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Research Article

In vivo therapeutic and antioxidant activities of aqueous extract of *Anogeissus leiocarpus* (DC.) Guill. et Perr. in Salmonellosis induced in broiler chicks

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Abstract

Salmonella are bacteria that cause numerous illnesses in humans and animals. In poultry farming, they cause major economic losses for farmers. In fact, during the poultry production cycle, poultry are frequently infected by S*almonella* and are exposed to oxidative stress. To deal with these problems, farmers use antibiotics. Unfortunately, overuse of these molecules has led to resistance. The aim of this study was to evaluate the *in vivo* therapeutic and antioxidant activities of aqueous extract of the leaves from *Anogeissus leiocarpus* in Salmonellosis induced in broiler chicks. For the experimental study, 4 batches of 25 broiler chicks each were produced, including :

- Batch 1 : uncontaminated chicks not treated with plant extract (NC-NT).

- Batch 2 : chicks contaminated and not treated with plant extract (C-NT).

- Batch 3 : chicks contaminated and treated with plant extract (C-T. extr).

-Batch 4 : chicks contaminated and treated only with a standard antibiotic, oxytetracycline (C-T.OTC). The aqueous extract of *Anogeissus leiocarpus* gave the best results compared with oxytetracycline. These include the significant improvement in average weight gain and biomarkers of oxidative stress.

The results obtained from this study show that the aqueous extract of *Anogeissus leiocarpus* can be used as an alternative to antibiotics in poultry farming.

Keywords : *Anogeissus leiocarpus*, Extracts, *Salmonella typhimurium*, therapeutic activity.

INTRODUCTION

Salmonella are bacteria that cause numerous illnesses in humans and animals. They can infect livestock such as poultry, cattle, sheep, pigs and fish, as well as wild animals and pets.[1,2] Humans can be easily infected by consuming contaminated food or water.[3] In poultry farming, *Salmonella* causes major economic losses for farmers.[4,5] During the poultry production cycle, birds are commonly infected with *Salmonell*a by various routes, including contact with carrier animals such as rodents, cats and insects. In addition, contamination linked to poultry feed, litter, water and aerosol transmission also contribute to the transmission of *Salmonella*.[6]

In addition, contamination of poultry by bacteria, and in particular *Salmonella*, creates oxidative stress in the birds that is likely to produce highly reactive molecules resulting from oxygen metabolism.[7] A fairly high production of these highly reactive molecules can lead to an alteration in membrane lipids, which ultimately deteriorates the condition of poultry meat.[8,9,10] However, stress in broilers remains difficult to control for several reasons, including chick transport, high ambient temperature and mitochondrial dysfunction, toxins in poultry feed and variations in intestinal flora.[11,12,13]

To avoid huge economic losses due to microbial contamination and to protect broiler consumers against gastroenteritis and food poisoning, farmers resort to the use of antibiotics. Antibiotics used as feed additives to control enteric pathogens in broilers, particularly *Salmonella*, include small amounts of penicillin, tetracycline and chloramphenicol.[14] However, the therapeutic use of these antibiotics in poultry feed in general is being reconsidered due to increasing concern about antibiotic resistance.[15] Furthermore, antibiotic use is associated with the destruction of beneficial intestinal bacteria that help fight enteric pathogens.[16] This destruction of beneficial intestinal flora in poultry can lead to oxidative stress, which can affect meat quality. In addition, overuse of antibiotics is not without toxic effects for poultry consumers.[17] As a result, alternatives to antibiotics such as probiotics, prebiotics, symbiotics and postbiotics are increasingly being used in both humans and animals.

Anogeissus leiocarpus (DC.) Guill. & Perr. (Combretaceae), also known as chewing stick or axle tree, has a long history of traditional use to combat various human infections. The leaves of *A. leiocarpus* are used in the treatment of skin diseases, fever, diarrhea, malaria and stomach infections.[18] This plant is commonly used in the north of Côte d'Ivoire by livestock farmers to treat various animal diseases.[19,20] Their studies confirmed that *A. leicocarpus* is used in veterinary medicine, particularly in the treatment of parasitic diseases caused by *Haemonchus contortus.* The methanolic extract of *A. leiocarpus* stem bark showed anti-trypanosomiasis activity against four strains of *Trypanosoma* and leishmanicidal activity.[21,22]

The aim of this study was to evaluate the *in vivo* therapeutic and antioxidant activities of the aqueous extract of the leaves from *Anogeissus leiocarpus* in Salmonellosis induced in broilers.

MATERIALS AND METHODS

Plant material

The plant material consists of *Anogeissus leiocarpus* leaves harvested in July 2022 in Lataha, a village located in the Korhogo region (northern Côte d'Ivoire) and authenticated by the Centre National Floristique of the Université Felix HOUPHOUËT-BOIGNY in Cocody-Abidjan (Côte d'Ivoire).

Bacterial strain

A multi-resistant strain of *Salmonella typhimurium* of avian origin with a broad antibiotic resistance profile (AMP-CHL-STR-SUL-TE-AUG-CTX-CIP-NA) was used to induce Salmonellosis in chicks. It was supplied by the Microbiology Unit of the Biotechnology Laboratory, UFR Biosciences, Université Félix Houphouët-Boigny, Cocody.

Animal material

For this study, one-day-old COBB broiler chicks were supplied by the local farm Ouattara Ali Nanan Issa (FOANI).

Breeding method

For the experimental study, 4 batches of 25 broiler chicks each were produced, including :

- Batch 1 : uncontaminated chicks not treated with plant extract (NC-NT).

- Batch 2 : chicks contaminated and not treated with plant extract (C-NT).

