https://doi.org/10.48047/AFJBS.6.14.2024.8104-8123

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

In vivo anthelmintic activity of aqueous extract of *Carica papaya* seeds on gastrointestinal nematode parasites of cattle in Cameroon.

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Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.8104-8123

Abstract

The management of gastrointestinal parasites in cattle reared on grazing land remains a major concern, and one to which ethnopharmacopoeia is associated. This study highlights the *in vivo* effect of the aqueous extract of C*arica papaya* seeds on gastrointestinal parasites in cattle. Doses of 5, 50, 300 and 1000mg/kg of extract were administered once to 4 batches of 3 animals each compared with 2 other batches of 3 animals each subjected once to albendazole and distilled water respectively. Doses of 5 and 50mg/kg induced a significant ($P<0.05$) reduction in faecal egg excretion with efficacy ranging from 71 to 92%. Although the 4 doses (5, 50, 300 and 1000mg/kg) rebalanced the physiology via the blood count and formula, the larger doses (300 and 1000mg/kg) did not significantly influence parasite pressure ($P<0.05$). The right dose of papaya seed extract could be an alternative for combating digestive strongyles in cattle.

Key words

Carica papaya, anthelmintic, *in vivo*, gastrointestinal strongyles, cattle.

INTRODUCTION

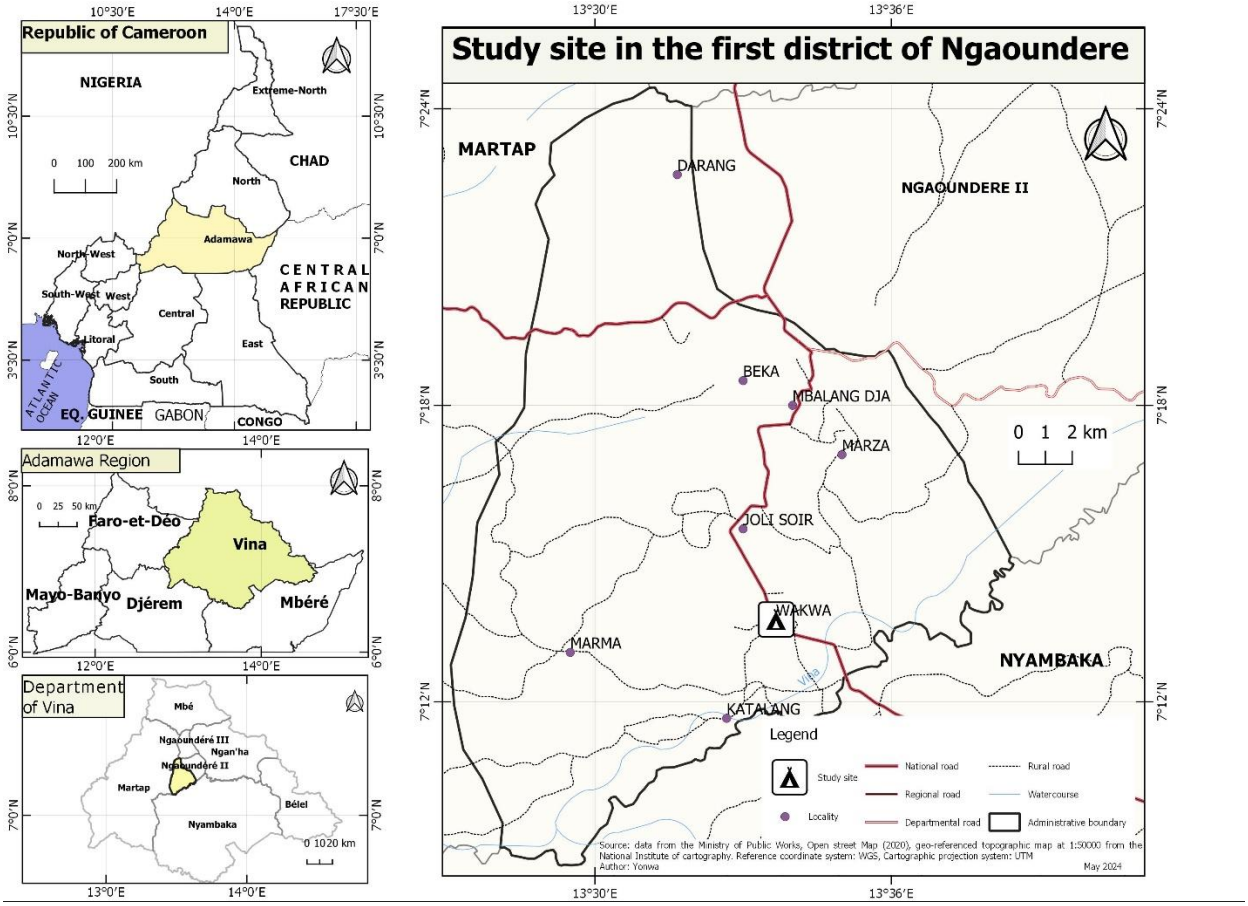
Cattle rearing, as it becomes more intensive, faces a number of obstacles, among which are digestive system parasites. Despite the progress made in treatment, control of these infestations relies essentially on the use of antiparasitic drugs. However, in developing countries in general, and in sub-Saharan Africa in particular, due to widespread poverty, an average farmer cannot afford to buy conventional products. Moreover, shortages of quality veterinary

