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In Vivo Anthelmintic Activity of Whole Plant Powder of Striga Hermonthica (Deli.) Benth

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SUMMARY

The fight against Gastro-Intestinal Nematodes is an urgent necessity for the sheep's productivity improvement in Burkina Faso. The anthelmintic activity of *S. hermonthica*'s whole plant powder was evaluated on Mossi sheep artificially infested with *H. contortus* L3 larvae. The experiment lasted 21 days with 2 phases of 4 days of treatment separated by 6 days in station. Two (2) treated lots of 6 sheep each received respectively 17g/kg of body weight and 10g/kg of body weight while two (2) control lots of 6 sheep each, one negative without treatment and one positive treated with levamisole 1/2 bolus for 25 kg were constituted. The faecal egg count (FEC) reduction rate was high during treatment and reached 84.49% for the 17g/kg body weight dose and 83.69% for the 10g/kg body weight dose at D21. Statistical analysis shows no significant difference between the two doses tested and between the two doses and the positive control, whereas the difference is significant ($P<0.05$) with the negative control. The mean hematocrit level ranged from 24 at D0 to 30.5 at D21 for the 17g/kg body weight treatment and from 25.83 at D0 to 31 at D21 for the 10g/kg body weight dose. The average daily gain (ADG) of the treated lots was not significant compared to the negative control ($P>0.05$).

KEYWORDS: Bioactive forages, Anthelmintics, *H. contortus*, *S. hermonthica*,

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INTRODUCTION

Parasitosis due to gastrointestinal strongyles is a worldwide problem that can lead to significant productivity losses in small ruminants (Okombé, 2011; Francesco and *al*, 2014). These parasites can cause mortality of up to 50% of lambs and economic losses beyond 50% of production capacity (Bodji and *al*, 2017). There are a large number of Gastro-Intestinal Nematodes's species that thrive under diverse climatic conditions and consequently there is a wide geographical distribution of nematodes threatening the sustainability of livestock farms around the world. *Haemonchus contortus* is one of the most widespread and pathogenic species causing severe anemia in small ruminants (Burke, 2007). This hematophagous parasite located in the abomasum of small ruminants can measure between 10 and 16 mm for the male and 18 and 30 mm for the female that can lay up to 5,000 to 10,000 eggs per day (Sicard and Robert, 2010; Mongellaz, 2019; Ruiz-Huidobro, 2018).

Gastrointestinal parasites's control has been based for several years on the use of synthetic anthelmintics which are unfortunately inaccessible and increasingly ineffective against these parasites (Kaboré and*al*, 2009). Also, these molecules have been found to be ecotoxic, thus presenting a high environmental risk (Saccareau, 2016).

Several control alternatives, including biological control through the use of medicinal plants with anthelmintic properties, have been developed. Studies in Burkina Faso and Benin have shown the anthelmintic activity of certain woody species (Kaboré, 2009; Awohouedji, 2014). But in Burkina Faso studies on the anthelmintic properties of herbaceous species such as *S. hermonthica* are rare. *S.*

hermonthica is an annual herb that parasitizes the roots of several cereals (Kiendrebeogo and *al*, 2006; Hamisou and *al*, 2020). Many studies have mentioned the use of *S. hermonthica* in human and veterinary pharmacopoeia and the presence of some secondary metabolites such as polyphenols, tannins and flavonoids in this plant (Djerro, 2002, Baba and *al*, 2012, Garba and *al*, 2019).

The objective of our study is to evaluate the anthelmintic activity of the whole plant powder of *S. hermonthica* through the measurement of the feacal eggs count in sheep artificially infested with L3 larvae of *H. contortus*.

MATERIAL AND METHODS

Plant material

The whole plant of *S. hermonthica* was collected in the villages of Katchari and Mamasiol in the urban commune of Dori early in the morning between the end of September and mid-October. Whole plant samples were dried in a room at DRREA-Sahel for 2 weeks at room temperature and then transported to CREAF Kamboinsin in the commune of Ouagadougou. One sample was identified at the National Herbarium of Burkina Faso (HNBF) under number 8759. The samples were then ground using a Retsch type SM 100 grinder with a mesh size of 5 before being used as whole plant powder for the *in vivo* test.

