Sjournals
Scientific Journals

Scientific Journal of Veterinary Advances (2018) 7(11) 254-262
ISSN 2322-1879
doi: 10.14196/sjva.v7i11.2608

CODEN (USA): SJVAAS

Contents lists available at Sjournals
Scientific Journal of **Veterinary Advances**
Journal homepage: www.sjournals.com

Original article

Valuation of anticoccidial activity of pulverulent extracted from Calotropis procera against coccidial infection in grasscutters

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ARTICLE INFO

Article history,
Received 17 October 2018
Accepted 15 November 2018
Available online 22 November 2018
iThenticate screening 19 October 2018
English editing 14 November 2018
Quality control 21 November 2018

Keywords,
Grasscutters
Coccidiosis
Calotropis procera
Pulverulent
Benin

ABSTRACT

Coccidiosis is a disease that is highly feared by breeders because it involves a high mortality rate among young people, which can reach 80 to 100% of the population. This work aims essentially to valorize medicinal plants at lower cost in the farms and to contribute to the improvement of breeding practices of grasscutters in Benin. The present work has made it possible, using 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%. The coprological analyzes to constitute the batches of the infested animals made it possible to record a rate of animals infested with 80%. With varying infestations between 500 and 4000 eggs per gram of droppings. The experimental treatment concerned 100 grasscutters, which are divided to four equal groups. All 25 grasscutters of group 4 were not infested, Those in groups 1-3 were medicated with 0.5, 0.9 and 1.3 g extract/kg body weight. The oocyst counts and mortality rates were recorded to evaluate the anticoccidial efficacy. According to our results, the 0.5 g/kg dose administered to the subjects showed that on day 5 the infested

individuals were not cured and 100% mortality was recorded in the 2000 groups ≥ EPG <4000 and EPG ≥ 4000. The 0.9 g/kg dose induced a decrease in the parasite load recorded on D5 in all the batches with a mortality rate of 44% for all 25 animals that received this dose and especially for the 2000 lot level ≥ EPG <4000 and EPG ≥ 4000. The best therapeutic effects were obtained with the administration of the dose d1,3 which induced a significant reduction (P<0,05) of oocysts in all subjects on day 4 with 4% of cases mortality (p<0.01).

1. Introduction

The domestication of game in general and the breeding of wild animal species (birds, mammals, reptiles, etc.) in particular, are essentially to fill a proteinic deficit in human and animal nutrition (Yewadan, 1992; Abé, 2009). In many countries, such as the USA, they are reared for recreation and the commercial product is a live animal. The meat are the most used in the human diet, is conceived as a complement to traditional breeding of domestic animals and is one of the solutions to the problem of malnutrition, which is faced by African rural population (Mensah et al., 2007). Livestock production is the country's second largest agricultural activity after crop production. It contributes 6% of GDP (Coudert, 2006). Benin is in an area of tropical climate characterized by high temperatures and more or less regular rains grouped over a few months of the year. These climatic factors condition the establishment and evolution of protozoan parasites in grasscutters (Mensah, 1992).

The grasscutter is one of the most popular game in West Africa, where an estimated 80 million animals are slaughtered each year, or about 300,000 tons of meat (Mensah, 1990; Fantodji et Mensah, 2000). The aroma, the flavor, the tenderness of the meat, make it a sought-after dish, paid at a high price in the big urban centers (Lawani, 1989). There are many ways to hunt this game: bushfires, traps, poison baits. Some of them are at the origin of serious plagues (desertification, famine, extinction of some animal and vegetable species, death of man). In addition, game is an important part of animal protein intake in many African countries (Asibey and Child, 1991; Malaisse, 1997). Among the animals whose scarcity does not pass unnoticed is the grasscutter (Thryonomys swinderianus Temminck, 1827). The grasscutter alone accounts for nearly 60% of offal. The grasscutter is mostly infested with Coccidia, whipworms, strongyles and sometimes cestodes. Coccidiosis is an important cause of mortality in llamas and alpacas (Kierszenbaum, 2006).