products are frequent and cause enormous losses to farmers (Mpoame *et al*., 2003). These losses cause some farmers to replace veterinarians with clams of incompetence (Mboera and Kitalyi, 1992; Mensah *et al*., 2006; Charlier *et a*l., 2014). Because of the presence of substandard drugs in Africa markets, parasites resistance to anthelminthics become a long-standing problem in Africa as the concentrations required to clear the parasites are hardly attained during treatments (Prichard, 1990; Sutherland and Leathwick, 2011). Farmers also make uncontrolled used of antibiotics and other basic drugs for almost all symptoms in animals wthout proper diagnosis and prescription. Uncontrolled ingestion of antibiotics through meat milk, contributes to bacterial resistance, which is responsible for the exhaustion of antibiotic families (Trémolieres *et al*., 2006). This behaviour increases the demand for antibiotics resulting in pharmaceutical pollution which constitutes a serious threat to the environment (Luo *et al*., 2014; Rehman *et al*., 2015). This is because the anthelmintics and antibiotics used are not fully metabolised. They end up unchanged in the dung, with dramatic effects on the insects that feed on the dung, and hence on the environment. Moreover, the prevalence of veterinary drug residues in foodstuffs of animal origin is less than 1% in Europe, but as high as 94% (Mensah *et al*., 2014) in some African countries. These residues are responsible for a series of known and unknown/new biological and chemical hazards in food and the environment, such as allergies, cancers, changes in intestinal flora, bacterial resistance and inhibition of fermentation processes in the dairy industry (Mensah *et al*., 2014). Faced with the cost of repeated interventions, large-scale medical prophylaxis campaigns do not seem feasible for the farmers (Thys and Vercruysse, 1990). It is therefore essential to find control methods that are inexpensive, accessible to farmers and environmentally friendly. According to Anjaria (1996), 85% of developing countries use phytotherapy as a priority. However, the effectiveness of plant extracts has yet been scientifically proven. Recourse to traditional pharmacopoeia therefore appears to be a valid alternative. Although some progress has been made in quantifying extracts from certain plants for use against gastrointestinal parasites (Mpoame and Essomba, 2000), the efficacy of most local medicinal plants (cited by agro-breeders, easily cultivated and identified in our previous work), particularly *Carica papaya*, a tropical plant whose fruit, root, leaves and seeds are used in human medicine (Okeniyi *et al*., 2007) and veterinary medicine (Satrija *et al*., 1994; Ahouandjinou, 1994) against gastrointestinal parasites in cattle has not yet been quantified (Tchoumboué *et al*., 1996). The aim of this study was to assess the antiparasitic efficacy of aqueous extract of pawpaw seeds against bovine nematodes, which has not been reported upon till date.

MATERIALS AND METHODS

Study site

The work took place in the veterinary laboratory of the Wakwa (fig. 1) Agricultural Research Centre. Located in the peri-urban zone, Wakwa is a village in the commune of Ngapoundere 1er in the Adamaoua region and the Vina department, 10 km south of the town of Ngaoundéré. Its geographical coordinates are: 7 ° 15'908 'North, 13 ° 32'912"East and 1143 m altitude.



Source: Data from the Ministry of Public Works, Open street Map (2020), geo-referenced topographic map at 1:50000 from the National Institute of cartography. Reference coordinate system: WGS, Cartographic projection system: UTM.

Figure 1: Location of study site

Extraction of secondary metabolites

The part used in this study were seeds and that was not detrimental to the survival of the plant. Fresh seeds were collected from mature pawpaw fruits in Vina Division, Adamawa region, Cameroon. They were dried in a ventilated room away from dust and sunlight and then ground to a powder. 100g of seed powder was mixed with 1000 mL of distilled water and boiled. The

decoction was then filtered with Wattman paper. The filtrate was dried in an oven and the extract obtained was kept refrigerated at 4°C. The yield is given by the formula $R= \frac{E}{MS} * 100$ where R is the extraction yield; E is the quantity of extract; MS is the quantity of dry matter in the product.

