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МАТЕРИАЛЫ

МЕЖДУНАРОДНОЙ НАУЧНО-ПРАКТИЧЕСКОЙ КОНФЕРЕНЦИИ «СКРЯБИНСКИЕ ЧТЕНИЯ», ПРОВОДИМЫХ СОГЛАСНО ПЛАНУ НАУЧНЫХ МЕРОПРИЯТИЙ В РАМКАХ ДЕСЯТИЛЕТИЯ НАУКИ И ТЕХНОЛОГИЙ И ПОСВЯЩЁННЫХ ПРАЗДНОВАНИЮ 300-ЛЕТИЯ РОССИЙСКОЙ АКАДЕМИИ НАУК

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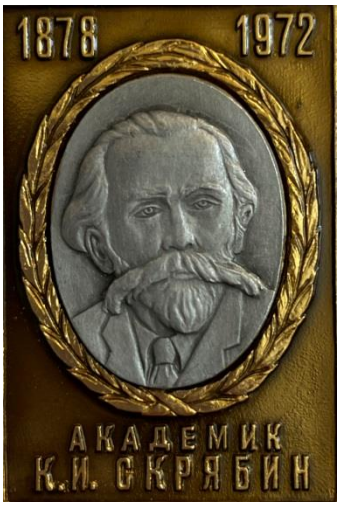

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Оглавление

Таким образом, сохраняя традиции К.И. Скрябина, кафедра живет современной, полной событий жизнью, пользуется заслуженным авторитетом в области паразитологии и всей ветеринарной науки.

ANTICOCCIDIAL EFFECTS OF *CALOTROPIS PROCERA* POWDER ON COCCIDIOSIS OF RABBITS IN STATIONARY REARING

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Abstract

The problem of diarrhea in rabbit farms is a major concern for breeders, who are often powerless to deal with this plague.

Diarrhea and coccidiosis are two diseases in rabbits that are very much feared by breeders because they lead to high mortality in young rabbits, which can reach 90 to 100% of the population. The present study mainly aims to valorize medicinal plants at a lower cost in the farms and to contribute to the improvement of rabbit farming practices in Benin.

Our study allowed, with the help of the extraction of the latex extract, to obtain from a volume of 42 cl of latex, a total weight of 396 g of *Calotropis procera* extract with a yield of 31.42%.

Coprological analyses to establish lots of infested animals resulted in a 90% rate of infested animals. With infestation intensities varying between 500 and 6000 eggs per gram of excrement. The experimental treatment involved 100 rabbits, 25 of which were not infested, at doses of 0.5, 0.9, and 1.3 g of *Calotropis procera* extract per kg of body weight. The 0.5g/kg dose administered to the animals showed that at D4 the infested animals were not cured and 100% mortality rates were recorded in lots $2000 \geq OPG < 5000$ and $OPG \geq 5000$. The 0.9g/kg dose induced a decrease in parasite load recorded at D4 in all lots with a mortality rate of 44% in all 25 animals receiving this dose and especially in lots $2000 \geq OPG < 4000$ and $OPG \geq 5000$. The best therapeutic effects were obtained with the administration of dose d1.3 which induced a significant reduction ($P < 0.05$) in oocysts in all subjects at D5 with 4% mortality ($p < 0.01$).

Depending on the determination of the toxic dose of *Calotropis procera* for rabbits, this plant with anticoccidial virtue may well be an alternative solution in the control of coccidiosis in rabbits. It could also slow down the reduction of zootechnical performances and the important mortality of rabbits (90 to 100% of the population)

induced by coccidiosis, which explains the considerable economic losses that it generates in this sector.

Keywords: Rabbits, Coccidiosis, Pulverulent, *Calotropis procera*, Benin

INTRODUCTION

Coccidiosis is the main cause of parasitic diarrhea in young people over 3 weeks of age. It is caused by parasites called Eimeria. Most often, coccidiosis remains subclinical, i.e. the affected animal does not show any clinical signs and in particular no diarrhoea. However, subclinical coccidiosis can interfere with animal performance. Clinical coccidiosis is sporadic and manifests itself by diarrhoea of increasing severity: initially serous and dark green, it becomes blackish, more or less associated with blood clots, colic and pain on defecation. If the diarrhoea degenerates, in the terminal phase, there is dehydration, a sharp drop in general condition and death in over 90% of cases.

The breeding of short-cycle animal species (rabbits, poultry, aulacodes, etc.) in particular, whose meat is the most widely used in human nutrition, is seen as a solution to the ever-increasing importation of frozen meat products and to the problem of malnutrition faced by rural African populations.