- Batch 3 : chicks contaminated and treated with plant extract (C-T. extr).

-Batch 4 : chicks contaminated and treated only with a common antibiotic, oxytetracycline (C-T.OTC). Oxytetracycline is an antibiotic used by many farmers to treat *Salmonella* diarrhea.

The batches of uncontaminated chicks were kept far enough apart from the diseased batches to avoid horizontal contamination between them. At the start of the experiment, all the chicks in each batch were numbered from 1 to 25 and weighed. In addition, a clinical examination was carried out on each batch of chicks to ensure that they showed no signs of gastroenteritis.

All chicks were fed the same standard broiler feed supplied by the Société de Fabrication d'Aliments Composés Ivoiriens (SOFACI). The different batches of chicks were fed the same quantities of feed, well packaged in clean 25 kg bags.

Animals were handled according to standard protocols for the use of laboratory animals. The studies were conducted in accordance with the ethical guidelines of the Animal Experiments Monitoring and Control Committee as described in "European Community Guidelines, EEC Directive 86/609/EEC" (EEC, 1986), on the use of animals in scientific research.

Preparation of the aqueous plant extract of *Anogeissus leiocarpus*

The leaves of *A. leiocarpus were* washed, cut and dried in the shade for a fortnight. Once dried, these plant parts were ground to obtain a powder. 100 g of this powder was mixed with 1 litre of distilled water. The mixture was homogenised at room temperature in the laboratory using a magnetic stirrer for 24 hours. The homogenate obtained was filtered twice on cotton wool and once on Whatman paper (3 mm). The filtrate obtained was evaporated in an oven (Med Center Venticell) at 50°C to give a powder that constitutes the total aqueous extract.[23]

Preparation of the bacterial *inoculum*

To induce Salmonellosis in chicks, an inoculum was prepared. After incubation for 18 hours on GSS agar, 2 young colonies of the multi-resistant *Salmonella typhimurium* strain were used to inoculate 10 ml of Mueller-Hinton broth, which was then incubated at 37°C for around 4 hours to obtain a preculture with an estimated bacterial load of $1.5.10^5$ CFU/ml.

Contamination of chicks.

For the induction of Salmonellosis in batches 2, 3 and 4, 1 ml of the prepared bacterial suspension was administered orally to each chick while holding the beak closed for a few seconds to avoid rejection of the inoculum.[24] The chicks were contaminated on the eighth day of the experiment (D_0), i.e. after a week of acclimatisation on the farm.

Treatment of chicks

Treatment of the chicks with the aqueous extract began on the day the first clinical signs of Salmonellosis appeared and lasted two weeks (14 days). The treatment was carried out according to the method described by Ouattara.[25] The chicks were treated every day as follows

- Each day at 07 AM, each chick in batch 3 received 1 ml of the aqueous extract of *Anogeissus leiocarpus* at 25.5 mg/ml for a chick of 300 g (0.3 kg) body weight (i.e. 85 mg/kg bw).

- The chicks in batch 4 received oxytetracycline (OTC). Each chick in this batch also received 1 ml of this antibiotic prepared at 6 mg/ml (i.e. 20 mg/kg bw).

Evaluation of bacterial loads in chick faeces after induction of Salmonellosis

To monitor the effect of the aqueous extract of *Anogeissus leiocarpus* and the efficacy of the treatment, the quantity of bacterial colonies present in the faeces of the chicks was evaluated. Fresh faeces from each batch of chicks were collected in sterile bottles with a clean spatula every day before treatment. The samples were collected in accordance with the recommendations of the International Organization of Animal Diseases for taking samples and sending them for laboratory diagnosis. Chicken faeces were collected following macroscopic observation, which helped to distinguish those showing symptoms of *Salmonella* infection by parameters such as appearance of clinical signs such as diarrhea, feacal excretion of *Salmonella* and colour. The bacteriological analysis used to isolate *Salmonella* strains was carried out in accordance with standard NF EN ISO 6579 (ISO-6579, 2002), which comprises 4 stages : pre-enrichment, enrichment, isolation and biochemical identification.

Pre-enrichment

A 10 g mass of each sample was placed in a sterile 120 mL flask containing 90 mL of buffered peptone water. The flask

containing the pre-enrichment culture was then incubated at 37°C in an oven for 24 hours.

Selective enrichment

The pre-enrichment culture is collected using single-use 1 mL pipettes. A volume of 0.1 mL and 1 mL of pre-enrichment culture is taken and added respectively to 10 mL of Vassiliadis Rappaport broth and to 10 ml of Müller-Kauffman Tetrationate broth in tubes to provide enrichment. Tubes were incubated at 42 °C and 37 °C for 24 hours respectively for Rappaport Vassilliadis (RV10) broth and Müller-Kauffman Tetrationate (MKT) broth.

Isolation

From each of the two tubes (RV10 and MKT), Hektoen agar and Xylose-Lysin-Deoxycholate (XLD) agar in Petri dishes were streaked and incubated at 37 °C for 24 hours. The suspected colonies were tested for Gram control, oxidase and catalase tests were carried out to confirm that they belonged to the Enterobacteriaceae family.

Research into other biochemical characteristics (biochemical identification)

Research into other biochemical characteristics consisted of inoculating a reduced Leminor rack made up of 4 culture media, namely Kligler-Hajna, urea-indole, mannitol-mobility and Simmons citrate and incubating the whole at 37°C for 24 h. These media are used to demonstrate whether or not glucose and lactose are fermented, with or without the production of gas and hydrogen sulphide ; whether or not urease or indole are produced ; and whether the carbon in citrate and mannitol is used.