***In vivo* test**

Experimental set-up and treatment

Cattle from the experimental farm of Wakwa Agricultural Research Centre were used for the experiment. The following criteria were defined for the choice of animals:

- Coproscopy positive (EPG ≥ 200);
- Not have been on deworming medication for 3 months prior to the experiment;
- Be between 2.5 and 3 years old;
- Have a diarrhoea index of 1 or 2;
- Have been naturally infested;
- Have access to pasture;
- Have a body condition score of at least 3 on 5.

Analyses were performed at the veterinary laboratory of the Wakwa Agricultural Research Centre (CRA) located in Ngaoundere, Vina Division, Adamawa region, on 18 cattle that met the inclusion criteria. They were divided into 6 batches of 3 subjects per batch as shown in Table 1. Diluted in distilled water, the extract was administered orally, see Table 1.

Table 1: Administration of the different doses of aqueous extracts of *Carica papaya* seeds to the 6 batches of cattle.

Extract/Doses	**PC**	**NC**	**5mg/kg**	**50mg/kg**	**300mg/kg**	**1000mg/kg**	**Total**
AECPS	3	3	3	3	3	3	**18**
PC: Positive Control; NC: Negative Control; AECPS: Aqueous Extract of *Carica papaya*.Seeds							

Each trio of animals received the corresponding doses: PC treatment (positive control) received albendazole at a dose of 5mg/kg; NC treatment (negative control) received distilled water and the other batches received the aqueous extract of *Carica papaya*.seeds as indicated in table 1 above. Treatment was carried out only once. For an animal weighing 250kg, for example, doses of 5 to 50mg/kg correspond to 13 to 129g of powder and 1.25 to 12.5g of the aqueous extract of *Carica papaya* seeds. Throughout the experimental period, the animals were provided with natural pasture and water *ad libitum*.

Faecal sampling

Every after 5 days and before the animals left for the pasture, faecal samples were collected using the method defined by Hansen and Perry (1995). Briefly, this involved:

- Restraining the animal, by two herdsmen,
- The collector stood behind the immobilised animal after wearing gloves,
- Lifted the animal's tail with one hand and then skilfully insert the fingers of the second hand joined together in the shape of a cone into the animal's rectum,
- He collected 50 to 100g of faeces into a plastic bag,
- He give each faecal sample the code of the animal sampled,
- Then packed all the samples into a cooler for transport to the laboratory,
- We preserved the samples at 4°C when the analysis was not immediate.

Preparation of Willis solution

We dissolved sodium chloride in pure water at a dose of 40 g of salt per 100 ml of water (40%). A densimeter was used to check the concentration of salt in the solution.

Blood sampling

At the jugular vein, compression was first applied with one hand proximal to the puncture site. The needle was then inserted first perpendicular to the animal in order to pierce the skin, and then inclined at an angle of approximately 30 degrees as it penetrates the vein. The needle of the vacutainer system was inserted into the cap of the tube until it is pierced, and 5mL of blood collected. During collection, we insure that the volume of blood/anticoagulant ratio in the tube met the standard recommandations because if there is too little or too much blood, the results of the analyses may be distorted given that excess EDTA causes an artefactual change in the shape of the red blood cells. The volume of blood usually collected is at least 2 mL, and usually 5 mL. When a vacutainer is used, it can be safely filled to the maximum, 5mL (Schalm *et al*., 2000; Chuzel, 2003).

Coproscopy and experimental monitoring

An initial quantitative coproscopy was performed on 30 animals in order to accept or reject candidates. 24 hours before each treatment, faecal samples were analysed and weighed to record the number of eggs per gram (EPG) of faeces at day zero (D0) and the masses to determine the corresponding concentration. The number of eggs per gram of faeces was

determined using Willis solution. At the same time quantitative pre- and post-treatment coproscopy and blood samples and weights were taken. The various pre- and post-treatment samples were used to assess various parameters.

Parameters measured

The following parameters were measured:

- **The effect of the aqueous extract on infestation intensity.**

This was evaluated by the EPG as described by Thienpont *et al* (1995) using the following formula**: EPG =** $\frac{n1+n2}{2} * 100$ with n_1 being the number of eggs counted in cell 1 of the slide; n_2 the number of eggs counted in cell 2 of the slide.

- **The effect of the aqueous extract on reducing parasite pressure between samples.**

The reduction in egg excretion, which makes it possible to assess the variation in the level of parasite egg excretion at a given time (t) compared with a previous situation, was assessed by the reduction rate (R). This rate is calculated using the formula of Bauer *et al* (1986): **R (%)= [(initial EPG - EPG at time t) / initial EPG] *100**.