There are any uncertainties concerning the treatment of coccidiosis in grasscutters and early literature not easily accessible. Apart from products containing sulphonamides, breeders very frequently used papaya seeds in dried and milled form or whole and fresh as a dewormer, before their efficacy has been scientifically tested in Benin (Sacramento et al., 2010). Surveys carried out in southern Benin, revealed in 67% of the farms, the presence of widespread diseases, of acute evolution, characterized by sudden deaths and digestive symptoms (Adjahoutonon, 2005). Because of the overall zootechnical losses caused by Coccidia, it has become imperative to carry out a rational fight against this pathology (Youn and Noh, 2001).

Economic losses caused by coccidiosis as a general principle are enormous. Avian coccidiosis, including decreased productivity, usage of coccidiostat, and vaccines, caused an economic losses are estimated to amount to $3 billion annually (Dalloul and Lillehoj, 2006; Lai et al., 2011; Williams, 1999; Zhang et al., 2012b; Michels et al., 2011). But the emergence of problems related to drug resistance and drug residues of antibiotics in the chicken meat has stimulated us to seek safer and more efficacious alternative control strategies (Allen, 2007; Lai et al., 2011). At present, treatments based on medicinal plants are not valued because of the lack of a proven scientific approach that can invalidate or confirm their effectiveness and the analysis of the mechanisms of action of the active ingredients of these plants remains sparse (Kasonia et al., 1993; Kerharo and Adam, 1974; Kumar, 1994). Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in

combination to treat or to diagnose and prevent illnesses or maintain well-being (Adjanohoun and Ake Assi, 1979; Le, 1997).

Calotropis procera is found in Africa, north and south of the Sahara, from Senegal to the Central African Republic, in East Africa, India, Pakistan, Latin America and the Caribbean. Its distribution is irregular, locally common and gregarious (Arbonnier, 2002). In West of Africa, per os aqueous decoction leaves are indicated for the treatment of cough, filariasis and anasarca. In case of toothache, the latex is applied locally to the diseased tooth. Root powder is recommended for women with obstructed labor. The infusion and decoction of the leaves are used respectively in the treatment of arterial hypertension and edema, external use, the use of latex is constant as antiseptic and sedative (Arbonnier, 2002; Muthu et al., 2006).

Its use in India in the treatment of skin diseases such as severe bullous dermatitis sometimes leads to hypertrophic scarring (Muthu et al., 2006). In addition, this plant is used in case of abortion, as anthelmintic, against colic, cough, whooping cough, dysentery, headache, in the treatment of rope, jaundice, sore gums and mouth, infertility, swelling and ulcers (Adjanohoun et al., 1989). The leaves, barks and roots are used to combat various ailments on the Mossi plateau: rheumatic pains, headaches, diarrhea, syphilis, epilepsy, dermatitis, asthma etc. (Parvais, 2000). The latex used externally is antiseptic, healing, anti-filariasis and anti-protozoan (Nacoulma, 1996).

Overwise, research has shown that parts of Calotropis procera (roots, leaves, stems, juices) are particularly used in West Africa in the treatment of many conditions including cardiovascular and digestive diseases, rheumatic pains and diseases, burns and wounds (Adjanohoun, 1980; Ake Assi, 1991; Arbonnier, 2002; Koudougou, 2004). These results lead us to verify if there is an effectiveness of the extract of the latex of Calotropis procera on the coccidiosis of the grasscutters.

2. Materials and methods

2.1. Field of study

The latex of Calotropis procera was harvest from the stem and fruits of the plant in the commune of Abomey-Calaviin Atlantic Department. The experimental phase took place at the Laboratory of Ethnopharmacology and Animal Health. The Laboratory of Ethnopharmacology and Animal Health under the label (LESA) which is created since 2007 in the Department of Animal Production (DPA) of the Faculty of Agricultural Sciences (FSA) of the University of Abomey-Calavi (UAC). In recent years, the laboratory has focused its work on animal diseases in the tropics as well as the valorization of medicinal plants of biodiversity as a new source of treatment, the mechanisms of action of bioactive plants.