After 24 h incubation at 37°C, the number of surviving *Salmonella* colonies in each Petri dish was determined and recorded. The results were converted into the number of colonies per gram of faecal material.

Determining average chick weight gain

The chicks were weighed individually before the experiment (on the day of germ inoculation, i.e. $\mathbf{D_0}$) and during the experiment, i.e. from $\mathbf{D_1}$ to $\mathbf{D_{14}}$. The average weight of the chicks per batch was determined using the following formula :

$$\mathbf{P_m} = \frac{\mathbf{\Sigma\ P_i}}{\mathbf{N}}$$

$\mathbf{P_m}$ = Average chick weight (g)/lot
$\mathbf{\Sigma\ P_i}$ = Sum of individual chick weights per batch (g)
N = Number of chicks per batch

These average weights per batch were used to determine the average weight gains of chicks per batch as follows :

$$\mathbf{GP_m = P_{mj} — P_{mp}}$$

$\mathbf{GP_m}$ = Average weight gain (g)/lot
$\mathbf{P_{mj}}$ = Average daily weight (g)
$\mathbf{P_{mp}}$ = Average previous weight (g)

Determination of oxidative stress parameters in chickens

At the end of the treatment **(D14)**, the chickens were fasted for 12 h, plucked and bled. The blood was collected and centrifuged at 3,000 rpm for 15 minutes. The serum (supernatant) was then isolated and stored at -18°C prior to analysis. The homogenate from each organ (liver, kidney, heart, lung and spleen) was prepared in 15% phosphate-buffered saline (i.e. 15 g of organ in 100 mL of solution) and centrifuged at 3,000 rpm for 15 minutes. The supernatants from these organs were then collected. These sera and supernatants were used to analyse biochemical markers linked to oxidative stress such as catalase, peroxidase, nitric oxide and malonaldehyde.

Measurement of catalase activity

The method of Dimo was used to assess catalase activity.[26] Briefly, 10 µL of serum or tissue homogenate was added to 150 µL of phosphate buffer (pH 7.4). Then 40 µL of H2O2 (50 mM) was also added. After 1 min, 400 µL of potassium dichromate (5%) prepared in 1% acetic acid was added to the reaction solution. The mixture was heated in boiling water for 10 min and immediately cooled. The absorbance was then recorded at 570 nm using a spectrophotometer. The enzymatic activity of catalase was expressed in mmol/min per millilitre of serum or gram of tissue.

Measurement of peroxidase activity

Peroxidase levels were determined in tissues as described by with slight modifications.[27] Two hundred and fifty microlitres of organ homogenate or serum were taken, to which 500 µl of 10 mM KI solution and 500 µl of 40 mM sodium acetate were added. The absorbance of potassium periodide was read at 353 nm, indicating the amount of peroxidase. Next, 10 µl of 15 mM H O_{22} was added and a change in absorbance over 5 minutes was recorded. The enzymatic activity of the peroxidase activity was expressed in µmol/min per millilitre of serum or gram of tissue.

Nitric oxide measurement

The level of nitrite, a reflection of nitric oxide (NO) production, was estimated using the Griess reagent. Briefly, equal volumes of 170 µL of sample or nitrite (0.1 M sodium nitrite in water for the curve standard) and 170 µL of sulphanilamide solution (1% sulphanilamide in 5% phosphoric acid) were mixed and incubated in the dark at room temperature for 5min. After incubation, 170 µL of naphthylethylenediamine N-1 dihydrochloride solution (0.1% naphthylethylenediamine N-1 dihydrochloride in water) was added and incubated as in the previous condition. NO concentration was estimated by measuring absorbance at 520 nm with a spectrophotometer and results were expressed as micromoles of NO per gram of tissue or per millilitre of blood using a sodium nitrite calibration curve.[28]

Determination of malonaldehyde

Malonaldehyde (MDA) is an end product of lipid peroxidation, so the amounts of MDA can be used to assess the degree of lipid peroxidation. The amount of MDA was measured in tissue or serum using thiobarbituric acid using the modified method of Serge. Briefly, 50 µL of homogenate, 250 µL of 1% orthophosphoric acid and 250 µL of a precipitant mixture (i.e. 1% thiobarbituric acid and 1% acetic acid) were mixed. The resulting mixture was homogenised, heated in a water bath for 15 min and immediately cooled. It was then centrifuged at 3000 rpm for 15 minutes and the supernatant was recovered and its absorbance recorded at 532 nm using a spectrophotometer. The MDA content was calculated as a function of the molar extinction coefficient, and expressed in terms of micromoles of MDA per gram of tissue or per millilitre of blood.

Statistical analysis

The data were subjected to analysis of variance using Graph Pad Prism 9.5.1 software. The results were analysed using analysis of variables (ANOVA) and Tukey's multiple comparison test for comparison of means. The difference is considered significant at a probability level of $p < 0.05$. A p value less than or equal to 0.05 was considered significant. Statistical differences (p less than or equal to 0.05) are indicated in the tables and figures by an asterisk (*). Statistically moderately significant differences (p less than or equal to 0.01) are

represented by two stars (**). Highly significant statistical differences (p less than or equal to 0.001) are represented by three stars (***) and highly significant statistical differences (p ≤0.0001) by four stars (****).

RESULTS AND DISCUSSION

Results

Figure 1 shows the bacterial load in chick droppings from the different batches. There was an absence of bacterial germs in the faeces of chicks in batch 1 (uncontaminated and untreated chicks) throughout the experiment. For the three other contaminated batches (batch 2, batch 3 and batch 4), the bacterial load in the faeces increased progressively until D_3. In fact, for these batches 2, 3 and 4, the *Salmonella* load reached 229.10^3 cfu/g faeces, 235.10^3 cfu/g faeces and 220.10^3 cfu/g faeces respectively for batches 2, 3 and 4 at D_3.