- **Treatment efficacy.**

It was calculated according to the method of Présidente (1985) which considers the average EPG before and after the treatments according to the formula: **E% = [1 - (T1/T2) (C2/Cl)] * 100**. Where E% = efficacy rate; T1 = EPG on the nth day after treatment; T2 = initial EPG of the treated batch; CI = EPG on the nth day after treatment of the control batch; C2 = initial EPG of the control batch.

- **The effect of aqueous extract on weight gain after treatment.**

Weighing was carried out early in the morning before departure for grazing. Average daily gains were calculated using the formula proposed by Lhoste *et al* (1993): **ADG = (Wf - Wi) * 1000 / ΔT** Where ADG = average daily gain (g/d); W_f = final live weight (kg); W_i = initial live weight (kg); ΔT = time between two weighings.

- **The effect of the extract on the Blood Count and Blood Formula (BCBF).**

It was carried out using a Mindray BC 20s flow cytometry device which is an automated equipment that uses a laser. When the cells in the sample pass through the laser, they absorb

light, which is used to count the cells. It has a number of important advantages, including ease of the work, speed, accuracy, reliability and the analysis of several haematological parameters (Dawson *et al*., 2000; Holsteg *et al*., 2002; Weiss, 2002).

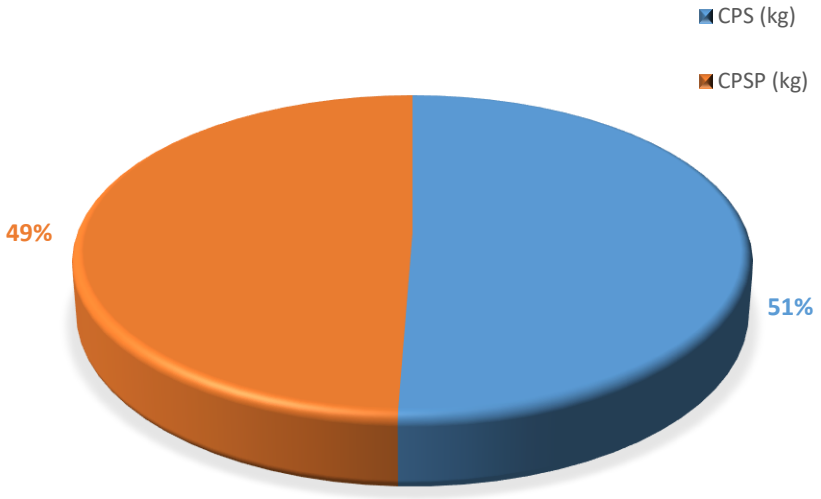
Statistical analysis

The mean of the eggs per gram of faeces, weights and blood count, and blood formula data for all batches were first processed using Excel version 2016, then compared using analysis of variance (ANOVA) and separated using the Statistical Package for Social Sciences (SPSS) software at 5% level of significance.

RESULTS

Yields

The powder yield (seeds to powder) obtained was 97.6% and the extract yield (powder to extract) yield obtained was 9.66%.



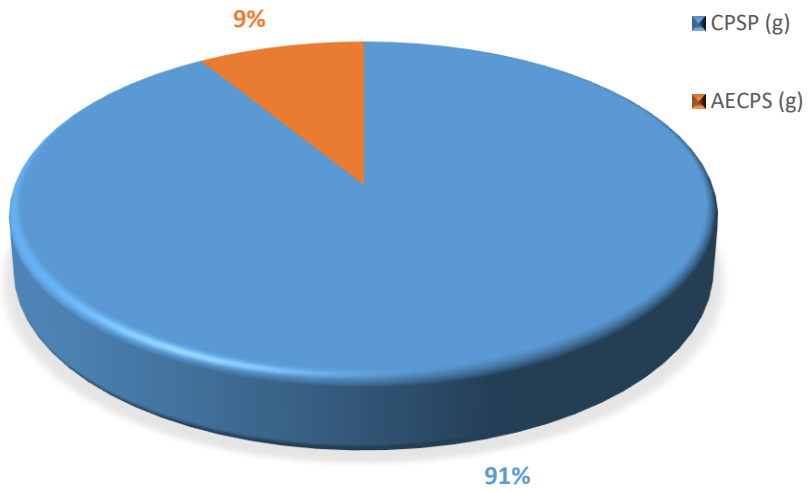


Figure 1: Percentage of powder obtained from papaya seeds **Figure 2:** Percentage of extract obtained from papaya seed powder

CPS: *Carica papaya* seeds; GCSP: *Carica papaya* seed powder; AECPS: *Carica papaya* seed aqueous extract.

Effect of the aqueous extract of *Carica papaya* seeds on parasite pressure in cattle.

Table 2: Effects of aqueous extract of *Carica papaya* seeds on the excretion of gastrointestinal parasites in cattle.

