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REGULAR ARTICLES

Evaluation of the in vivo anthelmintic properties of *Mitragyna inermis* (Willd.) as a livestock dewormer against parasitic hematophagous worm *Haemonchus contortus* infections in different breeds of lambs

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Abstract

Gastrointestinal (GI) nematodes remain a major constraint on livestock production throughout the world. This study assessed the in vivo efficacy of the powder of *Mitragyna inermis* leaves in three breeds of lambs, namely, West African dwarf lambs (WAD), West African long-legged lambs (WALL), and F1 lambs (cross of a WALL ram with a WAD ewe), artificially infected with 3000 L3s of *Haemonchus contortus* in a controlled experiment. Fecal sample examination, serological analysis, and necropsy were carried out to determine the egg count, worm burden, and worm fecundity reduction. A dose of 3.2 g/kg body weight (BW) *M. inermis* was administered per the oral route for three consecutive days and repeated 2 weeks later. Compared with the control, the powder of *M. inermis* leaves (> 60%) and albendazole (100%) significantly reduced ($p < 0.01$) fecal egg counts (FECs) in the three breeds of lambs. The posttreatment reduction in FECs fluctuated from 56.99 to 78.75% for WAD lambs, 38.39 to 66.39% for WALL lambs, and 35.55 to 63.11% for F1 lambs (WALL × WAD). Significant differences ($p < 0.05$) were observed in packed cell volume values before and after infection. *M. inermis* reduced the egg-laying capacity of female adult worms by up to 60% and eliminated more than 80% of the adult worms of *H. contortus* in lambs. Furthermore, albendazole reduced the worm count and fecundity of female worms by greater amounts than *M. inermis* (100%). The findings of this study showed that *M. inermis* is a good source of bioactive compounds for drug development. According to this result, a 3.2 g/kg BW dose of the plant could be applied for the control of GI nematodes in small ruminants.

Keywords Breeds of lambs · *Haemonchus contortus* · In vivo · *Mitragyna inermis* · West Africa

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Introduction

Parasitic nematodes continue to represent a serious challenge to the health, welfare, and productivity of grazing ruminants because of the major production losses that they cause worldwide (Morgan et al. 2013). Helminths reduce the level of meat, milk, and manure output and reduce asset value through increased mortality, especially of young stock (Da Silva et al. 2013; Mavrot et al. 2015). The incidence and severity of various helminth parasitic diseases are more widespread in tropical regions, including developing countries such as Benin (Salifou et al. 2013). Among gastrointestinal (GI) strongyle infections, haemonchosis due to *Haemonchus contortus* has a wide distribution, pathological importance, and strong predominance in different western African tropical countries.

Moreover, several surveys have underlined the wide distribution, pathological importance, and predominance of *H. contortus* in West Africa (Squire et al. 2013; Okorafor et al. 2015). In Benin, the overall prevalence of *H. contortus* in sheep was estimated to be 92.5% (Salifou et al. 2013).

The usual mode of control of these parasitic diseases based on repeated use of drug treatment is currently impaired by the increasing development and diffusion of resistance against a number of anthelmintics in worm populations (Kaplan and Vidyashankar 2012; Geurden et al. 2015). This worldwide problem explains the current interest in the search for alternative solutions to reduce the reliance on chemical control (Kommuru et al. 2014). In almost all developing countries, such as those in Africa, parasite control by grazing management, such as rotational grazing, is impractical for smallholder farmers and pastoralists due to complications in land ownership and restrictions on the size of individual farms (Soro et al. 2013). Although parasite-resistant indigenous breeds are common in these locations, young, poorly fed animals often succumb to parasitism (Morgan et al. 2013). In particular, there is renewed interest in ethnoveterinary medicine and plants with anthelmintic properties in both temperate and tropical countries (Waller 2006). Therefore, communities in sub-Saharan Africa rely on ethnoveterinary medicine instead of synthetic drugs (McCorkle et al. 1996; Nordeng et al. 2013), involving the use of medicinal plants endowed with anthelmintic properties to control animal parasitic diseases. These medicinal plants have the advantage of being available, inexpensive, and accessible for local populations, especially those with low income (Hammond et al. 1997; Cooper et al. 2015).

Ethnobotany and ethnomedical studies are recognized as the most reliable methods for medicinal plant identification and screening (Alowanou et al. 2015a). Therefore, during recent decades, many medicinal plants from sub-Saharan Africa, including the republic of Benin, that are potentially endowed with anthelmintic properties have been inventoried (Koné and Kamanzi 2007; Kaboré et al. 2007; Djoueché et al. 2011; Attindéhou et al. 2012a; Dassou et al. 2015). Among these anthelmintic plants is *Mitragyna inermis* Willd. O. Ktze (Rubiaceae), recorded in many prospective studies and belonging to the Beninese flora.