From D_3 onwards, *Salmonella* loads in the faeces of chicks in batch 2 (contaminated and untreated chicks) continued to increase, reaching a load of 268.10^3 CFU/g of faecal matter at D_7 and remaining almost constant until D_{14} with 266.10^3 CFU/g of faecal matter. In batch 3 of contaminated chicks treated with the extract, the number of Salmonella colonies in the faeces fell from 235.10^3 CFU/g faecal material on D_3 to 48.10^3 CFU/g faeces at D_7 then to a zero load at D_{10} which remained constant until D_{14}. As for the chicks of batch 4 contaminated and treated with oxytetracycline, the *Salmonella* load in the faeces also decreased but less than in batch 3. In fact, for batch 4, the number of salmonella colonies fell from 220.10^3 CFU/g of faecal matter (D_3) to 88.10^3 CFU/g of faecal matter (D_3) then to loads of 30.10^3 and 27.10^3 CFU/g faecal matter on days D_{10} and D_{14} respectively. However, the aqueous extract of *Anogeissus leiocarpus was* more active against the growth of *S. typhimurium* germs than oxytetracycline.

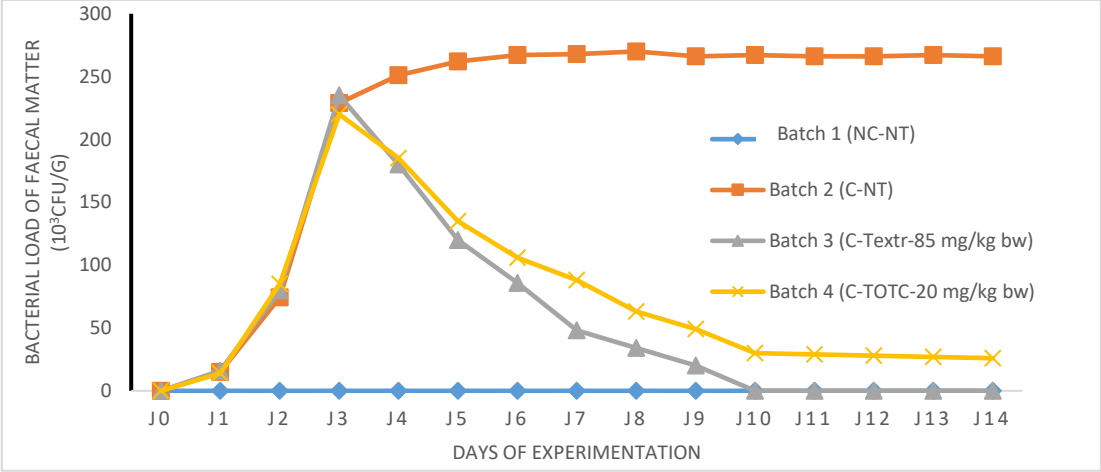

Figure 1 : Evolution of bacterial loads in chick droppings in the different batches

Figure 2 shows the variation in average weight gain in the different batches of chicks. The results showed that the average weight gains were not really significant between days D_1 and D_3 in the different batches of chicks, with gains of between 25 ± 0.5g and 27.04 ± 0.6g. From D_3, the average weight gains for batch 1 (uncontaminated and untreated chicks) increased progressively from 27.03 ± 0.6 g (D_3) to 41.6 ± 1.02 g (D_{14}). The results showed a fall in the average weight gain of chicks in batches 2, 3 and 4 compared with batch 1 from the appearance of the first signs of pathology (D_3), albeit in different proportions. For batch 2, the average weight gain fell from 27 ± 0.5 g (D_3) to 19 ± 0.6 g (D_{10}) and then to 15 ± 0.32 g (D_{14}). Also, for batch 4, average weight gains fell from 26.5 ± 0.22 g (D3) to 19.9 ± 0.35 g (D_{10}) then to 19 ± 0.5 g (D_{14}). As for the chicks in batch 3 treated with the aqueous extract of *Anogeissus leiocarpus*, there was an initial drop in average weight gain from D_3 to D_{10} (22.03 ± 0.25 g) then an increase in this gain from this day to D_{14} (26.5 ± 0.6 g).

