***Original Research Article***

**Evaluation of the in-vivo antiparasitic effects of the aqueous extract of the leaves of *Alchornea Cordifolia* (AEAC)(Euphorbiaceae) as an alternative in poultry farming in Côte d'Ivoire.**

**Abstract**

 Chickens are highly susceptible to coccidiosis, which is one of the reasons for the decline in their zootechnical performance. Conventional methods of combating coccidiosis involve the use of synthetic anticoccidants, which are highly risky. The search is on for alternatives, including natural plants. *Alchornea cordifolia* is a plant found in Côte d'Ivoire's flora and recognised in traditional medicine for its many therapeutic properties. A phytochemical screening revealed the presence of sterols and polyterpenes, polyphenols, quinone compounds, alkaloids and catechic tannins. The LD50 assessed in accordance with OECD guideline 423 showed that this plant is non-toxic when taken alone at doses of 2000 and 5000 mg/Kg bw in wista rats. The effect of the aqueous extract of *Alchornea cordifolia* leaves was assessed on weight gain, feed conversion, lesion indices and clinical appearance. This enabled us to see that the extract had no negative effect on the zootechnical parameters. Evaluation of the efficacy of the aqueous extract of *Alchornea cordifolia* leaves in the treatment of coccidiosis caused by Eimeria in broilers showed that this extract had similar anticoccidial properties to those of the reference anticoccidian. Indeed, at the end of the 7 days of treatment, the infestation rate jumped in the 3 batches, i.e. from 102,300 OPG to 200 OPG for the control batch. Then from 86350 OPG to 4200 OPG for the batch treated with 5g/l EAAC. Finally, from 21400 OPG to 3500 OPG for the batch treated with 10g/l of AEAC. A reduction in coccidia eggs was observed in all 3 batches. This study shows that Alchornea cordifolia leaf extract could be used in the treatment of coccidiosis.

**Key words: *Alchornea cordifolia*, aqueous extract, anticoccidial activity, poultry farming,** **Oocysts per gram (OPG).**

**I.** INTRODUCTION

Coccidiosis is a protozoan, obligate parasite belonging to the apicomplexan phylum, a group of pathogens of high economic, veterinary and medical importance (Aitfella, 2012). Coccidiosis is one of the poultry diseases that limit poultry production, food security and economic development. To combat the disease, some of our farmers use veterinary medicines, whose poorly controlled and often inappropriate use has led to resistance in Eimeria to some common anticoccidials (Dakpogan et al., 2012). In view of this resistance and the many harmful consequences for consumer and chicken health, other alternatives are needed (Kouamé et al., 2004) ; (F. S. Ouattara-Soro et al., 2023).. These alternatives include medicinal plants. These include Alchornea cordifolia. It is often used as an anti-inflammatory, antifungal, antibacterial, analgesic and antioxidant (Traoré, 2005 ; N'bra, 2017).

 The aim of this study was to evaluate the in vivo antiparasitic activity of the aqueous extract of Alchornea cordifolia leaves in broiler rearing.

1. **MATERIALS AND METHOD**
* **MATERIALS**

**Plant material**

 The plant material consisted of *Alchornea cordifolia* leaves, which were dried in the shade and then ground. The powder obtained was used to prepare the aqueous extract.

**Animal material**

 The animal material consisted of Wistar strain Rattus norvegicus (Muridae) rats weighing between 100 g and 130 g, which were used to assess acute toxicity.

Fifty-one (51) one-day-old Gallus gallus domesticus chicks of the coob 500 strain weighing an average of 43 g from IVOGRAIN were placed on 23 August 2022 and monitored from day 1 to day 32.

* **METHOD**

**Aqueous preparation of *Alchornea cordifolia* leaves**

The aqueous extract of *Alchornea cordifolia* leaves was prepared following the method used by Bagré et al 2011. A total of 100 g of leaf powder was dissolved in 1000 ml of sterile distilled water and macerated using a flask containing a bar magnet placed on a magnetic stirrer for 24 h at room temperature at a speed of 3000 rpm. The macerate obtained was filtered on poplin cloth, then on cotton wool and finally on Wattman paper. The filtrate was dried in an oven at 40°C to obtain the dry extract.