2.2. Methodology

The study was conducted in two stages: a phase of *in vivo* infestation of grasscutters with coccidia, a phase devoted to the use of powder on grasscutters and an analysis phase at the Laboratory of Ethnopharmacology and Animal Health. (LESA) of the Faculty of Agricultural Sciences (FSA) of the University of Abomey-Calavi (UAC).

2.3. Experimental apparatus

The plant material consists essentially of the latex of Calotropis procera. At first we proceeded to harvest the latex from the stem and fruits of the plant. To do this:

- We have three (03) test tubes and a hill.
- We have worn gloves to avoid direct contact with the product due to its toxicity. Once a sufficient amount of latex was removed, the tubes were sealed and put in a cooler. This precaution was taken to avoid coagulation of the latex. Then the cooler was sent to the laboratory and the latex was stored in the refrigerator for 48 hours at a temperature of 4 °C.
- For extract the latex extract, we diluted 14 µl of crude latex in 140 µl of distilled water in a volumetric flask. This proportion was selected taking into account the usual dilution method (50 mL for 500 mL of solvent (distilled water).
- After dilution, the mixture was stirred to homogenize the product.
- Then we filtered the product using a composite device (cotton, Erlenmeyer and sintered glass).

- The filtrate obtained was placed under the conditions of the Rotavapor water bath 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.
- Extraction of the extract of the latex of Calotropis procera is done in eight days.

2.4. Grasscutter infestation procedure

To be certain that the purchased animals are apparently healthy, their poop has been subjected to a coprological analysis. Then, it was a question of buying the viscera grasscutter from poaching for purposes of intestinal content. Once the oocysts were found in the intestinal contents from the results of coprology, the *in vivo* infestation of the animals in station was carried out. In the station, an infestation of 75 apparently healthy animals from intestinal contents taken from poached animals. Twenty-five (25) other non-infested animals were kept for control, to do this, we mixed this intestinal content with the meals of the animals to be infested. A coprological test every 48h allowed us to obtain 100% infestation rate.

2.5. Experimental method

The animal material consists of 100 grasscutters from farms located in the commune of Abomey - calavi. These animals had an initial average weight of 1.732 kg. Once in the station, the animals were put in communal lodges after observing a crawl space of two weeks. Their diet consisted of fodder Panicum maximum var c1. The adaptation period lasted two weeks. The animals were divided into 4 batches containing 5 subgroups of 25 grasscutters each according to a completely randomized experimental block device. Each batch in five groups of five grasscutters following their level of faecal excretion. Excretion levels were 500 ≥ EPG <1000; 1000 ≥ EPG <2000; 2000 ≥ EPG <4000; EPG ≥ 4000; EPG = 000. Each batch consisted of 4 treatments with 5 grasscutters per treatment. The 4 treatments of the same batch were in the same block distributed in different boxes. The four treatments corresponded to three doses of calotropisprocera latex and one untreated dose. D0.5 doses, d0.9; d1.3 corresponded to 0.5, 0.9 and 1.3 mL/kg body weight, respectively, of the administered Calotropis procera latex extract. The dose of d0.0 corresponded to no dose of treatment with a product. Oral administration of the latex extract lasted five days. It started on the 14th day after infestation of animals. Post-treatment follow-up in two weeks at.

Collection of data: The experiment was conducted over 6 weeks. During this period, the distribution of fodder and the collection of food refusals (food and wasted food) were done daily. Animal weightings performed before and at the end of the infestation (day 14) made it possible to evaluate the variation of the weight during the infestation and the amount of dose of latex extract to be administered during the treatment.