Animal material

Sheep

Twenty-four (24) Mossi sheep between 12 and 24 months of age and with an initial weight between 16.6 and 27.7 kg were used for the in vivo tests. All animals were paid at the livestock market of Kaya, capital of the Centre-Nord region. The animals were not dewormed during the three months preceding the experiment.

L3 larvae

The infesting L3 larvae of *H. contortus* were obtained by culturing fresh eggs in the sterilized's feacal of sheep mixed with wood's sawdust for 14 days at 31° C. After 14 days the culture was mounted in gauze and then placed on the Baermann device to get the L3 larvae.

METHODS

Experimental device.

Thirty (30) days before the beginning of the experiment, all animals were artificially fed 3200 *H. contortus* larvae orally through a naso-esophageal tube in two phases of 1600 larvae each, spaced one week apart. One week before the start of the in vivo tests, the animals were weighed and the degree of infestation was determined and then the animals were divided homogeneously into 4 lots of 6 sheep each according to the weight and the feacal eggs count (FEC). The lots included, 2 test lots treated with two different doses of *S. hermonthica* whole plant powder, 1 untreated negative control lot and 1 positive control lot treated with levamisole.

Table I. Experimental setup

Lots	Number	Weight (kg)	FEC	Dose (g/kg ofbody weight)	Concentrated food (g/animal)
***S.hermonthica*A**	6	18.78	5 200	17	200
***S. hermonthica*B**	6	19.67	2 500	10	200
Negative control	6	16.37	2 500	No	200
Positive control	6	16.50	3 900	Levamisole (1/2 bolus pour 25 kg)	200

The experiment lasted 21 days during which all animals received concentrate (SN Citec cattle feed) in the form of pellets in the morning at 6:00 am. For the treated lots, the concentrate was mixed with the equivalent amount of the set treatment dose. In the afternoon, all animals were fed corn bran for the duration of the experiment. The animals were provided with water and the mineral lick ad libitum during the experimental period. The treatment in the form of *S. hermonthica* whole plant powder was administered to the treated lots on the first four days (D1, D2, D3, D4) and then repeated on the 7th day after the end of the first treatment (D11, D12, D13, D14). At the end of the first treatment the animals grazed on natural pasture for 6 days before the second treatment was repeated. After the last day of the second treatment, the animals were still grazing on natural pasture until the 21st day of the experiment.

MEASURED PARAMETERS

The main parameters collected for this study are: clinical data concerning the general condition of the animals, parasitological and hematological data.

- During the period of the experiment, the clinical data concerning the general condition of the animals after the administration of the whole plant powder of *S. hermonthica* were evaluated by direct observations and recorded on a form.
- The weight growth of the animals of different lots was measured from individual weighing at D0, D4, D7, D14 and D21. Weighing was done to jeun using a MARECHALLE-PESAGE cattle scale for small ruminants equipped with an electronic indicator.
- Parasitological data were obtained by individual sampling of feces directly from the rectum of the animals at D0, D4, D7, D14, D21. Individual coproscopies were performed and

H. contortus egg excretion was quantified by the Mac-Master technique by triturating 3g of feces in 42 ml of NaCl density 1.2 to allow egg flotation. The sensitivity for one egg found was 50. The Feacal Eggs Count (FEC) was thus determined.

FEC: number of eggs obtained in the two compartments X 50

The Feacal Eggs Count Reduction Rate (FECR%) was determined according to the formula used by Kaboré. (2009):

$$FECR(\%) = \left(\frac{FEC\ before\ treatment - FEC\ after}{FEC\ beforetreatment}\right) x\ 100$$

-

The hematocrit rate variation of the animals in each lot was determined by drawing blood through the jugular vein of the animals using heparinized tubes at D0, D7, D14, and D21 of the experiment. Then each sample was used to fill a capillary tube with 4/5th hematocrit. The filled capillary tubes were centrifuged at 9000 rpm for 5 minutes using a HAEMATOKRIT 210 hematocrit centrifuge. In conjunction with the blood collection with heparinized tubes, a collection with dry tubes was performed on D0 and D21 of the experiment for each animal. The dry tubes were centrifuged using a SIGMA 3-15K centrifuge at 3000 rpm for 10 minutes and the serum was collected in 1.8 ml cryotubes for biochemical analysis.