**Phytochemical analysis**

Phytochemical screening was carried out using the methods described by Lazureski et al (2007), Abo (2013) and Mea et al (2017) in the Animal Physiology and Phytotherapy laboratory of the Biosciences UFR (UFHB).

**Acute toxicity by gavage of the aqueous extract of the leaves of *Alchornea cordifolia*** This experimental study was adapted from that described in guideline 423 (OECD 423, 2001). Wistar strain Rattus norgevicus rats weighing 100 g to 130 g, in three batches of three animals each, were used. Increasing doses prepared from the aqueous solution of Alchornea cordifolia were administered orally (gavage) to two groups of 6 rats at a rate of one dose per group and 1 group of 6 rats received distilled water. The animals were observed: The aim of this study was to evaluate the in vivo antiparasitic activity of the aqueous extract of the leaves of Alchornea cordifolia in broiler rearing.

**Induction and treatment of coccidiosis**

Fifty-one one-day-old chicks were randomly divided into 3 batches. Each batch consisted of 17 chicks.

- Batch 1 served as a control and the chickens were reared using conventional prophylaxis.

- In batch 2, the chickens were reared using conventional prophylaxis but instead of a conventional anticoccidial, AEAC was used at 5 g/l without antibiotics.

- In batch 3, the chickens were reared according to conventional prophylaxis but instead of a conventional anticoccidial, EAAC was used at 10 g/l without antibiotics.

The presence of coccidia was checked microscopically before artificial infestation. The chickens were contaminated through their drinking water after being deprived of water for one night.

The treatment was carried out at 2 levels, a preventive treatment and a curative treatment. The preventive treatment began on day 2 of the rearing cycle. The chicks in batches 2 and 3 received AEAC instead of antibiotics. On the other hand, curative treatment for coccidiosis began on day 22, i.e. 5 days after infestation, which was on day 17. Batch 2 and 3 received a 5 g/l and 10 g/l AEAC solution respectively instead of antibiotics and anticoccidials, while the control batch also received a 1 g/l conventional anticoccidial solution. Both treatments lasted according to the prophylaxis. The chickens were weighed individually every 7 days. Droppings were analysed on days 7, 14, 18, 21, 24 and 27 (days 7, 14, 18, 21, 24 and 27) and digestive tract lesion scores were also analysed at the end of treatment.

The bedding of the batches was constantly renewed to reduce the risk of recontamination. Monitoring focused on the criteria used to assess the level of parasitism of Eimeria strains, namely weight gain, feed conversion, coccidiosis mortality, oocyst excretion, intestinal lesions, clinical index and faecal appearance.

**Statistical analysis**

The results were subjected to analyses of variance using appropriate statistical software (ANOVA OneWay). Graphical representation and data processing were carried out using EXCEL and XLSTAT.

1. RESULTS
2. **Phytochemical screening**

 Phytochemical screening revealed the presence of sterols and polyterpenes, polyphenols, quinone compounds, alkaloids and catechic tannins.

**2. ACUTE TOXICITY**

A single gavage dose of 2000 mg/kg bw to female rats had no effect on their behaviour. However, administration of a single dose of 5000 mg/kg bw to the rats caused a reduction in motor skills, leading them to gather in a corner of the cage for 30 minutes. After 30 minutes the behaviour normalised. No rat mortality was observed at any dose during the fourteen (14) days of observation. All animals survived the 14 days of observation, implying that the LD50 is greater than 5000 mg/kg bw. According to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS, 2003), the aqueous extract of Alchornea cordifolia leaves is non-toxic by the oral route in Wistar rats (Table I).

**Average daily earnings**

The average daily gains of batches 2 and 3 treated with EAAC were 33.51 and 31.98 g respectively. That of the controls was 33.15 g. Statistical analysis shows that the average gains of the treated chickens were not significantly (P >0.05) different from those of the controls (Figure 1).

**Consumption index**

The feed conversion ratio of the control and the batches treated with 5g and 10g AEAC was 1.4, 1.42 and 1.4 respectively. The feed conversion ratio of the 2 batches treated with AEAC increased over the course of the study. However, this increase was not significant (P >0.05) compared with the control batch (Figure 2).