M. inermis is a riparian tropical plant found in Africa and Asia and is widely used in traditional medicine to heal a wide variety of human and animal diseases (Alowanou et al. 2015a). Ethnobotanical surveys carried out on medicinal plants of the African flora reported that different parts of *M. inermis*, mostly the leaves, are used for gastrointestinal helminthiasis control by small-scale farmers from West Africa, especially in Benin (Attindéhou et al. 2012b), Ivory Coast (Koné and Kamanzi 2007), and Burkina Faso (Kaboré et al. 2007). In Cameroon (Central Africa), only the bark of *M. inermis* is used by small-scale farmers to control helminth infections in ruminants (Djoueché et al. 2011).

A previous in vitro study reported that a crude ethanol extract of *M. inermis* bark showed significant anthelmintic activity against the egg hatching and larval development of *H. contortus* (Diehl et al. 2004). In a more recent study, an acetone/water extract of *M. inermis* leaves showed significant anthelmintic activity on the larval migration of *H. contortus* (Alowanou et al. 2015b).

The current study was therefore performed to extend, by in vivo experiments, the information obtained in vitro on the possible efficacy of *M. inermis* leaves against *H. contortus* in the different breeds of lambs found in Benin.

Materials and methods

Study area

The study was carried out at the Agricultural Research Center of Agonkanmey of the National Agricultural Research Institute of Benin (CRA_A/INRAB) located in the Abomey-Calavi district. This district (altitude 17.4 m; 06° 24′ north; 02° 20′ east) situated in southern Benin is characterized by a climate of Guinean type with two rainy seasons (mid-March to mid-July and mid-October to mid-November) and two dry seasons (mid-November to mid-March and mid-July to mid-October).

Plant collection and preparation

Leaves of *M. inermis* were collected in December 2015 (dry season) in southwestern Benin. The plant was authenticated at the National Herbarium of Benin, where a voucher specimen is deposited under the number AA6529/HNB. The leaves were subsequently dried indoors at room temperature for seven days and then powdered using a laboratory grinder, and the powder was kept at room temperature until use.

Animals and feed

45 lambs (12.6 ± 0.91 kg of body weight (BW), three to four months of age) belonging to three breeds, namely, 15 West African dwarf lambs (WAD), 15 West African long-legged lambs (WALL), and 15 F1 lambs crossbred from a WALL ram and a WAD ewe, were individually housed in pens equipped with shade and a food and water supply. The animals were selected and bought in one of the famous private agropastoral farms of Benin located in Ouassa-Péhunco in the department of Donga (northern part of the Republic of Benin). The lambs were identified with a numbered neck collar, weighed, dewormed with albendazole 10%® (HIPRA) in an oral suspension at a single dose of 5 mg/kg BW and then with Cypertop (LAPROVIT) in a spray against ectoparasites two weeks before the start of the experiment, and maintained in total enclosure. During the quarantine period, the health of

the lambs was closely monitored, and any ailment that might have an adverse effect on the lambs was treated promptly. To avoid secondary infection, the experiment was performed under a cut-and-carry system of feeding. Animals were fed with *Panicum maximum* var. C1 as the basal diet and were supplemented with 200 g of cassava peelings (*Manihot esculenta*) in the morning and 100 g of commercial pelleted feed in the afternoon during the quarantine and the experimental periods. Mineral blocks rich in essential minerals were placed in each box to meet the mineral requirements of the lambs. Free access to water was also provided.

Parasites

Feces obtained from an artificially infected donor lamb with pure strain of *H. contortus* were collected and cultured at room temperature for 10 days. The L3s were then extracted from the fecal mass using a Baermann apparatus.

Treatments, experimental procedure, and measurements

Three weeks before the start of the experiment, animals were randomly allocated to individual boxes, numbered, and distributed in three experimental groups of five lambs per breed, as follows: two control groups (positive and negative) and one dose (3.2 g/kg live weight) of the plant leaf powder. All 45 lambs were infected on day 0 with 3000 third-stage larvae of *H. contortus*. Different treatments were applied when the fecal egg counts (FECs) reached at least 3000 EPG on day 26 postinfection for all lambs. Into each breed of lambs, three types of treatments were applied: 10 ml of tap water administered to the untreated lambs (control), 5 mg/kg BW albendazole 10%® administered to lambs treated with synthetic drug (albendazole was used as a positive control), and 3.2 g/kg BW *M. inermis* leaf powder dissolved in 250 ml of water in a large bottle and provided to the animals. The powder of *M. inermis* leaf was administered per the oral route once a day for three consecutive days, which was repeated two weeks later. Thus, the treatment was performed on days 26, 27, and 28 postinfection and repeated 14 days later on days 40, 41, and 42 postinfection. Albendazole 10%® was administered in a single dose to animals in the positive control group. All experimental animals were autopsied on day 48 postinfection, and different data were collected. Note that on day 0, FEC examination was performed to verify the lack of any egg excretion and the efficacy of albendazole treatment.