2.6. Collect of the data

The collection of dung and coprology are done three times a week. Thus, the method of coprological analysis used is the quantitative method based on the technique of Mini-Flotac developed by Cringoli et al. (2010) and Holdsworth et al. (2004). This method is based on the use of Fill-Flotac (collector for homogenization of dung in the flotation liquid and filtration of the homogenate) and Mini-Flotac (reading disc comprising 2 chambers of 1 ml) whose the upper part rotates for microscopic observation, leaving only a very thin film to be observed, which greatly increases the reading comfort. The procedure was as follows:

- 18 ml of a saturated saline density solution (1200) 1: 10 dilution ratio was added in the Fill-Flotac;
- The conical collector of the Fill-Flotac was filled with a sample of 2 g of fresh droppings and then homogenized;
- Using the filling holes the two flotation chambers were filled with the fecal suspension until a small meniscus was formed. In order to avoid the formation of air bubbles, the chambers were filled with the Mini-Flotac apparatus maintained on a slope;
- After 10 minutes, the key was used to spin the disc
- Equipped with the adapter the Mini-Flotac was placed on the door object of the microscope for counting eggs.

The results of this coprology analysis are also expressed in Eggs Per Gram (EPG) where an EPG of 5 equals one egg contained in the Mini-Flotac cell counting in both chambers and 10 counting in a single chamber.

2.7. Statistical analysis of the data

Descriptive statistical analyzes in terms of mean and standard deviation were used to calculate weight variation and egg data per gram of faeces (EPG). The R software allowed us to compute and compare statistical analyzes from ANOVA1 criteria. Excel 2010 software allowed us to design charts and tables.

3. Results

3.1. Extraction results

Table 1 highlights the various results from the extraction process of the C. procera latex extract. Indeed, 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 extract mass of C. procera latex powder was observed on the first test (138 g) and the other two tests gave 130 g and 128 g, respectively.

Table 1
Extraction result.