Table I: Results of phytochemical screening of the aqueous extract of Alchornea cordifolia

|  |  |  |
| --- | --- | --- |
| **Compounds sought** | **Test or reagents** | **Results** |
| Sterols and polyterpenes | Liebermann | **+** |
| Polyphenols | Ferric chloride | **+** |
| Flavonoids | Cyanidine | **-** |
| Saponosides | Vigorous agitation | **-** |
| Quinonic compounds | Borntraeger | **+** |
| Alkaloids | Dragendorff | **+** |
| Bouchardat | **+** |
| Tannins | Catechics | Stiasny | **+** |
| Galliques | Hydrochloric acid | **-** |

**(+)** : Presence of compound

**(–)** : Absence of compound

Table II: Number of rats and mortality rate as a function of the dose of Alchornea cordifolia extract

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Lots | EAAC dose(mg/kg PC) | Number of rats tested | Number of dead rats | Mortality rate |
| 1 | 2000 | 3 | 0 | 0% |
| 2 | 5000 | 3 | 0 | 0% |

Duration of treatment

Body mass(g)

32 days

 Figure 1 : Evolution of weight gain in treated chickens

Duration of treatment

32 days

Consumption index

Figure 2 : Change in feed conversion rate for treated chickens

(n=17)

**1. Mortality rate**

During the rearing of the chickens, no mortality was observed in the 3 experimental batches. The overall mortality rate of the chickens during the experiment was 0%.

1. Oocyst excretion

**- Analysis before artificial infestation and treatment**

From the first to the 7th day of rearing, coprological analysis of the droppings revealed no presence of coccidia in the treated batches or the control. However, from the second week onwards, coccidia were present at low levels in all batches. In fact, in the batches treated with AEAC 5 g/l and 10 g/l, the number of eggs per gram (OPG) was 50 and 7250 respectively, whereas in the control batch, the results showed 650 OPG (figure 3).

- Analysis after artificial infestation and treatment

After 5 days of infestation, coprological analysis showed a high presence of coccidia in the droppings of all 3 batches. In the control batch, 86350 Oocysts Per Gram were counted, whereas in the batch treated with 10 g/l AEAC, a lower number of 21400 OPG was observed. For the batch treated with 5 g/l AEAC, a higher number of 102,300 OPG was counted.

On day 26, i.e. 4 days after treatment, a sudden drop in coccidia eggs in the droppings was observed in the different batches. In the control batches and those treated with 5 g/l AEAC, the number of OPG had fallen from 86350 to 1600 and from 102300 to 1800 respectively. However, at the dose of 10 g/l AEAC, there was a slight decrease from 21400 to 13900 OPG. By the end of the 7-day treatment period, the infestation rate had become low in all 3 batches. Thus, after microscopic observation, an OPG equal to 200 was counted for the control batch, 4200 for the batch treated with 5 g/l AEAC and an OPG of 3500 for the batch treated with 10 g/l AEAC (figure 4).

1. **Clinical index**

Chickens from the 3 infected batches showed clinical signs characteristic of coccidiosis, namely prostration with a ball-like posture, drooping wings and ruffled feathers. The droppings from the chickens in the 3 infected batches were highly variable, ranging from normal to bloody.

Times

Control

OPG

Figure 3 : Changes in OPG before artificial infestation

OPG

Time

 Figure 4: Changes in OPG after artificial infestation

1. **Lesion index**

After autopsy at the end of the treatment, lesions of the intestines and duodenum and dilatation of the caeca were observed in the control batch. On the other hand, in the batches treated with the aqueous extract of *Alchornea cordifolia*, no lesions of the intestines were observed, but slight lesions of the duodenum were noted. Slightly dilated caeca

1. **DISCUSSION**

Phytochemical screening has shown that the aqueous extract of fresh *Alchornea cordifolia* leaves contains sterols and polyterpenes, polyphenols, quinone compounds, alkaloids and catechic tannins. The extract is believed to be rich in phytochemical compounds responsible for the plant's therapeutic properties. These results differ from those of Saraka et al (2018), who used ethanolic extraction to reveal the presence of flavonoids, gall tannins and an absence of quinone compounds in fresh *Alchornea cordifolia* leaves. This difference could be explained by the solvent used to extract the chemical compounds. The solvent determines the nature of the chemical compounds in the extract.