EPG/Day	D0	D5	D10	D15	D20	D25	D30
EPGNC	200.00±50.00	383.33±57.74	216.67±28.87	250.00±50.00	133.33±28.87	616.67±28.87	583.33±57.74
EPGPC	$333.33±28.87^{a}$	$116.67±28.87^{ab}$	$400.00±86.60^{bc}$	$183.33±28.87^{acd}$	$83.33±28.87^{ace}$	$750.00±50.00^{abcdef}$	$500±40,80^{abdef}$
5mg/Kg	$750.00±50.00^{a}$	$150.00±50.00^{ab}$	$100.00±50.00^{ac}$	$183.33±28.87^{ad}$	$250.00±50.00^{ace}$	$750.00±132.29^{bcde}$	$700.00±50.00^{bcde}$
50mg/Kg	$950.00±86.60^{a}$	$366.67±28.87^{ab}$	$283.33±57.74^{ac}$	$283.33±76.38^{ad}$	$150.00±50.00^{abcde}$	$783.33±76.38^{abcde}$	$750.00±50.00^{abcde}$
300mg/Kg	$300.00±50.00^{a}$	$600.00±86.60^{ab}$	$300.00±86.60^{bc}$	$333.33±76.38^{bd}$	$150.00±50.00^{abcde}$	$1016.67±76.38^{abcde}$	$933.33±76.38^{abcde}$
1000mg/Kg	$516.67±76.38^{a}$	$233.33±76.38^{ab}$	$783.33±76.38^{ab}$	$350.00±86.60^{abc}$	$100.00±50.00^{abcd}$	$433.33±76.38^{bd}$	$383.33±57.74^{abd}$

Note: The values in the table are presented as the mean ± standard deviation. The small letters compare the averages on the lines. Means containing the same letter indicate a significant difference between them at the 5% level of significance. EPGNC: number of eggs per gram of faeces of negative control, EPGPC: number of eggs per gram of faeces of positive control

Table 2 shows the variation in EPG over time as a function of *Carica papaya* dose administered to the cattle. This table shows that after treatment, EPG fell from the positive control batch to the treated batches. At a dose of 5mg/kg, these values decreased from 750.00 ± 50.00 to 100.00 ± 50.00 at D10. This phenomenon was observed at 50mg/kg dose where these EPGs fell significantly ($p<0.05$) in all 6 analytic phases from 950.00±86.60 to the lowest of 150.00±50.00 at D20. From day 25 onwards, a timid recovery in egg excretion was noted in batches treated with 5mg/kg (D0=D25), 50mg/kg (D0>D25), 300mg/kg (D0<D25) compared with the positive control whose EPG recovery was more than double (2.3 times) that of D0 from D25 onwards, from 333 to 750 respectively.

Effect of aqueous extract of *Carica papaya* seeds on the reduction of gastrointestinal parasite oviposition in cattle.

Table 3: Rate of reduction in oviposition of bovine gastrointestinal parasite after treatment with the aqueous extract of *Carica papaya* seeds

AECPS/Days	**D5**	**D10**	**D15**	**D20**	**D25**	**D30**
5mg/Kg	80,00	86,67	75,6	66,67	0,00	6,67
50mg/Kg	61,47	70,21	70,21	84,21	17,58	21,05
300mg/Kg	-100,00	0,00	-11,00	50,00	-238,67	-211
1000mg/Kg	54,84	-51,74	32,17	80,62	16,09	25,78
EPG PC	65,17	-20,12	45,05	75,08	-125,23	-50,15
The values in the table are presented as percentages.						

Doses of 5 and 50mg/kg induced a lasting reduction (up to D20) in the excretion of gastrointestinal parasite eggs in cattle. As for doses of 300, 1000 and albendazole, the reductions were not consistent.

The aqueous extract of *Carica papaya* seeds treatment efficacy.

Table 4: Time-/dose-dependent variation in treatment efficacy with the aqueous extract of *Carica papaya* seeds

AECPS/Days	**D5 (%)**	**D10 (%)**	**D15 (%)**	**D20 (%)**	**D25 (%)**	**D30 (%)**
5mg/Kg	89,56	87,65	80,48	49,87	67,53	67,98
50mg/Kg	79,88	72,42	76,17	76,26	73,24	72,92
300mg/Kg	-4,44	7,41	11,2	24,81	-9,96	-6,69
1000mg/Kg	76,42	-40,50	45,74	70,86	72,75	74,54
EPG TP	81,81	-11,22	56,04	62,52	26,87	48,49

Unlike the batch treated with albendazole, which showed only 3 levels of efficacy greater than or equal to 50% over 6 coproscopic analyses, the batches treated with 5 and 50mg/kg showed efficacy of 71 to 92% over at least 4 phases of coproscopic analysis.