- The degree of anemia of each animal was determined by evaluating the FAMACHA score by comparing the coloration of the ocular conjunctiva of each animal with a card showing different colorations of the ocular conjunctiva in relation to the degree of anemia of the animal.

STATISTICAL ANALYSIS

The collected data were entered into Excel 2016 which was used to calculate the means and standard deviations of the different measured parameters as well as the animals's Feacal Eggs Count reduction (FECR) rate. Then the data of the different days of follow-up were subjected to a one-factor analysis of variance (ANOVA I) followed by a multiple comparison of means at the threshold of 5% by the method of Tukey's using the interface Rstudio Version 1.4.1717 with the packages Rcmdr Version 2.7-1 of the software R Version 4.1.0.

Prism 5.0.0.288 software was used to produce the graphics.

RESULTS

Clinical and parasitological data

No apparent clinical signs of toxicity (salivation, skin reaction, diarrhea) in the animals during the treatment time were observed. The evolution of FEC showed a significant decrease over time compared to the negative control P (<0.05) (Figure1).

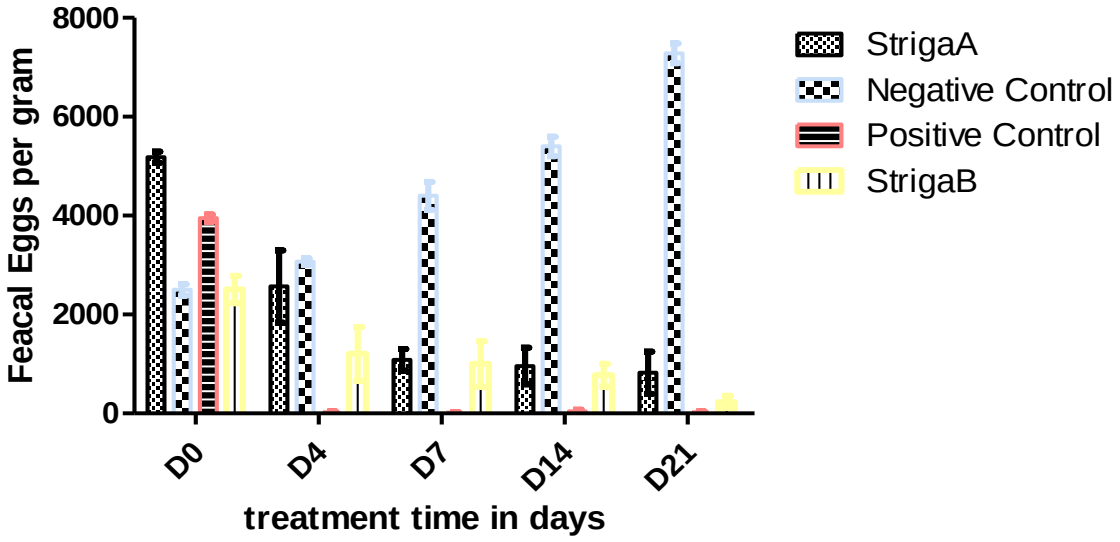

FIGURE 1:Animals treated with different doses of *S. hermonthica* FEC evolution. StrigaA: dose 17g/kg body weight; **StrigaB**: dose 10g/kg body weight.

FECAL EGGS OF H. *CONTORTUS* REDUCTION RATE

A significant reduction of fecal excretion rate of *H. contortus* eggs compared with the negative control P (<0.05) was observed from the first day of follow-up and throughout the experiment period (Table II).

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Table II. FEC Reduction rates of the animals in the different lots.