The study of acute toxicity by oral administration of the aqueous extract of the leaves of *Alchornea cordifolia* showed that this extract did not cause any mortality in rats at the respective doses of 2000 and 5000 mg/kg bw. Oral administration of the crude extract appeared to be well tolerated by rats. Consequently, the LD50 of AEAC would be greater than 5000mg/kg body weight in rats, which is in line with previous results obtained with EAAC in female mice (Traoré, 2004). According to the OECD's globally harmonised classification system (OECD, 2001), the aqueous extract of the fresh leaves of *Alchornea cordifolia* can be classified in category 5 and considered as a non-toxic substance by the oral route.

The average weights of the 2 batches treated with 5g/l and 10g/l AEAC increased. However, this increase was not significant (P>0.05) compared with the control treated with 1g/l SUPERCOX PLUS. The chickens in the 3 batches, i.e. the control batch, the batch treated with 5g/l of EAAC and the batch treated with 10g/l of AEAC had approximately the same average weight. This means that the chickens are growing well. These results differ from those of Kouakou et al (2010). These authors obtained a significant difference between the weight gain of the batch treated with Thonningia sanguinea (Balanophoraceae) and the batch treated with a classical anticoccidian on hens. The difference in results is certainly due to the plant used, which has different pharmacological effects.

The results showed that average daily gains increased in all batches. The weight gains of the batches treated with AEAC at 5 and 10g/l were 33.51 and 31.98g respectively. That of the controls treated with SUPERCOX PLUS at 1g/l was 33.15g. Statistical analysis showed that these average gains of the treated chickens were not significantly (P>0.05) different from those of the controls. In fact, the chickens were fed the same amount of feed ad libitum and growth proceeded normally. Contrary to the work of Chaaba (2014), the batches treated with Artemisia herba alba on turkeys at different doses gave a difference in weight gain from that of the control. This difference showed that EAAC does not negatively affect weight gain in treated broilers.

The feed conversion ratio of the 2 batches treated with AEAC increased over the course of the study, i.e. the chickens did not suffer from a lack of appetite. However, this increase was not significant (P>0.05) compared with the control batch. This result differs from that of Essomba (2003). Indeed, this author showed deteriorations in the feed conversion ratio in chickens infested with coccidiosis.

The mortality rate of the chickens during the experiment was 0%. This is contrary to the work of Bakli (2020). This work showed mortality during rearing after infestation of the chickens. According to Patra et al (2010), the highest mortality rate was between the sixth and eighth day after infection, due to excessive blood loss through diarrhoea. This was not the case in this study, although the chickens did experience bloody diarrhoea, the chickens in all 3 batches responded well to the treatment. No mortality occurred. Thus, the results are similar to those of Kouakou et al (2010) who found no mortality in their chicken experiment.

From the first to the 7th day of rearing, coprological analysis of the droppings revealed no presence of coccidia in the treated batches or the control. This is in line with the results of Essomba (2003). This result could be explained by the fact that the incubation time for coccidia was greater than one week. However, from the second week onwards, the presence of coccidia with a low infestation rate was observed in all batches. This is also in line with the results of Essomba (2003). The presence of coccidia at two weeks of rearing suggested that their incubation time had been reached.