Livestock production is the country's second most important agricultural activity after crop production and contributes 6% to GDP (APRM, 2006).

Benin is located in a tropical climate zone characterised by high temperatures and more or less regular rainfall grouped over several months of the year. These climatic factors influence the establishment and evolution of protozoan parasites, particularly in rabbits.

Rabbits are mainly infested by coccidia, trichures, strongyles and sometimes cestodes. Apart from sulphonamide-based products, farmers frequently used papaya seeds in dried and crushed form or whole and fresh as a means of controlling animal parasites before their effectiveness was scientifically tested in Benin (Sacramento T. I. et al, 2010).

Surveys carried out in southern Benin revealed that 67% of farms had widespread acute diseases characterised by sudden death and digestive symptoms (Adjahoutonon, 2005).

Because of the overall zootechnical and economic losses caused by coccidiosis in rabbits and the public health problem caused by the use of synthetic products in the fight against animal diseases in general and coccidiosis in rabbits in particular, it has become imperative to carry out a rational and ecological fight against these diseases.

At present, treatments based on medicinal plants are not widely used because of the lack of scientific evidence to confirm or refute their efficacy, and there is little analysis of the mechanisms of action of the active principles of these plants (Kasonia K. et al., 1993). Some research work has shown that the parts of Calotropis procera (roots, leaves, stems, juices) are particularly used in West Africa in the treatment of numerous ailments, notably cardiovascular and digestive system diseases, rheumatic pains, and ailments, burns and wounds in humans (Adjanohoun E. L., 1980; Ake Assi L., 1991). Other work carried out on aulacod coccidiosis has also shown that Calotropis procera is an alternative in the fight against coccidiosis. The results obtained inspired us to evaluate the efficacy of *Calotropis procera* latex extract on coccidiosis in rabbits.

MATERIALS AND METHODS

Study setting

The present study was carried out in the Laboratory of Ethnopharmacology and Animal Health under the label (LESA) created in 2007 in the Department of Animal Production (DPA) of the Faculty of Agronomic Sciences (FSA) of the University of Abomey-Calavi (UAC).

For several years, the laboratory has been focusing its work on animal pathologies in tropical zones, as well as on the use of medicinal plants from biodiversity as a new source of treatment, and the mechanisms of action of bioactive plants.

Methodology

The study was conducted in three stages:

- a first step which is the in vivo infestation phase of rabbits with coccidia oocysts;
- a second step devoted to the extraction of the *Calotropis procera* powder under laboratory conditions;
- a third step devoted to the administration of *Calotropis procera* powder to experimentally infested rabbits at the Laboratory of Ethnopharmacology and Animal Health (LESA) of the Faculty of Agricultural Sciences (FSA) of the University of Abomey-Calavi (UAC).

Experimental set-up

The plant material was essentially *Calotropis procera* latex. First, we harvested the latex from the stem and fruits of the plant. To do this:

- We provided ourselves with three (03) test tubes and a slope.
- We wore gloves to avoid direct contact with the product, given its toxicity. Once a sufficient quantity of latex had been collected, the tubes were hermetically sealed and placed in a cooler. This precaution was taken to avoid coagulation of the latex. The cooler was then transported to the laboratory and the latex was stored in the refrigerator for 48 hours at a temperature of 4°C.

To get the latex extract we diluted 14 cL of crude latex in 140 cL of distilled water in a volumetric flask. This proportion was chosen to take into account the usual dilution method (50 mL for 500 mL of solvent (distilled water).

Once the dilution was made, the mixture was shaken to allow homogenization of the product.

The product was then filtered using a composite device (cotton, Erlenmeyer, and sintered glass).

The filtrate obtained was put in the water bath conditions of the Rotavapor for 1 hour at a temperature of 80°C.

The new filtrate obtained was introduced into an oven for seven days at a temperature of 60°C. After this time the dry extract was collected and weighed.

The extraction of the *Calotropis procera* latex extract was carried out over eight days.

1. Rabbit infestation protocol

In order to be sure that the purchased animals were apparently healthy, their droppings were subjected to a coprological analysis.