Lots	FEC reduction rate			
	D4	D7	D14	D21
StrigaA	49.91d	78.83de	82.12de	84.49de
StrigaB	57.81de	64.03de	70.64de	83.69de
Positive Control	99.36e	99.57e	98.78e	99.35e
Negative Control	-24.01c	-77.28b	-118.39b	-195.07a

StrigaA: dose 17g/kg body weight; **StrigaB**: dose 10g/kg body weight.

WEIGHT EVOLUTION

The animals weight evolution in the different treated lots was not significant P (>0.05) compared to the both controls lots.

A low average daily gain was observed during the treatment (Table III).

Table III. Animals weight evolution in the different lots.

Lots	ADG(D0-D4)	ADG(D0-D7)	ADG(D0-D14)	ADG(D0-D21)
StrigaA	137.5g±0.08a	101.4g±0.02a	57.8g±0.07a	52,8g±0.04a
StrigaB	62.5g±0.5a	161.4g±0.4a	-15.7±0.16a	21.4g±0.11a
Positive Control	280g±0.09a	218.5g±0.06a	32,1g±0.02a	37.1g±0.03a
Negative Control	227.5g±0.1a	161.4g±0.09a	-10±0.04a	-25.7±0.01a
P		0.224		

EVOLUTION OF THE HEMATOCRIT RATE LEVEL

An improvement of the hematocrit rate with a rate between D0 of 24 and D21 of 30.5 for the lot treated with 17g/kg of body weight and a hematocrit rate at D0 of 25.83 and D21 of 31 for the lot treated with 10g/kg of body weight were noticed. However, there was a non-significant change in the hematocrit level compared to the controls and between the different treated lots (P>0.05) (Figure2).

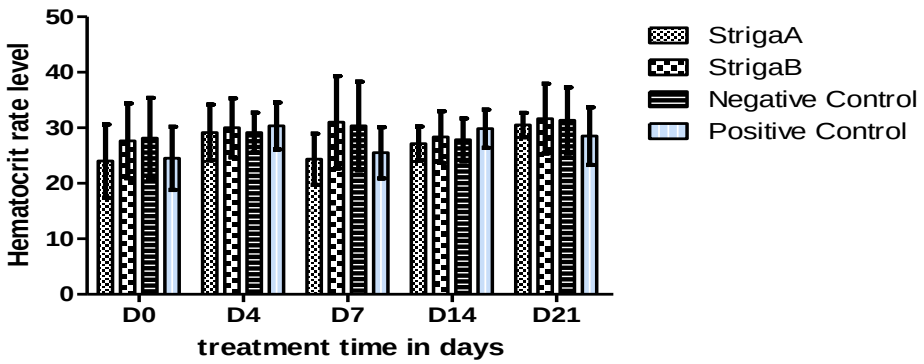

FIGURE 2: The hematocrit rate level evolution during treatment.

EVOLUTION OF FAMACHA

Figure 3 shows us a slight increase in FAMACHA score in animals at D21 compared to D0. Statistical analysis of the

comparison of means of the FAMACHA score shows a strong significance at D14 compared to the negative control (P<0.05).