Fecal egg count

From day seven to day 48, individual fecal samples were extracted directly from the rectum twice a week for FEC determination, which was expressed as trichostrongylid eggs per gram (EPG), according to a modified McMaster technique (Hansen and Perry, 1995).

Packed cell volume determination

Blood was drawn by puncture of the jugular vein and deposited in tubes with EDTA as an anticoagulant for packed cell volume (PCV) determination by microcentrifugation, according to the microhematocrit method (Hansen & Perry, 1995).

Counting of adult worms during necropsy

Immediately after animals were humanely slaughtered, the abomasum was removed and opened, and the luminal content was removed. After washing the abomasum, the stomach mucosa was placed in a saline solution at 37 °C for two h to collect the worms embedded in the mucosa. Worms present in the stomach were counted according to a 10% aliquot technique (Hounzangbé-Adoté et al. 2005a). The stage and sex of the *H. contortus* worms were identified according to the identification key of Van Wyk and Mayhew (2013). To compare the fertility of the adult females of *H. contortus*, the number of eggs per uterus was counted directly on 10 female worms per lamb from each genetic group, according to the method described by Kloosterman et al. (1978). Moreover, experimental management and data collection were approved and conducted in accordance with the guidelines of the Ethical Committee of the University of Abomey-Calavi (EC approval 2016/1134/UAC/Benin).

Data analysis

The values (x_i) of FECs were transformed into log ($x_i + 1$) before analysis. For the egg excretion and the PCV values, including those for the controls, comparisons were performed using two-way analysis of variance (ANOVA) on repeated measurements followed by the post hoc HSD test of Tukey-Kramer, which uses the agricolae package in R software (R Core Team, 2013). The same test was applied to compare the final worm counts between lambs receiving *M. inermis* and albendazole and those that were not treated. Finally, significant differences in the fertility of female worms measured by the number of eggs per female were determined using a two-way ANOVA, with the two factors being the treatment and individual female worms from each group of lambs. The percentage of fecal egg count reduction (FECR) was calculated using arithmetic means and the formula of Coles et al. (1992):

$$\mathrm{FECR} = [1 - \{\mathrm{C1}/\mathrm{C2}\}] \times 100,$$

where C1 is the mean posttreatment FEC for the lambs treated either with leaf powder of *M. inermis* (animal test) or albendazole (positive control) and C2 is the mean

posttreatment FEC for the untreated lambs (negative control).

Results

Effect of treatments on PCV values from WAD lambs

The mean PCV values for the experimental WAD lambs are shown in Fig. 1. For all animals, the PCV fluctuated within the normal range of 25–44.5% (Hoffman 2006). At two weeks before infection, significant differences ($p < 0.05$) in PCV values were recorded between all the groups (Fig. 1). A progressive decline in PCV in all infected lambs was observed from the second week postinfection up to the fourth week postinfection, without significant differences ($p > 0.05$) between the treated and untreated groups (Fig. 1). However, compared with the untreated group (negative control), all the treated (*M. inermis* and albendazole) groups exhibited a significant increase ($p < 0.05$) in PCV values from day 26 to day 42 posttreatment (Fig. 1). A slight increase in PCV values was observed at the end of the experiment in the albendazole group (41.32%) compared with that in the *M. inermis* group (39.9%) (Fig. 1).

Effect of treatments on PCV values from F1 lambs

The mean PCV values for the experimental F1 lambs (WALL × WAD) are shown in Fig. 2. The ANOVA on repeated measurements for PCV values (from day − 18 to day 42) revealed overall significant differences between the groups ($p < 0.01$; Fig. 2). In contrast to all treated animals (*M. inermis* and albendazole), untreated animals (control) displayed considerable fluctuation in the PCV values at two weeks before infection. A progressive decline in PCV values (41.7 to 35.18) in all infected lambs was observed from the first week and second week postinfection for the *M. inermis* (39.6 to 34.5%) and control groups (41.7 to 35.18%), respectively, and for the albendazole group (39.14 to 34.5%) up to the fourth week postinfection, without significant differences ($p > 0.05$) between the treated and untreated groups (Fig. 2). Animals that were treated with *M. inermis* and albendazole postinfection showed a significant increase in PCV ($p < 0.05$) at weeks five and six postinfection (Fig. 2) compared to that of the control animals. However, the difference between the two treated groups of lambs was not significant ($p > 0.05$).