Then, the viscera of rabbits from the slaughter of rabbits for trade were purchased for a sampling of the intestinal contents. Once the oocysts were found in the intestinal contents following the coprological results, the in vivo infestation of the rabbits on the station was carried out.
An infestation of 75 apparently healthy rabbits on the station was carried out from the intestinal contents collected from affected rabbits.
A further 25 rabbits were kept uninfested to serve as controls.
For this purpose, we mixed these intestinal contents with the meal of the rabbits to be infested. Coprological examinations allowed us to obtain a 100% infestation rate with variable infestation intensities.
The animal material consisted of 100 rabbits from farms located in the commune of Abomey-Calavi. These animals had an average initial weight of 0.535 kg.
Once in the station, the young rabbits were placed in communal boxes after a two-week sanitary vacuum had been observed. Their diet consisted of *Panicum maximum* var c1 fodder and a feed supplement consisting of industrially produced pelleted feed.
The adaptation period lasted two weeks.
The rabbits were divided into 4 lots containing 5 subgroups of 25 rabbits each in a completely randomized block set-up.
Each lot was divided into five groups of five rabbits according to their level of fecal excretion.
Excretion levels were $500 \geq OPG < 1000$; $1000 \geq OPG < 2000$; $2000 \geq OPG < 4000$; $OPG \geq 4000$; $OPG = 000$.
Each lot consisted of 4 treatments with 5 rabbits per treatment.
The 4 treatments of the same lot were in the same block and distributed in different boxes.
The four treatments corresponded to three doses of *Calotropis procera* and one untreated dose.
The doses d0.5, d0.9; d1.3 corresponded to 0.5; 0.9, and 1.3 mL/Kg (PV) of *Calotropis procera* latex extract administered respectively.
The dose d0.0 corresponded to no product treatment dose.
The per os administration of the latex extract lasted five days. It started on day 14 after the infestation of the rabbits.
Post-treatment check-up in a fortnight.

a. Data collection

The experiment itself was conducted over 6 weeks.
During the experiment, feed distribution and collection of feed refusals (leftover and wasted feed) were done daily.
Weighing the animals before and at the end of the infestation (D14) allowed to evaluate the weight variation during the infestation and the amount of latex extract dose to be administered during the treatment.
Dung collection and coprology were performed three times a week.
Thus, the coprological analysis method used was the quantitative method based on the Mini-Flotac technique developed by Cringoli et al (2010).

This method is based on the use of the Fill-Flotac (collector allowing the homogenisation of the droppings in the flotation liquid and the filtration of the homogenate) and the Mini-Flotac (reading disc comprising 2 chambers of 1 ml), the upper part of which rotates for microscopic observation, leaving only a very thin film to be observed, which offered an easy reading.
The procedure was as follows:
- 18 ml of a saturated saline solution (1200) 1:10 dilution ratio was added to the Fill-Flotac;
- the conical manifold of the Fill-Flotac was filled with a 2 g sample of fresh poop and homogenised;
- Using the filling holes the two flotation chambers were filled with the faecal suspension until a small meniscus was formed. In order to avoid air bubbles, the chambers were filled with the Mini-Flotac device held at an angle;
- After 10 minutes, the spanner was used to rotate the disc
- the Mini-Flotac was placed on the microscope slide with the adapter for egg counting.
The results of this coprological analysis are expressed as Eggs Per Gram of droppings (OPG) where an OPG of 5 is equivalent to one egg contained in the Mini-Flotac cell when counting in both chambers and 10 when counting in one chamber.
The animals were necropsied at D14 of infestation and at D5 of treatment to identify any gross lesions. It consisted of placing the animal in dorsal recumbency and fixing it on a board. The skin was incised along defined cut lines, then the planes, taking care not to damage the internal organs. The thoracic cavity was opened. The intra-thoracic and intra-abdominal organs were then examined. The gastrointestinal tract, heart, liver, and lungs were examined in detail.

b. Statistical analysis of the data

Descriptive statistical analyses in terms of mean and standard deviation were used to calculate the weight variation and Eggs per Gram of feces (EPF) data.
R software was used to calculate and compare the results obtained from ANOVA1.
Excel 2010 was used to design the graphs and tables.

RESULTS

Table 1 presents the various results from the extraction process of the C. procera latex extract.

Table 1 : Extraction results

	Tests			
Plants	1	2	3	Productivity%
C. procera	138 g	130 g	128 g	31,42

In fact, on the 42 cl of extracted latex, a mass of 396 g was obtained with a yield of 31.42% of the extracts.
The highest mass of extracted C. procera latex powder was obtained in the first test (138 g) and the other two tests yielded 130 g and 128 g respectively.