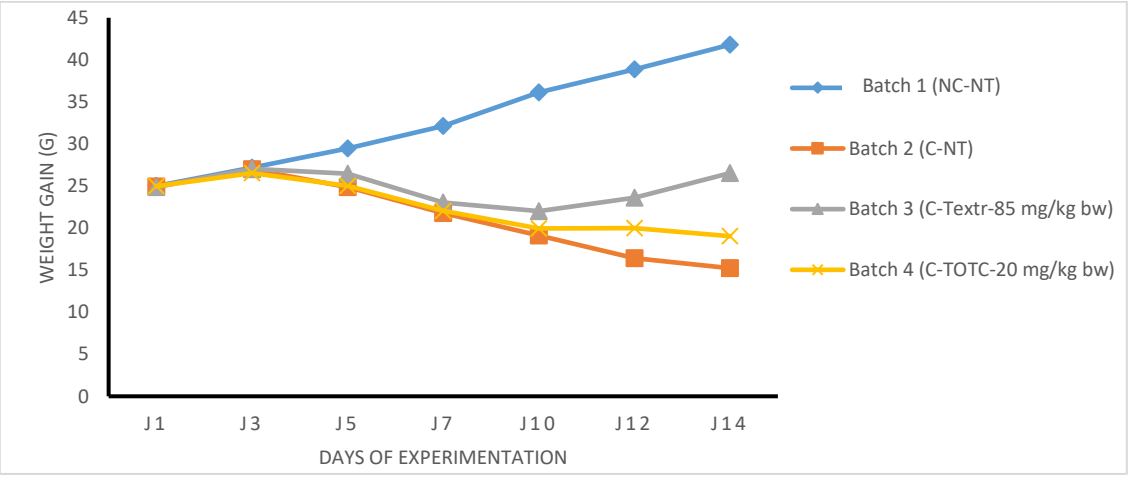

Figure 2 : Weight gain of chicks in different batches

Batch 1 (NC-NT): uncontaminated and untreated chicks; **Batch 2 (C-NT)**: contaminated and untreated chicks; **Batch 3 (C-T.extr)**: chicks contaminated and treated with plant extract; **Batch 4 (C-T.OTC)**: chicks contaminated and treated with oxytetracycline.

Table 1 shows the catalase activities in the serum and in the different organs of the chicks. Catalase activity varied from 0.21 ± 0.014 mmol/min/ml to 0.25 ± 0.02 mmol/min/ml in the sera of the different batches of chicks. However, the statistical study showed no significant difference ($p > 0.05$) between catalase activities in the sera of the different batches of chicks studied. In the livers, the non-significant ($p > 0.05$) catalase activities between all the batches of chicks ranged from 0.79 ± 0.03 mmol/min/g (batch 2) to 0.94 ± 0.07 mmol/min/g (batch 1). Thus, infection with the *Salmonella typhimurium* strain did not modify catalase activities in serum and liver in the different batches of chicks compared with batch 1 control. However, compared with the control batch (batch 1), infection with the *Salmonella typhimurium* strain (batch 2) significantly reduced catalase activities in the breasts of the kidneys, hearts, lungs and spleens. Compared with batch 1, this decrease in catalase activity was moderately significant ($p \leq 0.01$) in the breasts of the female rats (0.69 ± 0.01 03 mmol/min/g), while a highly significant decrease ($p \leq 0.0001$) was observed in the hearts (1.13 ± 0.07 mmol/min/g) and lungs (1.27 ± 0.11 mmol/min/g) of the batch 2 chicks. Treatment of the contaminated chicks with aqueous extract of *Anogeissus leiocarpus* (batch 3) resulted in an increase in catalase activity compared with batch 2 (contaminated and untreated chicks) in the same organs (kidneys, hearts, lungs and spleens). Also, compared with batch 1, treatment of infected chicks with the aqueous extract of *Anogeissus leiocarpus* resulted in a normalisation of catalase activities in the kidneys, hearts, lungs and spleens. In fact, no significant difference was observed between the catalase activities of the kidneys, hearts, lungs and spleens of batch 1 control and batch 3 (chicks contaminated and treated with aqueous extract of *Anogeissus leiocarpus*). In addition, treatment of the contaminated chicks with oxytetracycline was unable to normalise catalase activities in the kidneys, hearts, lungs and spleens, showing significant differences in decreases compared with batch 1.

Table 1 : Catalase activity in serum and in the various organs of chicks

Lots	Serum mmol/min/ml	Liver mmol/min/g	Kidney mmol/min/g	Heart mmol/min/g	Lung mmol/min/g	Rate mmol/min/g
Batch 1 (NC-NT)	0,24 ± 0,01	0,94 ± 0,07	0.93 ± 0.05	1,74± 0,06	1,79 ± 0,1	0,97 ± 0,15
Batch 2 (C-NT)	0,21 ± 0,014	0,79 ± 0,03	0,65*± 0,03	1,13****± 0,07	1,27 ****± 0,11	0,69**±0,01
Batch3 (C-T.extr)	0,25 ± 0,02	0,85 ± 0,04	0,79 ± 0,05	1,58 ± 0,10	1,70 ± 0,04	0,96 ± 0,2
Batch 4 (C-T.OTC)	0,22 ± 0,03	0,83 ± 0,07	0,67*± 0,06	1,14****± 0,04	1,33****± 0,07	0,72**± 0,01

Batch 1 (NC-NT): chicks uncontaminated and untreated; **Batch 2** (**C-NT**): chicks contaminated and untreated; **Batch 3 (C-T.extr)**: chicks contaminated and treated with plant extract; **Batch 4** (**C-T.OTC)**: chicks contaminated and treated with oxytetracycline. **Zero stars :** not significant ($p > 0.05$), **one star (*) :** significant ($p \leq 0.05$), **two stars (**):** moderately significant ($p \leq 0.01$), **three stars (***):** highly significant ($p \leq 0.001$), **four stars (****):** highly significant ($p \leq 0.0001$).

Table 2 shows the peroxidase activities in the serum and in the different organs of the chicks. Peroxidase activities in serum ranged from 0.38 ± 0.02 µmol/min/ml (batch 2) to 0.68 ± 0.02 µmol/min/ml (batch 3). Peroxidase activities in the various organs ranged from 1.24 ± 0.02 µmol/min/g (batch 2) to 1.88 ± 0.03 µmol/min /g (batch 3). Analysis of the table shows a highly significant decrease ($p \leq 0.0001$) in peroxidase activities in serum and other organs in batch 2 (contaminated and untreated chicks), with the exception of the lungs, compared with batch 1. In general, compared with batch 2, peroxidase activities increased when sick chicks were treated with aqueous extract of *Anogeissus leiocarpus* and oxytetracycline. Furthermore, no significant difference ($p > 0.05$) could be reported between the peroxidase activities in the sera, livers, kidneys and spleens of batches 1 and 3 compared with these same activities in the hearts and lungs, where significant differences were obtained. Treatment of chicks suffering from Salmonellosis with the aqueous extract of *Anogeissus leiocarpus* resulted in an increase and normalisation of peroxidase activities in the sera, livers, kidneys and spleens of batch 3. On the other hand, with the exception of the lungs, treatment of the sick chicks with oxytetracycline (batch 4) showed a decrease in peroxidase activities that was highly significant compared with the control batch (batch 1).