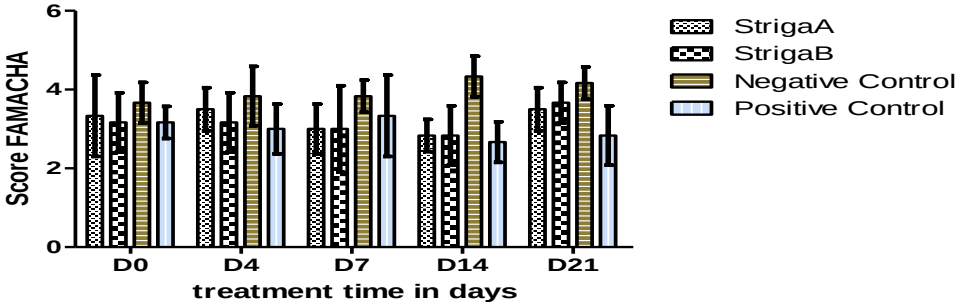

FIGURE 3: Evolution of FAMACHA score during treatment

EFFECT OF TREATMENT ON HEPATIC ENZYME METABOLISM AND RENAL

Treatment with both doses of *S. hermonthica* did not result in liver and kidney damage in animals from different lots. Indeed, the values of Aspartate Aminotrasferase (ASAT) and Alamine Aminotransferase (ALAT) at D0 and D21 of the treated animals are all located within the range of the usual reference values for the hepatic function. Also, creatinine values, which is one of the most reliable biochemical markers of glomular function, were within the range of the different usual and reference values at D0 and D21 (Table IV). Statistical analysis of these parameters at D0 and D21 and between treated batches and negative controls was not significant (P>0.05).

Table IV. Effect of treatment on liver and kidney function

Treatment		Creatinine (µmol/l)	ASAT (UI/l)	ALAT (UI/l)
Striga A	D0	94.4±14.3a	128±51.1a	20±7.3a
	D21	78.7±5.4a	147.8±43.8a	21±4.8a
Striga B	D0	100.6±14.7b	122.2±25.9a	20.8±4.7a
	D21	81.9±8ab	114.5±22.7a	16.5±2.5a
Negative Control	D0	78.85±8.45a	119.66±36.10a	20.66±5,98a
	D21	83.8±6.36ab	125.16±38.20a	20.83±3.12a
Positive Control	D0	79.06±12.29a	130.4±32.99a	23±7.41a
	D21	81.25±7.56ab	129.5±63.17	19.33±2.5a
P		0.007	0.94	0.62
References Values		160-168 **A;** 53-115**C**	71-209**B**	30±4 **A**

A: Kaneko and *al*., (2008); **B:** Ramos and *al*, (1994); **C:** Debreuil and *al.*, (2005); **P**: Probability; **a, b**: no difference between the columns, **StrigaA:** dose 17g/kg body weight**; StrigaB:** dose 10g/kg body weight**.**

EFFECT OF TREATMENT ON ENERGY AND MINERAL METABOLISM

Table V shows that the treatments with the two doses of *S. hermonthica* powder did not lead to an imbalance of the energetic and mineral metabolism with a normal range obtained for all the values of the different parameters at D0 and D21. The statistical analysis of the different parameters at D0 and D21 and between the treated lots and the negative controls was significant (P<0.05) except for the triglyceride parameter which was only measured at D21 (P>0.05).

Table VI. Effect of treatment on energy and mineral metabolism

Treatment		Glycemic (mmol.l)	Cholesterol (mmol/l	Calcium (mmol/l)	Triglyceride (g/l)	Totals Proteins(g/l)
Striga A	D0	3.1±0.8ab	3,7±0,47b	3,9±0,4c	ND	138.7±20.76c
	D21	4.3±1.09b	2,2±0,37a	2,5±0,5a	0.5±0.1a	92±8.22a
Striga B	D0	3.3±0.83ab	3.1±0.94ab	3.7±0.6bc	ND	136.3±28.15bc
	D21	3.3±0.45ab	2.5±0.51a	2.6±0,5a	0.3±3a	101.7±4.93ab
Negative Control	D0	2.3±0.97a	2.9±0.51ab	3.27±0.6ac	ND	132.1±30.62bc

	D21	3.3±0.33ab	2.1±0.37a	2.76±0.70ab	0.5±0.23a	96.9±8.7a
Positive Control	D0	2.2±0.36a	2.9±1.05ab	3.19±0.29ac	ND	119.08±14.5ac
	D21	3.4±0.55ab	2.1±0.24a	2.79±0.27ab	0.4±0.1a	105.1±8.8ac
P		0.001	0.0008	0.0004	0.3	0.0001
References values		0.8-2.4 **D** 3.5-3.80 **E**	1.68±1.3 **E**	3.04±0.07**A**	0.5±0.19 **E**	130.2±38.9 **E**

A: Kaneko and *al*., (2008), **D**: Deghnouche and *al*., (2011); **E:** Ndoutamia and Ganda (2005), **P**: Probability, **a, b, c**: difference between the columns, **ND**: Not Dosed, **StrigaA:** dose 17g/kg body weight**; StrigaB:** dose 10g/kg body weight.