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

3.2. Dynamics of animal infestation rate by period

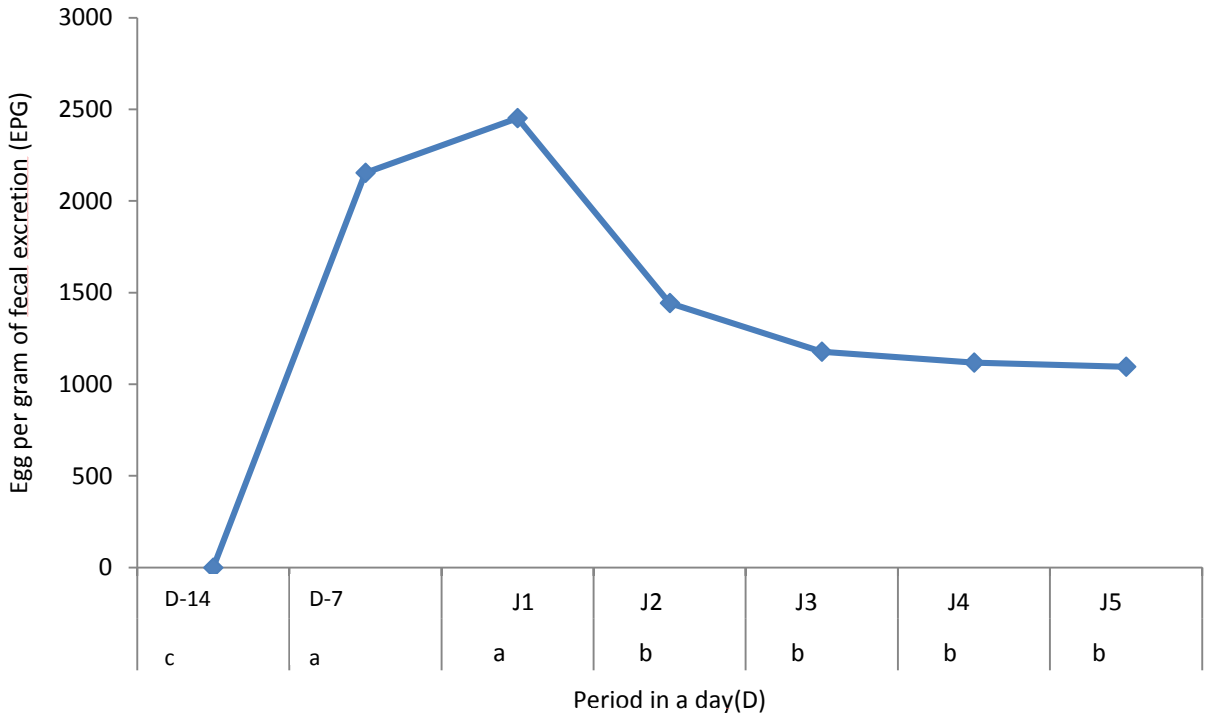

Fig. 1. Infestation Rate for nineteen days.

At the beginning of the experiment, the rate of coccidiosis infestation with grasscutters was 0%. One week later, the rate increased significantly ($p<0.05$) on Day 7 (82.66%) and Day 1 (100%) (Figure 1). On the other hand, the second day after the first treatment (J1) with the aqueous extract of the C. procera latex powder, a significant decrease ($p<0.05$) of the infestation rate was noted. This decrease in infestation rate was continuous up to Day 5 with the last four treatments (D2, D3, D4 and D5) but the difference was significant only ($p>0.05$) (Figure 1).

Quantitative coproscopy performed on the 100 grasscutters, gave a rate of infestation of 75% for coccidian oocysts.

3.3. Effect of different treatments on faecal excretion of eggs in grasscutters

The results of the constitution of the experimental group made it possible to establish a therapeutic scheme and the results of which are presented in Table 2.

Table 2
EPG changes according to treatment.

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

From this table, it appears that the subjects who received the dose of 0.5 did not experience a cure with a cure rate of 0%. However, subjects receiving doses of 0.9 and d1.3 recorded healing cases of 15 and 23 subjects with a cure rate of 68.18 and 95.83%, respectively.

Table 3
Treatment.

N°	Dose (g/kg b.wg)	Number of grasscutters submissiv to treatment	Number of grasscutters return to health	Recovery rate (%)
1	d0,5	21	0	0
2	d0,9	22	15	68,18
3	d1,3	24	23	95,83
Total		67	38	-
Average rate				56,71

4. Discussion

Quantitative coproscopy performed on the 100 grasscutters, gave a rate of infestation of 75% for coccidian oocysts. Compared with the results of over researchers as Adjahoutonon (2005) who obtained 72.73% as infestation rate for oocysts of Coccidia, Coulibaly (2006) with 67% infestation rate, this diagnosis revealed a

predominance of Coccidia. Infestation with Coccidia seems to begin when hygiene rules are not followed in livestock buildings; in this case the rate of infestation increases as pointed out by Tamegnon (2001). Coccidia are prevalent throughout the year in the tropics. Indeed, in southern Benin, we have an abundance of water points and plant wealth. The problem is mainly ecological because these areas are favourable to the proliferation of parasites. Thus, outdoor temperatures allow the sporulation of oocysts: excessive humidity, lack of ventilation, overcrowding of premises, quality of food (Bodji et al., 2007).

Our results were confirmed by Abé (2009) who found the oocysts of Eimeria sp in the feces of the grasscutter in the district of Abidjan, in Ivory Coast.

4.1. Effect of different treatments on fecal excretion of eggs in grasscutters

When the infestation rate is high and the dose of d0.0 is administered, we can record a high mortality rate but this is not the case with low infestation. Calotropis procera has been used against many diseases but little is known about its anticoccidial activity (Pines et al., 2000). In the present study, the anticoccidial efficacy of Calotropis procera extract was investigated. The data indicate that the dose of D0.5 had no therapeutic effect on Coccidia (demarcation dose) and behaved like the dose d0.0. These results show that there is no therapeutic action of the plant on Coccidia at a dose of 0.5 g/kg body weight. The dose of 0.9 indicate a therapeutic effect but not optimal because of the recorded cases of mortality.