Table 2 : Peroxidase activities in serum and in the various organs of chicks

Lots	Serum µmol/min/ml	Liver µmol/min/g	Kidney µmol/min/g	Heart µmol/min/g	Lung µmol/min/g	Rate µmol/min/g
Batch 1 (NC-NT)	0,64 ± 0,02	1,70 ± 0,02	1,81 ± 0,03	1,47 ± 0,02	1,81 ± 0,01	1,79 ± 0,03
Batch 2 (C-NT)	0,38****± 0,02	1,49****± 0,03	1,54**** ± 0,02	1,24****± 0,02	1,73*± 0,01	1,56****± 0,01
Batch3 (C-T.extr)	0,68 ± 0,02	1,70 ± 0,04	1,78 ± 0,02	1,33****± 0,07	1,88* ± 0,03	1,82 ± 0,04
Batch (C-T.OTC)	0,42***± 0,04	1,51**** ± 0,02	1,60**** ± 0,02	1,25****± 0,03	1,75 ± 0,02	1,60****± 0,02

Batch 1 (NC-NT): chicks uncontaminated and untreated; **Batch 2** (**C-NT**): chicks contaminated and untreated; **Batch 3 (C-T.extr)**: chicks contaminated and treated with plant extract; **Batch 4** (**C-T.OTC)**: chicks contaminated and treated with oxytetracycline. **Zero stars :** not significant ($p > 0.05$), **one star (*) :** significant ($p \leq 0.05$), **two stars (**) :** moderately significant ($p \leq 0.01$), **three stars (***) :** highly significant ($p \leq 0.001$), **four stars (****) :** highly significant ($p \leq 0.0001$).

Table 3 shows the levels of nitric oxide (NO) in the serum and in the various organs of the chicks. There were no significant differences in serum nitric oxide (NO) levels between any of the batches of chicks. The average NO concentration was 0.20 µmol/ml for these different batches of chicks. Variable NO levels were also obtained in the chicks' organs (livers, kidneys, hearts, lungs and spleens). In these organs, NO levels ranged from 1.65 ± 0.09 µmol/g to 3.59 ± 0.04 µmol/g. However, with the exception of the kidneys where a highly significant difference ($p \leq 0.0001$) was noted (increase in NO levels), the induction of Salmonellosis showed no significant difference ($p > 0.05$) in NO production in the sera and organs of chicks in batch 2 compared with control batch 1. Also, compared with batch 1, treatment of the diseased chicks in batch 3 with the aqueous extract of *Anogeissus leiocarpus* did not alter the concentrations and levels ($p > 0.05$) in the sera and organs, respectively. Thus, in the presence of the aqueous extract of *Anogeissus leiocarpus*, NO concentrations and levels remained normal. Despite treatment with oxytetracycline, there was a highly significant increase ($p \leq 0.001$) in NO levels only in the chick kidneys (3.25 ± 0.18 µmol/g) compared with batch 1. As for the other organs, oxytetracycline treatment did not influence NO levels, giving a non-significant difference ($p > 0.05$) compared with batch 1.

Table 3 : Nitric oxide levels in serum and in the various organs of chicks

Lots	Serum µmol/ml	Liver µmol/g	Kidney µmol/g	Heart µmol/g	Lung µmol/g	Rate µmol/g
Batch 1 (NC-NT)	0,20 +0,02	2,98 ± 0,16	2,82 ± 0,16	1,74 ± 0,04	3,42 + 0,05	2,90 + 0,04
Batch 2 (C-NT)	0,21 ± 0,02	3,05 ± 0,08	3,51****± 0,08	1,80 ± 0,08	3,59 ± 0,04	2,98 ± 0,04
Batch3 (C-T.extr)	0,20 ± 0,04	2,95 ± 0,12	2,70 ± 0,06	1,65 ± 0,09	3,48 ± 0,003	2,87 ± 0,02
Batch 4 (C-T.OTC)	0,20 ± 0,02	2,99 ± 0,08	3,**25*****±** 0,18	1,66 ± 0,06	3,50 ± 0,02	2,93 ± 0,03

Batch 1 (NC-NT) : chicks uncontaminated and untreated ; **Batch 2 (C-NT)** : chicks contaminated and untreated ; **Batch 3 (C-T. extr)** : chicks contaminated and treated with plant extract ; **Batch 4 (C-T.OTC)** : chicks contaminated and treated with oxytetracycline. **Zero stars :** not significant ($p > 0.05$), **one star (*) :** significant ($p \leq 0.05$), **two stars (**) :** moderately significant ($p \leq 0.01$), **three stars (***) :** highly significant ($p \leq 0.001$), **four stars (****) :** highly significant ($p \leq 0.0001$).