Effect of treatments on PCV values from WALL lambs

The mean PCV values for experimental WALL lambs are shown in Fig. 3. A comparison between the different infected groups did not indicate any significant differences ($p > 0.05$) in PCV values on day − 18 to day − seven before infection or the first 2 weeks postinfection (Fig. 3). During the third week (day 19 to day 25) postinfection, the PCV values for all the infected groups declined significantly ($p < 0.05$). However, compared with the untreated group (negative control), the *M. inermis*-treated (36.04 to 38.1%) and albendazole-treated (38.04 to 39.58%) groups exhibited a significant increase ($p < 0.05$) in PCV values from day 26 to day 42 posttreatment (Fig. 3). Indeed, animals treated with *M. inermis* and albendazole had the highest PCV values among the groups during the last three weeks of the experiment (Fig. 3). However, the difference between the albendazole and *M. inermis* groups was not significant ($p > 0.05$).

Effect of treatments on FEC reduction from WAD lambs

The variation in the FECs in the albendazole, control, and *M. inermis* groups of WAD lambs during the period of the experiment is shown in Fig. 4. The experimental infection was carried out on day zero, and a prepatent period started

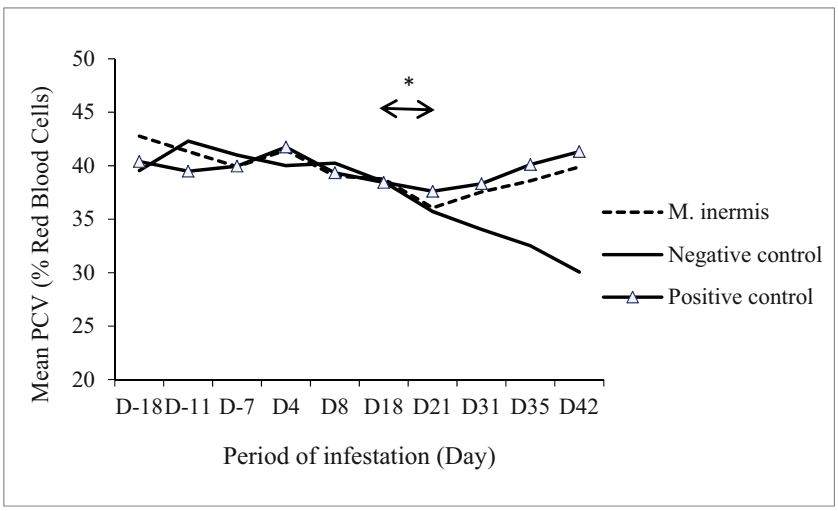

Fig. 1 Variation in packed red cell volume (PCV) for two months of experiment in the control groups (control +, control −) and WAD lambs treated with *M. inermis* at the dose of 3.2 g/kg BW, * indicating the prepatent period

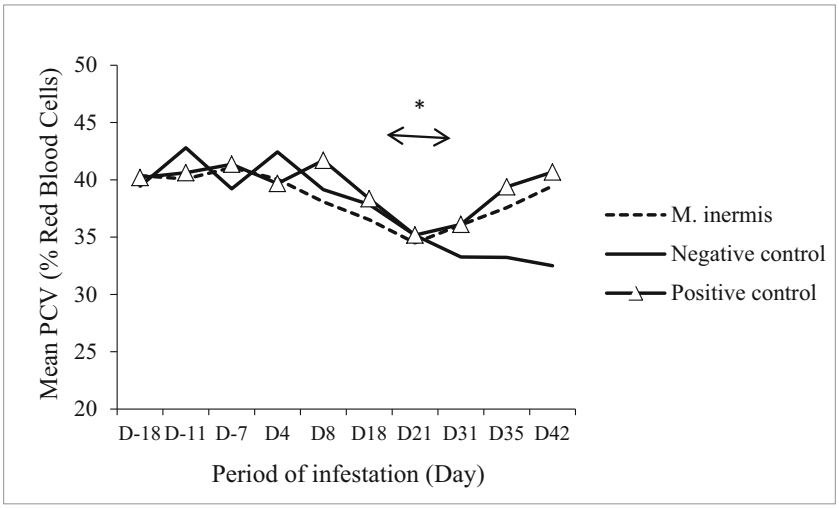

Fig. 2 Variation in packed red cell volume (PCV) in two months of experiment in the control groups (control +, control −) and F1 (WALL × WAD) lambs treated with *M. inermis* at the dose of 3.2 g/kg BW, * indicating the prepatent period

on day 18 postinfection for all groups. From this time onward, different trends in egg count reduction were observed according to the treatment group. Compared to the control lambs, lambs in the *M. inermis* and albendazole groups displayed 56.99% and 99.90% reductions in FEC, respectively, after the first treatment (day 16 to day 28) postinfection (Fig. 4). The albendazole group showed a significant difference ($p < 0.05$) from the *M. inermis* group (Fig. 4). However, the second treatment (day 40 to day 42) postinfection induced a supplementary reduction in FEC (21.76%) in the *M. inermis* group (Fig. 4).