Effect of the **aqueous extract of *Carica papaya* seeds on average weight gain after treatment.**

Table 5: Daily weight gain of cattle after treatment with the aqueous extract of *Carica papaya* seeds

DWG AECPS	**D5**	**D10**	**D15**	**D20**	**D25**
5mg/Kg	-1600	1600	-6800	600	1600
50mg/Kg	400	-400	-2000	-200	2000
300mg/Kg	400	200	-8200	600	3800
1000mg/Kg	1200	2000	-5000	-2800	5200
WPC	2400	800	2800	-5800	1000
WNC	4400	-400	1400	-5600	2200
The values in the table are presented as g/d (gram per day). DWG/AECPS: Daily weight gain by aqueous extract of *Carica papaya* seeds WPC: Weight of positive control; WNC: Weight of negative control					

Five days after treatment, weight gain of 1600g/d was observed in animals from batches treated with 5mg/kg of aqueous extract of *Carica papaya* seeds, then 600g/d from D15 and 1600g/d from D20. The greatest weight gain at 50mg/kg was 400g/d noted during the 5 days following treatment.

Effect of the aqueous extract of *Carica papaya* seeds on Blood Counts and Blood Cells

Table 6: Effect of the aqueous extract of *Carica papaya* seeds on the blood count and formula

AECPS	**D0**				**D5**				**D10**			
	5mg/kg	**50mg/kg**	**300mg/kg**	**1000mg/kg**	**5mg/kg**	**50mg/kg**	**300mg/kg**	**1000mg/kg**	**5mg/kg**	**50mg/kg**	**300mg/kg**	**1000mg/kg**
WBC (10^9/l)	8,33	8,77	10,57	9,37	10,07	10,23	8,80	10,23	9,60	10,30	9,53	13,27
Gran (10^3/µL)	3,50	4,23	3,52	3,67	3,70	3,67	2,93	3,60	3,57	3,20	3,18	4,93
Gran (%)	41,80	51,33	45,17	38,57	35,00	36,37	35,10	35,50	35,90	31,07	25,93	37,00
RBC (10^6/µL)	4,52	5,28	5,28	4,46	4,46	3,98	4,18	4,59	6,20	5,15	4,71	5,17
HGB (g/dL)	10,17	12,13	11,93	9,20	10,37	9,07	8,97	9,93	10,70	9,17	9,23	10,97
HCT (%)	28,20	29,00	30,07	27,90	28,00	21,20	22,20	25,90	34,93	29,17	26,37	30,80
MCV (μ^3)	62,40	61,83	55,20	61,50	61,53	53,40	53,10	56,37	63,93	56,27	55,83	59,10
MCHC-T (pg)	22,53	22,60	21,83	20,53	22,43	22,60	21,50	21,53	21,47	17,83	19,63	21,00
MCHC (g/dL)	37,13	36,73	39,77	36,77	38,57	42,50	40,80	38,37	32,30	31,97	35,43	35,80
PLT (10^3/µL)	152,33	109,67	93,67	309,67	194,67	199,33	131,33	111,67	361,67	207,00	110,33	251,67
	D15				**D20**				**D25**			
	5mg/kg	**50mg/kg**	**300mg/kg**	**1000mg/kg**	**5mg/kg**	**50mg/kg**	**300mg/kg**	**1000mg/kg**	**5mg/kg**	**50mg/kg**	**300mg/kg**	**1000mg/kg**
WBC (10^9/l)	10,27	9,07	8,67	11,23	5,50	6,00	6,17	7,40	5,97	7,83	6,63	8,43
Gran (10^3/µL)	3,33	2,77	2,89	3,83	1,77	2,47	2,06	2,93	1,77	1,83	2,21	2,20
Gran (%)	32,27	34,57	34,77	33,40	32,23	41,17	34,00	40,23	29,37	24,47	25,63	26,03
RBC (10^6/µL)	4,96	4,60	4,91	4,77	5,38	4,80	4,40	4,22	5,53	4,54	4,97	4,71
HGB (g/dL)	10,57	11,10	10,57	9,80	10,90	9,50	9,10	10,43	10,83	8,70	9,03	9,20
HCT (%)	28,43	31,10	30,93	26,83	31,87	27,33	25,13	30,67	32,73	27,40	28,07	29,03
MCV (μ^3)	57,23	60,50	62,87	56,07	59,07	57,03	56,77	58,30	58,87	60,03	56,40	61,33
MCHC-T (pg)	21,17	21,77	21,63	20,43	20,20	19,67	20,97	27,17	19,43	18,97	18,13	19,37
MCHC (g/dL)	37,07	36,20	34,73	36,53	34,30	34,63	37,27	34,10	33,13	31,73	32,37	31,67
PLT (10^3/µL)	168,00	600,00	789,67	93,00	146,00	216,33	156,00	177,00	107,67	159,33	328,33	117,67

