2006), which make a drug administered as a single dose to have little chances and short time of getting in contact with the worms. The lower anthelmintic efficacy of a single dose treatment compared with repeated doses suggests that, following dilution in the GIT, the enzymes require prolonged contact time with the worms in order to demonstrate effectiveness (Buttle et al., 2011). Thus, experiments with repeated treatment doses for 90 and 270 mg/kg of encapsulated bromelain are needed in order to see the possibilities of achieving effectiveness greater than 22.6%.

Similar to the findings of this study, Stepek et al. (2007) reported a variation in the enzyme activity of papaya latex with time in the different organs as the drug was passing through the GIT of mice. In the current study, it was noted that four hours after the drug administration the encapsulated bromelain had already passed the rumen thus very low activity was recorded in that GIT site. This confirms the report by Stepek et al. (2005) who indicated the limitation of bromelain in ruminants was their rapid movement in the GIT. Twelve hours after the drug administration, a build-up of activity in the large intestine was observed and this may be due to an increase in enzyme concentration following absorption of water in the small intestine (Stepek et al., 2007).

Apart from *H. contortus*, there are many other parasite species infecting the stomach and intestines of sheep and goats. These include *Teladorsagia (Ostertagia)* spp., *Trichostrongylus* spp., *Trichostrongylus*, *Nematodirus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Cooperia* spp., and *Strongyloides* spp. (Urquhart et al. 1996; Hutchinson, 2009). The observed build-up of encapsulated bromelain activity in the large intestines 12 hours after the drug administration shows that the drug will be effective against the intestinal nematodes residing in the large intestine of the host animal (Hutchinson, 2009). The omasum and abomasum showed the second and third highest encapsulated bromelain proteolytic activity 12 hours after the drug administration, respectively, indicating that the drug will be effective against nematodes such as *Haemonchus* spp. and *Ostertagia* spp., which are located in the abomasum of ruminants (Hutchinson, 2009). The present study demonstrated that encapsulated bromelain remained intact and proteolytically active within the GIT, which is consistent with the findings of Hale (2004). Therefore, encapsulated bromelain can act as an effective anthelmintic treatment for different strongyle residing along the GIT of small ruminants.

CONCLUSION

In conclusion, chitosan-encapsulated bromelain administered orally in a single dose up to 270 mg/kg is not associated with any adverse clinical signs of toxicity and mortality in goats. This compound was not very effective in reducing the burden of strongyle eggs in goats. Additionally, the encapsulated bromelain remained intact and proteolytically active along the gastrointestinal tract of goats, although the protease activity of it varied with time in the different organs as the drug was passing through the gastrointestinal tract. With regards to the low efficacy of single-dose bromelain observed in this study, further studies with repeated doses of encapsulated bromelain are needed.

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Authors' contribution

All authors conceived and designed the experiment. Wasso Shukuru and John Kagira performed the experiment. Naomi Maina performed the biochemical assays. All authors analyzed data and wrote the manuscript. All authors read and approved this manuscript.

Competing interests

All authors declare that they have no conflict of interest.

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