An outbreak of coccidiosis was noted in the 3 batches after artificial infestation and a decrease in coccidia in all batches infested after treatment. Coprological analysis showed a high presence of coccidia in the droppings of the 3 batches, control, treated with 5 g/l and 10 g/l AEAC respectively 86350, 102 300 and 21400 OPG after 5 days of artificial infestation. Four days after treatment, a sudden drop in coccidia eggs in the droppings was observed in the different batches, control, treated with 5 g/l and 10 g/l of EAAC of 1600, 1800, 1900 OPG respectively. At the end of the 7 days of treatment, the infestation rate became low in the 3 batches, i.e. 200 OPG for the control batch, 4200 OPG for the batch treated with 5g/l EAAC and finally 3500 OPG for the batch treated with 10g/l AEAC. A reduction in coccidia eggs was observed in all 3 batches. AEAC would therefore have an anticoccidial effect, this result being due to the different compounds contained in the extract. According to Bakli (2020), it has been reported that tannins complex with polysaccharides and that condensed tannins bind to the cell walls of microorganisms, preventing the growth and activity of proteases, leading to destruction of the molecular structure of coccidia. One of the molecular actions of tannins is to complex with proteins by so-called non-specific forces such as hydrogen bonding and hydrophobic interactions, as well as by the formation of covalent bonds. They are also thought to have the ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins. Their anticoccidial mechanism of action is thought to be due to the induction of oxidative stress against Eimeria species.

AEAC also contains phenolic compounds. The antimicrobial effects of phenols are expressed on the microbial cell wall by affecting its structure. Phenols interact with the cytoplasmic membrane and modify its permeability to H+ and K+ cations. The dissipation of ionic gradients leads to the alteration of essential processes in the cell, allowing the escape of cellular constituents, resulting in water imbalance, a collapse in membrane potential and inhibition of ATP synthesis leading to lysis of the parasite wall (Bakli., 2020). In addition, the number of coccidia in the batch treated with SUPERCOX PLUS was 200 OPG, i.e. lower than in the batches treated with the extract, which were 3500 and 4200 OPG. The slight difference in oocyst reduction between the synthetic anticoccidial used and the extract could be explained by the presence of a low concentration of the active ingredient contained in the plant extract compared with the synthetic anticoccidial (Ola-Fadunsin & Ademola., 2014).

Chickens from the 3 contaminated batches showed clinical signs characteristic of coccidiosis, namely prostration with a ball-like posture, drooping wings and ruffled feathers. The droppings from the chickens in the 3 infected batches were highly variable, ranging from normal to bloody. This was confirmed by Kouakou et al (2010), who observed the same clinical signs following infestation of the hens. The presence of bloody droppings is thought to be due to the presence of coccidia in the intestinal walls, which feed on the various nutrients.

Necropsy at the end of the treatment showed lesions of the intestines and duodenum and dilatation of the caeca in the control batch treated with SUPERCOX PLUS at 1g/l. On the other hand, the batches treated with AEAC at 5 and 10 g/l showed no lesions of the intestines but slight lesions of the duodenum, and a slightly dilated caeca was observed. The lesion indices due to the appearance of coccidiosis in the chickens were slight in the batches treated with the extract. This result would be due to the efficacy of the extract in the treatment of coccidiosis. The lesion indices therefore show that *Alchornea cordifolia* controls coccidia. Studies carried out by Saarinen et al (2001) showed that the use of plant extracts could reduce oxidative stress in chickens, thereby reducing lesions and delaying the development of the parasite.

1. **CONCLUSION**

 This study highlighted the anti-parasitic properties of *Achornea cordifolia* leaves. The phytochemical study carried out on the aqueous extract of Alchornea cordifolia leaves showed the presence of several chemical groups, whose presence in the extract would be responsible for many of the therapeutic properties attributed to the plant. The acute toxicity study showed that this plant is not toxic when taken alone, with an LD50 greater than 5000 mg/Kg CP. These results seem to support its safety for oral use in the traditional treatment of certain illnesses. Evaluation of the efficacy of the aqueous extract of *Alchornea cordifolia* at concentrations of 5 g/l and 10 g/l in the treatment of avian coccidiosis caused by Eimeria in experimentally-infested broilers showed that this plant has similar anticoccidial properties to those of SUPERCOX PLUS, a synthetic anticoccidial at a dose of 1 g/l. Doses of 5 and 10g/l of aqueous extract not only significantly reduced the number of coccidia, but also protected the intestinal mucosa against the harmful effects of coccidia. In addition, the extract showed no adverse effect on the feed conversion ratio or zootechnical parameters of treated chickens compared with control chickens. A study of subacute toxicity is recommended to improve the use of this plant in traditional medicine.

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