WBC: White blood cells; Gran: Granulocytes; RBC: Red blood cells; HGB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCHC-T: Mean Corpuscular Haemoglobin Content; MCHC: Mean Corpuscular Haemoglobin Concentration; PLT: Platelets

From this table, a significant reduction in granulocytes was observed with the 4 doses: 5mg/kg (42 to 29%), 50mg/kg (51 to 24%), 300mg/kg (45 to 26%), 1000mg/kg (39 to 26%). Values outside the norm at D0 were stabilised throughout the experiment to the bovine normal range, in particular MCV (40 and 60µm3), CCMH (30 and 36%).

DISCUSSION

Yield

The yield of the aqueous extraction of *Carica papaya* seeds (9.66%) is little different from that obtained by Azando *et al* (2011) with acetone, which is 9.77% for *Zanthoxylum zanthoxyloïdes*. However, it is significantly lower than the ethanolic values obtained by Hounzanbgé-Adoté (2005) of 12.6% for *Z. zanthoxyloides*, 12.8% for *Newbouldia laevis* and by Yonwa *et al* (2020) of 16.8% for *Callistemon rigidus*. This difference could be explained by the nature of the plant material or part and the solvent used.

Effect of the aqueous extract of *Carica papaya* seeds on parasite pressure in cattle.

During handling, a significant drop ($p<0.05$) in EPG below 250 up to day 20 was observed by the smallest doses (5 and 50mg/kg). This drop is in line with the results of Hounzanbé-Adoté *et al* (2008) who showed that pawpaw seeds maintained infestation in sheep at a relatively low level. Earlier work by the same author and his colleagues in 2001 on sheep showed that pawpaw seed powder had an antiparasitic effect at an optimum dose (100 mg/kg in lambs and 200 mg/kg in adults).

The timid resumption of egg excretion from D25 would indicate that papaya seed temporarily limits their excretion or eliminates part of the worm population. These findings are identical to those of Hounzanbé-Adoté *et al* (2008) who thought that the reduction in egg excretion observed in vivo was due either to the mortality of adult worms or to a drop in the prolificacy of female worms. Alternatively, there was rapid reinfestation on pasture, and this is in line with the work of Nfi *et al* (1999) who showed that the ineffectiveness of certain plants in ruminants could be attributed to pastures constantly infested by parasites. As a result, sporadic treatment with aqueous pawpaw seed extract appears insufficient to completely eliminate gastrointestinal parasite from cattle. It is also in this sense that Fabiyi (1973) showed that the practice of systematic treatments, one or even two treatments are not sufficient to completely eliminate the parasites.

Effect of the aqueous extract of *Carica papaya* seeds on the reduction of gastrointestinal parasite laying in cattle.

After a single dose, reductions reached 86.67% for 5mg/kg and 84.21% for 50mg/kg. These results indicate that the aqueous extract of *Carica papaya* seeds significantly ($p<0.05$) reduces digestive strongyles in cattle. Indeed, these results are better than those obtained by

Hounzanbé-Adoté *et al* (2008), who found that pawpaw seeds reduced the level of sheep strongyles gastrointestinal egg excretion by 60% at D13. However, in relation to the reduction by albendazole (75.08%), the results (albendazole rate wasn't above the extract from D5 to D20) are in contradiction with those of Nguessan *et al*. (2017) according to which the reduction rates of *Annona senegalensis* organ extracts during the first days were lower than that of albendazole. Furthermore, the results are contradicted by those of Githiori *et al*. (2006) according to which herbal remedies have, in most cases, lower redcution levels for parasitism than synthetic anthelmintics *in vivo* control tests. Nevertheless, compared to the work of Minaflinou *et al*. (2015), even after three successive days of treatment their results did not reach the aqueous extract of *Carica papaya* rates, instead they achieved 79% (treatment with only *N. laevis*); 79.03% (combination of extracts from both plants in a proportion of 0.3 g + 0.3 g/kg) and 46.53% (combination of extracts from both plants in a proportion of 0.6 g + 0.6 g/kg).

Treatment efficacy (E%) with the aqueous extract of *Carica papaya* seeds.