Effect of treatments on FEC reduction in F1 lambs (WALL × WAD)

The FECs (mean ± SEM) of the albendazole, control, and *M. inermis* groups of F1 lambs (WALL × WAD) are shown in Fig. 5. The experimental infection was carried out on day zero, and a prepatent period started on day 16 postinfection for all groups. The FECR results showed that *M. inermis* had the capacity to reduce fecal egg excretion in F1 lambs (Fig. 5). The difference between the two treated groups, the *M. inermis* and albendazole groups, was statistically significant ($p < 0.05$). The percentage of reduction was 35.55% in the *M. inermis* group and 98.42% in the albendazole group after the first treatment (day 16 to day 28) postinfection. After the second treatment (day 40 to day 42) postinfection, the FECR% was 63.11% and 100%, respectively, for *M. inermis* leaf powder and albendazole (Fig. 5).

Effect of treatments on FEC reduction in WALL lambs

The FECs (mean ± SEM) of the albendazole, control, and *M. inermis* groups of WALL lambs are shown in Fig. 6. The experimental infection was carried out on day zero, and a prepatent period started on day 17 postinfection for all groups. Analyses of the egg excretion values based on the ANOVA on repeated measures showed significant differences ($p < 0.01$)

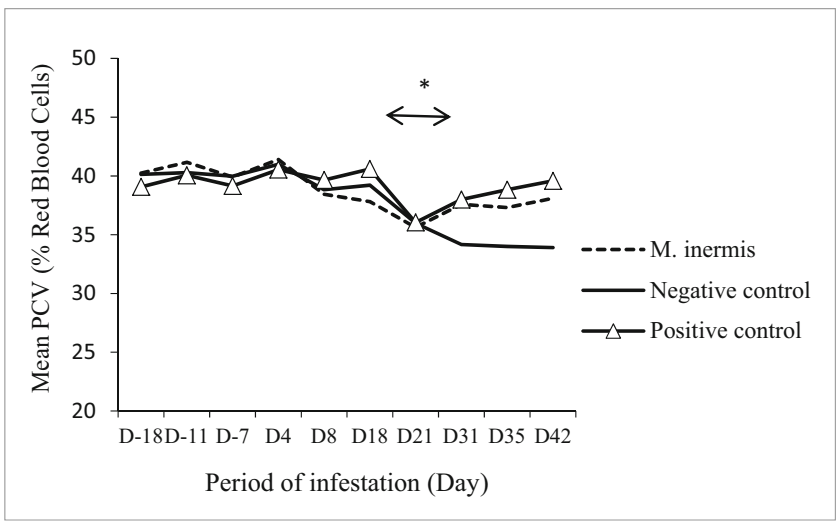

Fig. 3 Variation in packed red cell volume (PCV) in two months of experiment in the control groups (control +, control −) and the WALL lambs treated with *M. inermis* at the dose of 3.2 g/kg BW, * indicating the prepatent period

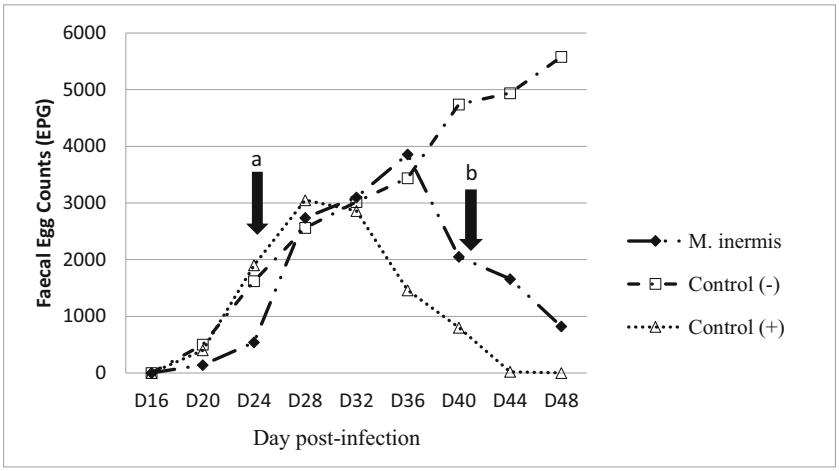

Fig. 4 Variation in fecal egg counts on two months of experiment in the control groups (control +, control −) and the group of WAD lambs treated with *M. inermis* (**a**, **b**). **a** Powder of *M. inermis* leaf applied at the dose of 3.2 g/kg BW on days 26, 27, and 28 postinfection and albendazole administered in a single dose on day 26. **b** Powder of *M. inermis* leaf at a dose of 3.2 g/kg BW repeated 14 days later on days 40, 41, and 42 postinfection

between the albendazole, control, and *M. inermis* groups (Fig. 2). A significant reduction ($p < 0.05$) in FEC was observed for animals treated with *M. inermis* leaf powder (38.39%) and for those treated with albendazole (99.17%) after the first treatment (day 16 to day 28) postinfection. Moreover, statistically, after the second treatment (day 40 to day 42), the difference in FECR between the two treated groups was significant ($p < 0.05$). Indeed, the FECR values were 66.39% and 100% for *M. inermis* and albendazole, respectively, at the end of the experiment (Fig. 6).