DISCUSSION

Whole plant powder of *S. hermonthica* has significantly reduced FEC of sheep artificially infested with L3 larvae of *H. contortus* at the different doses tested. The two doses of *S. hermonthica* powder, 17g/kg of body weight and 10g/kg of body weight, all resulted in a strong decrease in FEC from day 4 of follow-up with 49.91% and 57.81% reduction rates respectively. The decrease in FEC of both doses then continues throughout the treatment time and reveals an anthelmintic activity of the plant. Our results are consistent with those obtained on Djallonke kids treated with different doses of *Zanthoxylum zanthoxyloides* leaf powder and *Newbouldia laevis* with a strong decrease in FEC (Azando and *al*, 2011). Also aqueous extracts of *Annona senegalensis* stems, roots and leaves administered to Djallonké sheep at dose of 22 mg/kg live weight resulted in a decrease in FEC similar to our results although the nature of the remedy administered was not the same in our two studies (Bodji and*al*, 2017). In contrast to our results, Mossi breed sheep treated with dried leaves of *Calotropis procera* (Will). R. Br had a decrease in FEC reduction rate at D3 and D7 and then the level of FEC rate increased from D14 to D28 of the Follow-up (Kanazoé and *al*, 2017). The difference with our present study would be due to the content of secondary metabolites in *C. procera*. The Average Daily Gain (ADG) of the treated animals in our present study was low over the follow-up time and statistical tests showed no significant difference compared to the two controls performed. In Congo, goats treated with *Vitex thomasii* root powder did not obtain a statistically significant difference in weight evolution after 126 days of treatment which corroborates our results (Okombé, 2011). Our results are in agreement with those obtained on sheep treated with aqueous extracts of *Myrsine africana* by doses of 50 and 125 g/kg of weight but which did not obtain a significant difference between the different batches (Githiori and *al*, 2002). Treatment with both doses of *S. hermonthica* resulted in an improvement of the hematocrit level in animals of the different lots with a statistically significant difference ($P<0.05$). Indeed, we note a mean hematocrit level varying from 24 at D0 to 30.5 at D21 for the treatment at a dose of 17g/kg of body weight and from 25.83 at D0 to 31 at D21. In conjunction with the hematocrit, there was an improvement in the FAMACHA score in the animals of the different lot. Sheep treated with *Newbouldia laevis* leaf powder at a dose of 1.6g/kg body weight achieved improved hematocrit levels similar to our study (Olounladé and *al*, 2017). Sheep treated with Newbouldia laevis leaf powder at dose of 1.6g/kg body weight achieved improved hematocrit levels similar to our study (Olounladé and*al*, 2017). In contrast to our study, Mendonça-Lima and *al*. (2016) did not obtain a significant difference in hematocrit levels in goats treated with different doses of *Cratylia mollis* leaf decoction in Brazil. The area and/or the different species incriminated in these goats could explain this difference. During treatment, no signs of toxicity (salivation, skin reaction, diarrhea) were observed in the animals. The transaminases, creatinine and the different energy and mineral parameters measured at D0 and D21 are within the range of the usual reference values, which shows non-injury of the liver and kidneys of the animals treated with the whole plant powder of *S. hermonthica*.

CONCLUSION

The results of the present study show a significant reduction in FEC of animals artificially infested with *H. contortus* after treatment with the tested doses of *S. hermonthica* powder. Treatment with *S. hermonthica* powder resulted in improved hematocrit levels in treated animals between D0 and D21, but weight changes were not elevated in animals from different lots. The liver and kidneys of the animals were not affected by the treatment of *S. hermonthica* powder at the tested doses. Thus, *S. hermonthica* can be used as an alternative to chemical treatment of GIN in powder form.

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