The levels of malonaldehyde (MDA) in the serum and in the various organs of the chicks are shown in Table 4. Analysis of this table shows that, compared with batch 1, induction of Salmonellosis caused a highly significant increase in MDA levels in the livers and lungs of batch 2 chicks. On the other hand, the concentrations and levels of MDA in the sera, kidneys, hearts and spleens of the infected chicks in batch 2 were not influenced and showed a non-significant difference compared with those in batch 1. Treatment with the aqueous extract of *Anogeissus leiocarpus* succeeded in stabilising the concentrations and levels of MDA in the sera and organs of chicks made ill with the exception of the livers (0.13 ± 0.001 µmol/g) where a moderately significant difference ($p \leq 0.01$) was reported (batch 2) compared with batch 1. Treatment of chicks with Salmonellosis with oxytetracycline did not significantly alter the concentrations and levels of MDA in the breasts of the chicks' sera, kidneys, hearts and spleens compared with batch 1 ($p > 0.05$). On the other hand, despite treatment with oxytetracycline, MDA levels increased in the livers and lungs of chicks from batch 4 showing very highly significant differences ($p \leq 0.0001$) compared with batch 1 control.

Table 4 : Malonaldehyde levels in serum and in various chick organs

Lots	Serum µmol/ml	Liver µmol/g	Kidney µmol/g	Heart µmol/g	lung µmol/g	rate µmol/g
Batch 1 (NC-NT)	0,047± 0,002	0,14 ±0,001	0,11±0,004	0,23±0,004	0,24 ±0,004	0,12±0,002
Batch 2 (C-NT)	0,050 ± 0,001	0,21**** ±0,002	0,11±0,002	0,24±0,005	0,30****±0,005	0,13±0,005
Batch3 (C-T.extr)	0,045 ± 0,001	0,13*±0,001	0,11±0,001	0,22±0,001	0,24 ± 0,001	0,12±0,001
Batch 4 (C-T.OTC)	0,049 ± 0,001	0,180**** ± 0,001	0,11± 0,01	0,23 ± 0,01	0,27 ****± 0,01	0,12± 0,01

Batch 1 (NC-NT) : chicks uncontaminated and untreated ; **Batch 2 (C-NT)** : chicks contaminated and untreated ; **Batch 3 (C-T. extr)** : chicks contaminated and treated with plant extract ; **Batch 4 (C-T.OTC)** : chicks contaminated and treated with oxytetracycline. **Zero stars :** not significant ($p > 0.05$), **one star (*) :** significant ($p \leq 0.05$), **two stars (**) :** moderately significant ($p \leq 0.01$), **three stars (***) :** highly significant ($p \leq 0.001$), **four stars (****) :** highly significant ($p \leq 0.0001$).

DISCUSSION

The *Salmonella* load in the faeces of batch 1 chicks (uncontaminated and untreated chicks) remained zero throughout the experiment, indicating that these chicks were not contaminated with *Salmonella* germs. This absence of contamination can be explained by the control of this farm during our experiment, particularly with regard to good hygiene practices. It can also be explained by the better health and natural immunity of the chicks.

In the contaminated batches (batch 2, batch 3 and batch 4), *Salmonella* loads in the chicks' faeces increased progressively until D_3 (fourth day after induction) when the first clinical signs appeared. This observation shows the virulence over time of the *Salmonella typhimurium* strain used as the infecting agent to induce Salmonellosis in the chicks. *Salmonella* pathogenicity can be divided into several stages, including adhesion and invasion of intestinal epithelial cells, survival, multiplication within host cells and extraintestinal spread.[29] Similar studies were performed by some Researchers who evaluated the activities of *Albizia gummifera* aqueous extract against typhoid-induced Salmonellosis in rats. On the one hand, they confirmed the anti-*Salmonella* properties of the hydroethanol extract of *Canarium schweinfurthii* in experimental Salmonellosis in chickens ; and on the other hand, they began treating rats suffering from Salmonellosis practically four days after the disease was induced. While some began treating experimental Salmonellosis in chickens after five days of induction.[30] The differences observed between the start of treatment in relation to the appearance of the first clinical signs in the animals can be explained by several reasons. These include the dose of bacteria ingested, the type and health of the animal, the characteristics of the *Salmonella* strains used and, above all, the age of the host animal. To this could be added the effects of oxidative stress in farming systems, which can favour the appearance of the first clinical signs and therefore necessitate the start of treatment.

From D_3, the number of *Salmonella* colonies in the faeces of chicks from batch 2 (contaminated and untreated chicks) continued to increase, which can be explained by the absence of antibiotic treatment, which weakened the natural immunity of the contaminated chicks.

In batch 3 of contaminated chicks treated with the aqueous extract of *Anogeissus leiocarpus*, *Salmonella* levels in the chicks' faeces fell, thus justifying the presence in this extract of active molecules with anti-*salmonella* properties and especially against the *Salmonella typhimurium* strain.

As for the chicks in batch 4 that were contaminated and treated with oxytetracycline, *Salmonella* loads in the faeces also fell, confirming the anti-Salmonella activity of oxytetracycline, which is an antibiotic commonly used in poultry farming. The better activity of the aqueous extract of *Anogeissus leiocarpus* on *Salmonella* loads in chick faeces compared with that of oxytetracycline testifies to the presence of the essential active principles in this extract. However, unlike oxytetracycline, a pure chemical molecule, the aqueous extract of *Anogeissus leiocarpus is* thought to contain active ingredients that act synergistically. The ineffectiveness of oxytetracycline compared with aqueous extract of *Anogeissus leiocarpus* may be linked to the heavy use of this antibiotic in poultry farming, especially broiler rearing. Repeated heavy use of antibiotics leads to multiple resistance in bacterial strains.