Effect of treatments on adult worm viability

The reduction in adult *H. contortus* worms in the stomach mucosa depended on the treatment applied ($p < 0.01$) and on the breed of lamb ($p < 0.05$). Except for lambs from groups treated with albendazole (positive control), which did not have any worms in their stomach mucosa during a postmortem inspection, *M. inermis* leaf powder showed a high efficacy on adult worms' viability through a significant decrease ($p < 0.01$) in worm numbers (Table 1). However, there were more worms ($p < 0.05$) in the stomach mucosa of WALL lambs (994 ± 154 worms) and F1 (WALL × WAD) lambs (1012 ± 134 worms) than in WAD lambs (424 ± 157 worms) (Table 1).

Effect of treatments on female worm fertility

Compared to the control treatment, *M. inermis* leaf powder significantly reduced ($p < 0.001$) the number of eggs per female in adult female worms of *H. contortus* in the three breeds of lamb (Table 2). However, between the breeds of lamb, no significant difference ($p > 0.05$) in the decrease in egg number per female was found.

Discussion and conclusions

This study was undertaken to evaluate the in vivo effectiveness of *M. inermis* leaves against *H. contortus* in artificially infected sheep. The dose of 3.2 g/kg BW *M. inermis* leaf powder used during this experiment corresponds

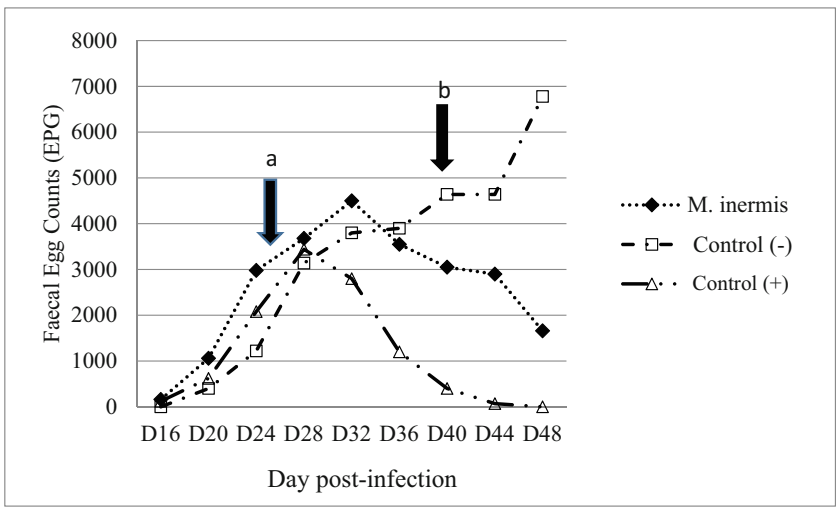

Fig. 5 Variation in fecal egg counts in two months of experiment in the control groups (control +, control −) and the F1 group WALL × WAD treated with *M. inermis* (**a**, **b**). **a** Powder of *M. inermis* leaf applied at a dose of 3.2 g/kg BW on days 26, 27, and 28 postinfection and albendazole administered in a single dose on day 26. **b** Powder of *M. inermis* leaf at a dose of 3.2 g/kg BW repeated 14 days later on days 40, 41, and 42 postinfection