Dynamics of the infestation rate of the animals according to the Period

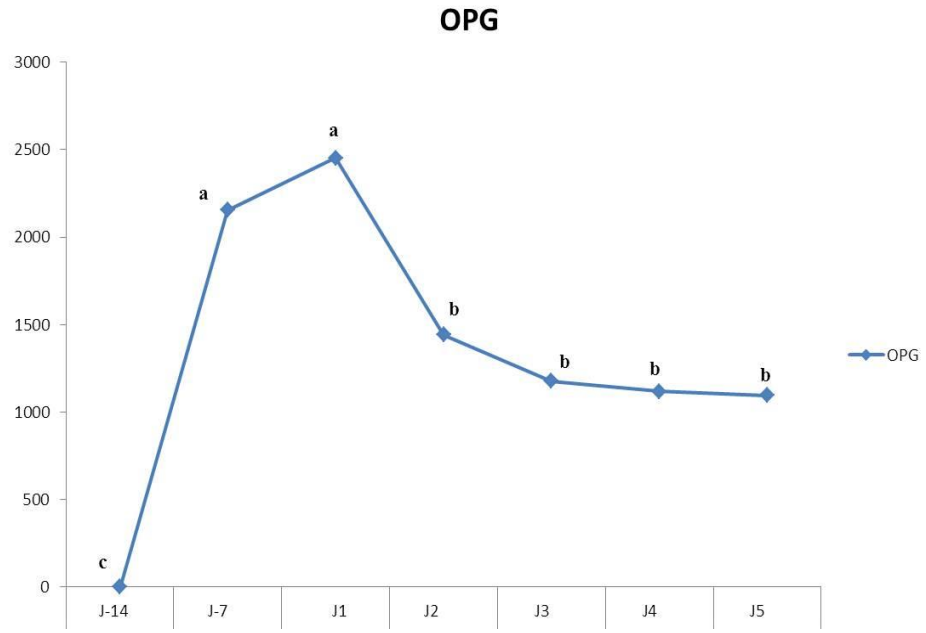

Figure 1: Infestation rate of animals over a 19-day period
NB: OPG = Eggs per Gram of faeces; J= day

At the beginning of the experiment, the coccidia infestation rate was 0%.
One week later, the rate increased significantly ($p < 0.05$) on D-7 (82.66%) and D-1 (100%), (Figure 15). On the other hand, on the second day after the first treatment (D1) with the aqueous extract of the C. procera latex powder, a significant decrease ($p < 0.05$) in the infestation rate was noted. This decrease in infestation rate was continuous until Day 5 with the last four treatments (D2, D3, D4, and D5) but the difference was only significant at ($p > 0.05$) (Figure 1).
Quantitative coproscopy of the 100 rabbits gave an infestation rate of 75%.

Effect of different treatments on fecal egg excretion in rabbits
The results of the experimental group were used to establish a treatment plan, the results of which are presented in Table 2:

Table 2: Treatments

N°	Dose	Number of rabbits receiving treatment	Number of cured rabbits	Cure rate (%)
1	d0,5	23	0	0
2	d0,9	24	14	58,33
3	d1,3	24	22	91,66
Totals		71	36	-
Average cure rate				50,70

From this table, it appears that the rabbits that received the d0.5 dose did not show any improvement in their health status and the cure rate was 0%. However, the rabbits that

received the d0.9 and d1.3 doses showed a cure rate of 58.33% and 91.66% respectively.

Table 3: Variation of OPG according to treatments

Lots	Doses (g/kg pv)	OPG (average - standard deviation)	Number of dead animals over 5 per subgroup	Mortality rate, %
500 ≥ OPG < 1000	d0,0	547,5714±46,59740 c	1	20
1000 ≥ OPG <2000		1101,4286±101,28431 c	2	40
2000 ≥ OPG < 4000		2596,1429±213,83582 b	5	100
OPG ≥ 4000		5107,5714±466,88343 a	5	100
OPG= 000		0,0000±0,00000 d	0	0
500 ≥ OPG < 1000	d0,5	731,5714±62,89339 d	0	0
1000 ≥ OPG < 2000		1352,2857±111,54913 c	1	20
2000 ≥ OPG < 4000		2729,8571±216,26815 b	5	100
OPG ≥ 4000		5914,2857±537,85007 a	5	100
OPG= 000		0,0000±0,00000 e	0	0
500 ≥ OPG < 1000	d0,9	190,2857±52,57605 c	2	40
1000 ≥ OPG < 2000		476,8571±102,93897 b	3	60
2000 ≥ OPG < 4000		1163,8571±230,74660 b	3	60
OPG ≥ 4000		2509,8571±469,42086 a	4	80
OPG= 000		0,0000±0,00000 d	0	0
500 ≥ OPG < 1000	d1,3	263,4286±77,85970 c	0	0
1000 ≥ OPG <2000		514,4286±145,81526 b	0	0
2000 ≥ OPG < 4000		1170,5714±293,05023 b	0	0
OPG ≥ 4000		2575,5714±554,37658 a	1	20
OPG= 000		0,0000±0,00000 d	0	0