Catalase is an enzyme that defends against reactive oxygen species. A number of researchers have shown that reduced catalase activity in animals can lead to an accumulation of highly toxic compounds and hydrogen peroxide that can cause stress. Moreover, a significant decrease in catalase activity indicates oxidative stress.[31] The results of the present study confirm those of these authors. Indeed, in this study, infection with the *Salmonella typhimurium* strain significantly decreased catalase activities in the breasts of kidneys, hearts, lungs and spleens compared with batch 1 control (uncontaminated and untreated chicks). Treatment of sick chicks with aqueous extract of *Anogeissus leiocarpus* resulted in an increase in catalase activities in kidneys, hearts, lungs and spleens compared with the contaminated and untreated batch of chicks (batch 2). The same treatment with aqueous extract of *Anogeissus leiocarpus* also normalised catalase activities in kidneys, hearts, lungs and spleens compared with the control batch 1 (NC-NT). In the present study, this significant increase in catalase activities demonstrates the antioxidant effect that this plant extract possesses. Indeed, the aqueous extract of *Anogeissus leiocarpus is* thought to act either by directly scavenging free radicals or by stimulating the production of antioxidant enzymes.[32,33]

Peroxidase is an enzyme that catalyses the reduction of hydroperoxides, including hydrogen peroxides, thereby protecting the cell from oxidative damage. According to some authors, infection with bacterial strains causes a significant reduction in peroxidase activity in chickens and rats. The same observations were made in our study. In fact, a significant decrease in peroxidase activity was observed in the sera and organs of chicks in batch 2 (C-NT) compared with batch 1 control. However, peroxidase activities increased significantly when the sick chicks were treated with the aqueous extract of *Anogeissus leiocarpus*. This extract even normalised peroxidase activities in sera and most organs compared with batch 1 control (NC-NT). This mechanism of action of our plant could be explained by the fact that the aqueous extract of *Anogeissus leiocarpus* should have stimulated an increase in peroxidase activities, which should consequently also have prevented an accumulation of excess free radicals and therefore prevented oxidative stress.[34,35]

Nitric oxide (NO) is a pathophysiological modulator produced by macrophages. This modulator can combine with oxygen, iron and thiols to exert bacteriostatic effects on *Salmonella*. However, its association with superoxides (O^{2-}) makes it bactericidal against Salmonella.[37] However, excess NO production can lead to oxidative stress. In this study, only the kidneys of contaminated and untreated chicks (batch 2) showed a highly significant increase in NO levels compared with batch 1 control (NC-NT). Treatment of the sick chicks with aqueous extract of *Anogeissus leiocarpus* reduced NO levels in the kidneys (batch 3) compared with batch 2 (C-NT). This treatment also led to a normalisation of NO levels in the kidneys compared with batch 1. These results may be explained by the fact that the aqueous plant extract of *Anogeissus leiocarpus* stimulates the chicks' immune system, and in particular macrophages, to fight against this strain of *Salmonella typhimurium*. Furthermore, the ability of this aqueous extract to regulate NO production is thought to be linked to both its anti-salmon and antioxidant activities. The antisalmonary and antioxidant activities of the aqueous extract of the leaves from *Anogeissus leiocarpus,* have already been demonstrated respectively. [36]

Malonaldehyde (MDA) is a good indicator of the level of lipid peroxidation. It is an end product of the oxidative degradation of unsaturated fatty acids. In addition, a bacterial infection, and therefore a *Salmonella* infection, can induce oxidative stress, which can lead to quantifiable lipid peroxidation. In this study, moderately significant and highly significant decreases were observed respectively in the livers and lungs of sick chicks treated with the aqueous extract of *Anogeissus leiocarpus* compared with batch 2 (C-NT). These results show that the aqueous extract of *Anogeissus leiocarpus* had a positive effect in

reducing MDA levels, thereby preventing the destruction of the membrane bilayer.

Generally speaking, in this study, the interesting antioxidant activities of the aqueous extract of *Anogeissus leiocarpus* are linked to the synergistic action of the secondary metabolites it contains. Indeed, several studies have already shown the presence of several groups of secondary metabolites in *Anogeissus leiocarpus* extracts, including phenolic compound. Some researchers have also confirmed that phenolic compounds, and flavonoids in particular, inhibit the formation of free radicals and prevent the oxidation of membrane proteins.[37,38]

CONCLUSION

Failure to control health risks in poultry farming is the cause of major economic losses due to bacterial infections such as Salmonellosis.The overuse of antibiotics to combat these infections has led to the phenomenon of bacterial resistance to these classic molecules. The use of medicinal plants is therefore seen as an alternative to conventional antibiotics. It is with this in mind that we proposed to carry out this study with a view to making our modest contribution to solving this problem.

At the end of this study, the aqueous extract of *Anogeissus leiocarpus*, used in the treatment of Salmonellosis induced in chicks during experimental rearing, gave the best results compared with oxytetracycline. These include significant improvement in average weight gain and biomarkers of oxidative stress. This plant has been shown to have a positive effect on feed conversion efficiency, improving digestibility and absorption.

The very interesting antioxidant activities of *Anogeissus leiocarpus* obtained in our previous studies could be linked to the synergistic action of the secondary metabolites it contains. Indeed, numerous studies have already revealed the presence of several groups of secondary metabolites, including phenolic compounds, in extracts of this plant.

In view of the results obtained from the present study, we can say that *Anogeissus leiocarpus* is of particular interest with a view to the development and use of phytobiotics as an alternative to antibiotics in poultry farming. We therefore believe that it would be useful to continue research on this plant to gain a better understanding of its antibacterial and antioxidant activities

As far as we are concerned, we believe that all these investigations could, in the long term, contribute to the development and marketing of inexpensive and accessible *A. leiocarpus-based* medicines, which could be effective in the fight against antibiotic resistance in poultry farming.

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Conflict of interests

The Authors state that they have no conflict interests in this article.

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