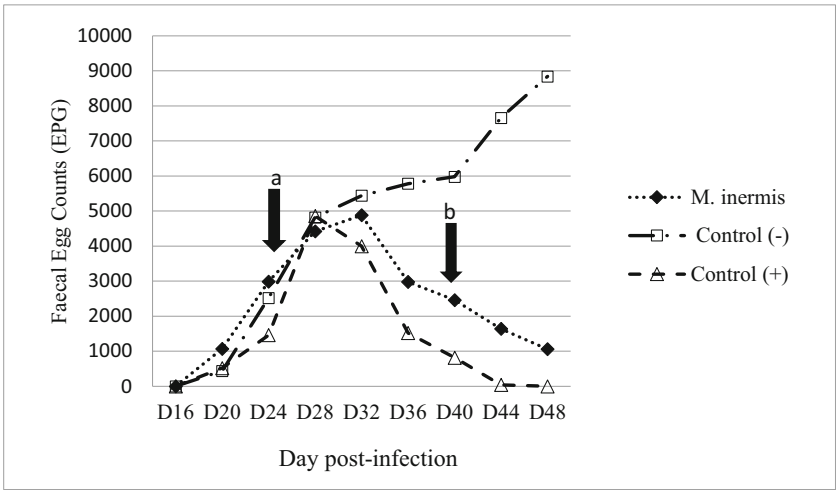

Fig. 6 Variation in fecal egg counts in two months of experiment in the control groups (control +, control −) and the group of WALL lambs treated with *M. inermis* (**a**, **b**). **a** Powder of *M. inermis* leaf applied at a dose of 3.2 g/kg BW on days 26, 27, and 28 postinfection and albendazole administered in a single dose on day 26. **b** Powder of *M. inermis* leaf at a dose of 3.2 g/kg BW repeated 14 days later on days 40, 41, and 42 postinfection

approximately to the fresh base of the plant leaf given to small ruminants in confinement by small-scale farmers, according to a survey conducted by Alissou (2013). This dose was afterwards used in in vivo experiments by Azando et al. (2011) and Dédéhou et al. (2014) with satisfactory outcomes. Albendazole, used as a standard control in a single dose of 5 mg/kg BW, showed significant effects on the FECR, adult worm viability, and fecundity of *H. contortus* that were stronger than the effects of the plant material, with an efficacy rate of 100% at the end of the experiment. These outputs clearly indicate the lack of resistance of worms facing this synthetic drug. In effect, the medicinal plants used until now in our research unit at a single dose of 3.2 g/kg BW showed slight anthelmintic effects (55 to 80%) on GI parasites (Azando et al. 2011; Dédéhou et al. 2014). Likely, with a second treatment of plant materials, we could increase the plant efficacy up to 80%. This result justifies the purpose of this project.

M. inermis leaf powder at a dose of 3.2 g/kg BW showed moderate efficacy (> 60%) compared to that of the controls in the reduction of FEC in three breeds of sheep. Globally, after the first treatment postinfection, the FECR in all sheep was slight. However, a significant reduction in FEC was obtained after the second treatment was applied. The light efficacy of plants may be due to the secondary metabolites recognized to have anthelmintic activities (condensed tannins, saponins, and flavonoids) included in plant material, which are progressively liberated in the rumen of animals (Alowanou et al. 2015b). Moreover, according to Santos et al. (2012), the ruminal flora is one of the factors that can have a strong influence on the activity of substances administered orally, as *M. inermis* leaf powder was. After the second treatment, normal doses of condensed tannins and flavonoids are likely diffused in the blood to induce many mechanisms (eggs hatching, L3 larvae ensheathment and migration, and worm's motility inhibition),

Table 1 Mean number (± SD) of adult *H. contortus* worms recovered from controls and lamb groups treated with leaf powder of *M. inermis* postinfection

Breed of lamb	Treatment	Dose/kg BW	Female[a]	Male[a]	Total worms[a]	Effect on viability[b]
WAD lamb	*M. inermis*	3.2 g	260 ± 110	164 ± 96.7	424 ± 157	a
	(−) control	0.0 g	1638 ± 28.5	524 ± 72.8	2162 ± 59.1	b
	(+) control	5 mg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	c
F1 (WALL × WAD) lamb	*M. inermis*	3.2 g	752 ± 108	260 ± 32.7	1012 ± 134	d
	Control (−)	0.0 g	1556 ± 34.1	794 ± 80.5	2350 ± 54	b
	Control (+)	5 mg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	c
WALL lamb	*M. inermis*	3.2 g	718 ± 128	276 ± 64.2	994 ± 154	d
	Control (−)	0.0 g	1616 ± 101	688 ± 99	2304 ± 150	b
	Control (+)	5 mg	0 ± 0	0 ± 0	0.0 ± 0.0	c

Control (−), negative control; control (+), positive control

[a] Values are mean ± SD, $n = 9$

[b] Different letters indicate a significant difference in values ($p < 0.05$)

Table 2 Mean number of eggs per female (± SD) in *H. contortus* recovered from control lambs and lambs treated with leaf powder of *M. inermis* postinfection

Breed of lamb	Treatment	Dose (g/kg BW)	Eggs per female[a]	Effect on fecundity[b]
WAD lamb	Control (−)	0.0	700 ± 50.1	A
	M. inermis	3.2	263 ± 31.8	B
WALL lamb	Control (−)	0.0	864.6 ± 36.9	A
	M. inermis	3.2	296.8 ± 28.1	B
F1 (WALL × WAD) lamb	Control (−)	0.0	787 ± 31.2	A
	M. inermis	3.2	280 ± 13	B

Control (−), negative control

[a] Values are mean ± SD, $n = 6$

[b] Different letters indicate a significant difference in values ($p < 0.05$)

leading to an additional reduction in FEC, as mentioned in some previous studies by Brunet et al. (2011) and Hoste et al. (2012). The activity shown by the plant agrees with earlier works that reported a significant effect of potential anthelmintic plants for FEC reduction of up to 60% (Hounzangbé-Adoté et al., 2005b; Grade et al. 2008; Vatta et al. 2011; Soro et al. 2013; García-Hernández et al. 2016).