Those results obtained at the dose of 1.3 g of powdery extract/kg could mean that the latex of C. procera has a very optimal effect on Coccidia and that the effect of this dose was observed on D4 in all infested subjects. Briefly, 1.3g of powdery extract/kg feed can reduce oocysts excretion and mortality rate. In sum, the dose of 1.3g of powdery extract/kg has more therapeutic effect on Coccidia than the dose of 0.9g of powdery extract/kg. The deaths recorded at a dose of 1.3g of powdery extract/kg were due to the various controls performed. Our results confirm the idea of Nacoulma (1996) who emphasized that the latex has a therapeutic action against protozoa.

It should be noted that a toxic dose was not determined during this study. In addition, the deterioration in zootechnical performance and the high mortality rate (80 to 100% of the population) induced by coccidiosis, mentioned by Buldgen (1996), explain the considerable economic losses caused by this condition that we have known. There are no anti-coccidial drugs approved specifically for grasscutters. Generally, benzene acetonitrile compounds (ponazuril, diclazuril, toltrazuril), sulfonamides, and amprolium have been used to treat or prevent coccidiosis in breeding (Metwaly et al., 2012; De Pablos et al., 2010).

According to Dieye et al. (1993) efficacy of various drugs and of the extract of the latex of Calotropis procera for treating clinical coccidiosis is unknown. Treatment for coccidiosis included amprolium hydrochloride (10 mg/kg) in a 1.5% solution orally daily up to 15 days, or sulfadimethoxine (110 mg/kg) orally daily for 10 days, and supportive therapy. However, some grasscutters died despite this therapy and had confirmed coccidiosis histologically (Cebra et al., 2007). In one instance, an entire herd of 30 alpacas that developed coccidiosis 20 days after introduction to a new farm was treated with amprolium hydrochloride, two died and four were euthanized. Two of the four that died had histologically confirmed coccidiosis (Cebra et al., 2007).

But, the benzene acetonitrile compounds in general have low toxicity compare at the extract of the latex of Calotropis procera according to Ma et al. (2011). They are used extensively to treat coccidiosis in breeding (birds, mammals, reptiles, etc) in countries where they are readily available in a convenient treatment form. Prophylactic treatment should be considered during winter when outbreaks of coccidiosis are common. Decoquinate may be added to feed at 0.5 mg/kg/day for 4 weeks (Cebra et al., 2014).

On the basis of the results of this study and results of others researchers (Mossa et Coll, 1991; Ouédraogo, 2001; Sen et al., 1988), Calotropis procera extract was effective as an anticoccidial to protect grasscutters against coccidiosis infection and may be recommended to be used as a coccidiostat drug, and some further studies in grasscutters breeding are still needed.

5. Conclusion

This study, which is part of a logic of contributing to the improvement of rearing and sanitary conditions of the grasscutter (Thryonomys swinderianus, Temminck, 1827), focused on the use of the latex of C. Procera as an anticoccidial in this field species in the Republic of Benin. These observations revealed the presence of oocysts of coccidia. These eggs are found in 100% in all subjects.

In summary, grasscutters are very sensitive to coccidiosis and the latex of C. procera can be recommended in the control of coccidiosis using a dose of 1.3 g/kg body weight. However, it seems necessary that further research can be undertaken to evaluate the total toxicity of the latex before being a recommendation drug in the fight against this pathology. Faced with this imperative, it is recommended that breeders apply the various sanitary prophylaxis programs correctly according to climates and rainy seasons.

At the end of this current study, it would be desirable to consider a more extensive study on the entire Beninese territory, this time by performing work on gastrointestinal parasites, hemoparasites and ectoparasites. Work should also be done on other health aspects such as bacteriology and virology.

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How to cite this article: Adenile, D.A., Akouedegni, C.G., Alowanou, G.G., Gbaguidi Sedegnon, A.V.A.M., Daga, F.D., Hounzangbe-Adote, S., 2018. Valuation of anticoccidial activity of pulverulent extracted from Calotropis procera against coccidial infection in grasscutters. Scientific Journal of Veterinary Advances, 7(11), 254-262.