The efficacy of albendazole was noted in 50% of all coproscopic analyses. However, the efficacy (71-92%) of the smallest single doses (5 and 50mg/kg) under conditions of hyperparasitemia (EPG>250) of the animals at D0 indicates that the aqueous extract of *Carica papaya* seeds has an antihelmintic action against bovine gastrointestinal parasite as confirmed by the work of Lohiya *et al* (2000) that pawpaw seeds can effectively kill helminths in infested pigs. In addition, the work of Kermanshai *et al*. (2001) showed that the seeds of many plants contain glucosinolates which are metabolised by myrosinases into isothiocyanates responsible for the antihelmintic action. According to this author, pawpaw seeds contain myrosinases in the endosperm and glucosinolates in their outer parts. The enzymatic activity releases benzylisothiocyante (BITC), which gives *Carica papaya* seeds their anti-parasitic properties. The results obtained here are at odds with those of Macedo *et al*. (2010), who looked at to the properties of *Eucalyptus staigeriana* and demonstrated its antihelmintic activity, but with an efficacy that does not reach the level of synthetic anthelmintics. In fact, the extract's effectiveness is thought to be due to the reduction in egg-laying worms or the disruption of the fecundity of adult female worms. It is in this sense that Minaflinou *et al*. (2015) stated that the control of gastrointestinal parasite by plants lies, among other things, in their ability to act on the viability or fertility of adult worms, to reduce egg excretion or to limit larval settlement by immobilising them or inhibiting their unsheathing thus interrupting in the life cycle of the parasites.

Effect of the aqueous extract of *Carica papaya* seeds on weight after treatment.

An increase of a few grams (400 to 1600g/d) was observed. This average daily gain (ADG) would be partly linked to the improvement in the physiological balance of the animals (establishment of erythropoiesis with stabilisation of the values of HG, MCHC, MCV, thrombocyte, . . .) and the restoration of the feeding behaviour of the animals because in the presence of the parasites, a behavioural disorder would affect the physiology of the host Chartier (2009) given that the pathogenic power of the parasites is exerted on voluntary ingestion with a reduction in ingestion of up to 20%.

Effect of the aqueous extract of *Carica papaya* seeds on Blood Counts and Blood Formulae

The significant drop ($p<0.05$) in granulocyte count observed here is thought to be related to the completion of their task. At D0, their rate was 41.80%; 51.33%; 45.17%; 38.57% respectively with doses of 5; 50; 300 1000mg/kg indicating their participation in the defences put in place by the host against the EPG≥250 parasite pressure. Their significant drop observed up to D25 (29.37%; 24.47%; 25.63%; 26.03%) would indicate that what they were mobilised for (antigen) had been reduced thanks to the aqueous extract of *Carica papaya* seeds. Drieu (2009) stated that when granulocytes fulfil their function they can disappear in a controlled and programmed manner by a mechanism known as apoptosis.

Fundamental erythrocyte constancy in the investigation of anaemia (and not the number of red blood cells), the mean globular volume was stabilised at around 58µm^3 leaving values above 63µm3 (macrocytosis) at D0. This would indicate a correction of the anaemia after treatment. This is consistent with the results of Dawson *et al*. (2000) and Cordonnier and Fontaine (2001), who found that in healthy cattle, the MCV should be between 40 and 60µm3 (normocytosis), with an average of 52µm3.

The extract also had a normochromic effect on the erythrocyte population by stabilising the mean corpuscular haemoglobin concentration (33g/1000ml). In addition, at D0, the batch that received 300mg/kg had thrombocytopenia (platelets<100*10^3/mm^3) (Meyer and Wardrop, 1991; Schalm *et al*., 2000; Dawson *et al*., 2000; Cordonnier and Fontaine, 2001) but throughout the experiment, this value rose to 131 (D5), 110 (D10), 789 (D15), 156 (D20) and 328 (D25), indicating that the animal's physiological equilibrium was restored by the aqueous extract of *Carica papaya* seeds. It is all these benefits that have led some authors to refer to the antiseptic (Iwu, 1983) or antihaemolytic (Pousset, 1981) properties of *Carica papaya*, which could prevent anaemia, whether or not linked to parasitism.

CONCLUSION

With a yield of 9.66%, the aqueous extract of papaya seeds significantly ($p<0.05$) controls the parasitic pressure of digestive strongyles on cattle. It reduced egg laying from 86.67 (5mg/kg) to 84.21% (50mg/kg). Compared with albendazole, which did not reach 50%, the efficacy of treatment with aqueous extract of papaya seeds was 71% for 5mg/kg and 92% for 50mg/kg. The significant drop ($p<0.05$) in the granulocyte reduction rate from 41.80% to 29.37%; from 51.33% to 24.47%; from 45.17% to 25.63%; from 38.57% to 26.03% respectively with doses of 5; 50; 300; 1000mg/kg indicates their participation in the defences put in place by the host against OPG≥250 parasite pressure.

ACKNOWLEDGEMENTS

We would like to thank the senior management of the Institute of Agricultural Research for Development for making the animal material available to us, as well as the entire team of the animal health section and the bovine programme of the Wakwa Agricultural Research Centre, Ngaoundere, who kindly participated in carrying out this work.

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