This study demonstrates the effect of *M. inermis* on adult parasites: decreasing egg-laying capacity and eliminating adult parasites. *M. inermis* at a dose of 3.2 g/kg BW eliminated more than 80% of adult worms of *H. contortus* from the abomasum. In addition, a reduction of up to 70% in female adult worm fecundity was achieved against this nematode, which is responsible for extensive production losses in small ruminants. Data from this study that found a significant reduction in adult worms of *H. contortus* of up to 80% are in agreement with data from other studies (Soro et al. 2013; García-Hernández et al. 2016). The decrease in adult worms and female worm fecundity induced by *M. inermis* could be associated with the ability of its bioactive compounds, especially condensed tannins, including those in the leaf of *M. inermis*, to exhibit vermifuge activity as a consequence of either worm mortality or worm paralysis according to Hoste et al. (2012). Moreover, the direct effects of condensed tannins are mostly related to structural damage in adult nematodes that prevents their appropriate feeding, movement, and mating, as demonstrated by Martínez-Ortíz-de-Montellano et al. (2013) in studies using an electron microscope. In addition, the reduction in FEC induced by *M. inermis* leaf was principally associated with a significant reduction in the fertility of female worms, as measured by egg counts in the uterus of the parasite. An observed reduction in egg count does not necessarily reflect a reduction in the parasite load because it could be related to a reduction in the fecundity of the parasites (Moreno et al. 2012). In concordance with the present results, previous studies (Paolini et al. 2005; Lange et al. 2006) examining the interactions between bioactive plants and nematode infections in small ruminants have been recorded, indicating that a decrease in the egg output of parasitic nematodes was not due to a reduction in worm number but mainly to a specific effect on the reproductive function of female worms.

Any animal with a PCV below 20% can be considered anemic and not resilient to a GIN, according to Byers and Kramer (2010). With regard to this criterion, the PCV values recorded in the present study (30 to 43%) indicate that the experimental animals were not anemic until the end of the experiment, perhaps because of the long time before the prepatent period began. In the *M. inermis* group, the PCV values that declined in the beginning of the prepatent period were established after treatment application. This observation could be explained by the effect of *M. inermis* occurring likely through a reduction in FEC or/and adult worms of *H. contortus*. Moreover, several works have reported that in *Haemonchus* spp. infections, there is a strong correlation between blood loss and both worm burden and egg production (González et al. 2011; Onzima et al. 2017).

This work was not directly focused on the comparison effect of resistance and/or resilience between the breeds of sheep infected with *H. contortus* but was performed to obtain scientific knowledge regarding the use of *M. inermis* leaf as a dewormer by the local population. However, regarding the control group where animals were not anemic (PCV > 30%), no mortality postinfection was observed until the end of the experiment despite a great worm burden (> 2000) and high FEC (> 5500). We can consider that animals were resilient to *H. contortus* infection regardless of the breed of sheep according to the report of Bisset and Morris (1996) and Bishop (2012). Moreover, the evidence for variation in resistance (low FEC) and/or resilience to GIN, particularly *H. contortus*, between breeds has been extensively documented in sheep (González et al. 2011; Chiejina and Behnke, 2011; Getachew et al. 2015). However, there is no known scientific literature about the variation in resistance/resilience to GIN

among the indigenous sheep breeds of Benin. Therefore, as an extension to this present work, our further studies will be focused on the aspects mentioned above.

In conclusion, the powder of *M. inermis* leaves at a dose of 3.2 g/kg BW showed significant efficacy on FEC, adult worm viability, and female worm fecundity in lambs, regardless of the breed, when applied by a 3-day administration that was repeated 2 weeks later. Therefore, plant preparation proved to be an alternative way to replace or complement the use of chemical drugs to achieve more sustainable control of haemonchosis in sheep from western Africa. Nevertheless, further in vivo studies are needed to assess the efficiency of *M. inermis* at the same dose against other predominant gastrointestinal nematodes in small ruminants in tropical regions, such as *T. colubriformis*.

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Compliance with ethical standards

Ethical clearance Ethical clearance was obtained from the guidelines of the Ethical Committee of University of Abomey-Calavi and registered under no. CEI-2016/1134/UAC/Benin. Data collection was conducted using the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP).

Conflict of interest The authors declare that they have no conflict of interest.

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