DISCUSSION

- Evolution of the infestation rate of the animals according to the period

The quantitative coproscopy carried out on the 100 rabbits gave an infestation rate of 75% for coccidial oocysts. These results differ from those of Adjahoutonon K., (2005) who obtained 72.73% as the infestation rate for coccidial oocysts in aulacodes as well as those of Coulibaly P. O., (2006) with 67% infestation rate. This diagnosis revealed a predominance of coccidia in rabbits. Coccidia infestation seems to start when hygiene rules are not followed in rabbit houses; in this case, the infestation rate increases as pointed out by Tamegnon A. (2001).

Coccidia are present all year round in tropical regions. Indeed, in the south of Benin, we have an abundance of water points and vegetation cover. The problem is mainly ecological because these areas are favorable to the pullulation of the parasites. Thus, the ambient temperatures favor the sporulation of oocysts: excessive humidity, lack of ventilation, overcrowding of premises, not very good quality of food (Bodji N. C. et al, 2007). Coccidiosis is widespread and is recorded in several animal species. The results of the work conducted by Abé C. R., 2009 who found Eimeria sp. oocysts in the droppings of aulacodes in the District of Abidjan, Côte d'Ivoire confirm this hypothesis. Moreover, lambs and kids are also very affected by coccidiosis, sometimes with a very contagious aspect (often more than 30% of the young affected).

Effect of different treatments on faecal egg excretion in young rabbits

With a high infestation rate and at the dose d0.0 administered, we can record a high mortality rate but this is not the case with low infestation rates.

The results of our work showed that the d0.5 dose had no therapeutic effect on coccidia (demarcation dose) and recorded the same figures as in the untreated batches with the d0.0 dose. These results then show that there is no therapeutic action of the plant on coccidia at the dose of 0.5 ml/kg live weight.

The results at dose d0.9 indicated a therapeutic effect but not optimal because of the mortality cases recorded.

On the other hand, the results obtained at dose d1.3 could mean that C. procera latex has a very optimal effect on coccidia and that the effect of this dose was observed at D4 in all infested subjects. On day 4, a 100% cure rate was not recorded, which could lead to an increase of the dose or a prolongation of the treatment duration.

We tried to increase the dose to d1.5 and to continue the treatment until day D6. However, we found that the rabbits abandoned the medicated feed, which no longer improved their condition. This justifies the use of d1.3 as the optimal dose and D4 as the optimal duration of treatment.

In sum, the d1.3 dose has a greater therapeutic effect on coccidia than the d0.9 dose.

The mortality cases recorded at dose d1.3 were due to the various controls performed.

Our results confirm the idea of Nacoulma O. G., (1996) who stressed that latex has a therapeutic action against protozoa.

It should be noted that we did not determine a toxic dose during our work.

Moreover, the deterioration of zootechnical performance and the importance of mortality (80 to 100% of the stock) induced by coccidiosis, mentioned by Buldgen A.

(1996), explain the considerable economic losses caused by this disease that we experienced.

Conclusion

Our study, which is in line to contribute to the improvement of breeding and sanitary conditions of rabbits, focused on the use of Calotropis procera latex as an anticoccidial agent in this species in the Republic of Benin.

These observations revealed the presence of Coccidia oocysts. These oocysts were found in 100% of all subjects.

In sum, it should be said that rabbits are very susceptible to coccidiosis and that the latex of C. procera can be recommended as an alternative method in the control of coccidiosis using a dose of 1.3 mL/Kg of live weight for four days. However, it seems necessary that further in-depth research be undertaken to determine the precise dose that would achieve 100% cure and to assess the total toxicity of the latex before it can be recommended as a drug for the control of this disease. In view of this imperative, it is recommended that farmers apply the various health prophylaxis programmes according to the climate and rainy season.

At the end of our study, it would be desirable to envisage a more extensive study on the whole national territory, this time by carrying out work on coccidiosis of other animal species including poultry.

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РАСПРОСТРАНЕННОСТЬ КНЕМИДОКОПТОЗА КУР В РАЗНЫХ ГЕОГРАФИЧЕСКИХ РЕГИОНАХ РЕСПУБЛИКИ АРМЕНИЯ

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Аннотация

В последние годы развитию птицеводства в Республике Армения мешает ряд инвазионных заболеваний, среди которых наиболее распространенным является