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Antisalmonellal and antioxidant potential of hydroethanolic extract of *Canarium schweinfurthii* Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks

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ABSTRACT

Objective: To evaluate the anti-infectious and antioxidant activities of hydroethanolic extract of *Canarium schweinfurthii* on broiler chickens infected by *Salmonella enterica* serovar Typhimurium, known to be threatening public health.

Methods: Animals were divided into six groups of eight animals per group: the normal control group, negative control group, positive control group and three test groups receiving the plant extract at 5, 19 and 75 mg/kg bw, respecively. The evolution of the disease as well as the effectiveness of the treatment were monitored by stool culture from the second day post infection until the end of the treatment. In addition, the effects of treatment on growth performances and feed conversion efficiency of broilers were evaluated. For the assessment of antioxidant status, enzymatic and non-enzymatic biomarkers such as catalase, glutathione peroxidase, malondialdehyde and nitric oxide were evaluated in the serum and tissues of animals.

Results: The infected chickens treated with oxytetracycline recovered on day 7 after treatment, while animals treated with 19 and 75 mg/kg of *Canarium schweinfurthii* extract recovered on day 9 and those with 5 mg/kg of the extract on day 10. *Salmonella* infection caused a decrease on catalase and glutathione peroxidase activities; the administration of various doses of *Canarium schweinfurthii* extract increased these enzymatic activities. Animals receiving the extract at 5 mg/kg showed a significant increase in catalase activity in serum, heart and lungs while all concentrations of the extract significantly increased glutathione peroxidase activity in the serum, liver and spleen. Concerning non-enzymatic biomarkers, *Salmonella* infection caused a significant increase of nitric oxide and malondialdehyde concentration in the liver and lungs. Treatment with 75 mg/kg of the extract significantly reduced nitric oxide concentration in the heart and lungs while each dose of the extract reduced and normalized the malondialdehyde level in the serum. Additionally, malondialdehyde production was significantly decreased in the liver, heart and lungs after administration of *Canarium schweinfurthii* extract at all doses.

Conclusions: The hydroethanolic extract of *Canarium schweinfurthii* attenuates oxidative stress, and is effective in the treatment of avian salmonellosis.

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1. Introduction

Salmonellosis caused by a Gram-negative bacteria called *Salmonella*, remains one of the most frequent foodborne zoonoses, constituting a worldwide major public health concern[1]. *Salmonella* are highly diversified with more than 2 600 serovars[2] among which some serotypes like *Salmonella* Gallinarum and *Salmonella* Pullorum are well adapted to birds and cannot be transmitted to the human. Avian salmonellosis caused by theses specific serovars is more severe but rare compared to those caused by *Salmonella* Enteritidis and *Salmonella* Typhimurium[3], which are very common. This pathology remains one of the limiting factors in the development of poultry farming because it causes heavy direct and indirect losses[4] mainly in developing countries of Africa and Asia[5]. When the chickens are infected by *Salmonella*, the infection is not only a source of foodborne human salmonellosis, but also a source of disease in the chicken itself. The large majority of salmonellosis in humans is carried by foodstuffs, mainly those of avian origin.

In humans, *Salmonella* can be divided into two major groups based on the disease symptoms: typhoidal *Salmonella* and non-typhoidal *Salmonella* (NTS). *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Choleraesuis account for the majority of NTS cases worldwide[6]. Ubiquitous NTS also found in poultry, globally causes more than 93.8 million cases of gastroenteritis and 155 000 deaths annually. In developing countries, they cause approximately 2.5 million cases of disease with approximately 4 100 deaths per year; these deaths include children younger than three years, people with malaria, and HIV-immunocompromised adults[7,8]. The commonest serotypes from poultry causing disease in humans are *Salmonella* Enteritidis and *Salmonella* Typhimurium[9] both belonging to the NTS. Similarly, typhoidal serotypes including *Salmonella* Typhi and *Salmonella* Paratyphi cause many deaths. The World Health Organization[10] estimated that 11-20 million people get sick from typhoid and between 128 000 and 161 000 people die from it every year.

Moreover, antimicrobial resistance in non-typhoidal *Salmonella* is considered one of the major public health threats related with food-animal production, including the poultry production chain and poultry meat, which is an additional concern in the management of salmonellosis[1]. In fact, the use of antibiotic agents to prevent or cure the diseases in chickens creates a selection pressure that favors the survival and emergence of antibiotic resistant pathogens. Resistance of *Salmonella* to commonly used antibiotics is increasing in the veterinary sector as well as public health sectors and this has emerged as a global problem. The multiple drug resistant *Salmonella* in chickens has been reported in various geographical regions of the world. In our country, Cameroon, as well as in other countries, several strains of *Salmonella* isolated from chicken have shown resistance to many antibiotics commonly used in human medicine[11,12] and some of these strains have been found in humans[11], testifying that some antibiotic-resistant *Salmonella* infections found in humans are acquired following the consumption of contaminated meat of animal origin.

Animals have similar connections to oxidative stress that happens in man. Like other species, birds rely on both exogenous and endogenous antioxidant defense systems[13] to fight against oxidants. *Salmonella* infection leads to the production of superoxide and nitric oxide (NO) radicals which react together and form peroxynitrite, a strong biological oxidant[14]. Consequently, pathological conditions characterized by oxidative stress can greatly result from *Salmonella* infection due to production of reactive oxygen species (ROS). ROS are the substances such as O_2-derived free radicals including hydroxyl ($HO^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), peroxyl ($RO_2^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals, or O_2-derived non radical species including hydrogen peroxide (H_2O_2)[14]. Over production or insufficient elimination of ROS can cause the oxidation of biomolecules like lipids, proteins and DNA. Lipids are the most likely susceptible to oxidation and the end product of lipid oxidation is malondialdehyde (MDA)[15]. In birds, endogenous antioxidant enzymes include superoxide dismutase, glutathione peroxidase (GSH-PX) and the product of the enzyme xanthine oxidase, uric acid[13]. During infection, inflammatory cytokines produced by the immune system can stimulate many types of cells such as host macrophages to synthesize huge amount of NO by an enzymatic protein that is known as inducible nitric oxide synthase (iNOS). It has been proven that the exploration of the biomarkers like MDA, NO and enzymatic antioxidant like catalase (CAT) and GSH-PX could help to assess the oxidative/antioxidative status in birds[16,17].

At a global scale, the main sources of *Salmonella* human infection include meat products especially the consumption of contaminated poultry meat, in spite of the success of *Salmonella* control programs applied in food-animal production of industrialized countries[1]. Both traditionally and pharmacologically, attention is now shifted to the use of plants as antimicrobial agents to cure the salmonellosis or to serve as a good source for new antibiotics[18,19]. Therefore, Waihenya *et al*.[20] and Ayachi *et al*.[21] showed respectively in their work that *Aloe secundiflora* and *Thymus vulgaris* are traditionally used for the treatment of human or avian salmonellosis. Plants are also recommended as an alternative to antibiotic growth promoters[22]. Since plants are reservoirs of many bioactive compounds, their exploration could help in the development of new and more effective drugs. Our previous study[23] revealed that the hydroethanolic extract of *Canarium schweinfurthii* (*C. schweinfurthii*) exhibited a crucial antibacterial activity against *Salmonella* Typhimurium, a non-typhoidal *Salmonella* having a public health significance. In this study, we therefore tested the antisalmonellal efficacy and antioxidant activities of this extracts on the *in vivo* model of broiler chicken orally infected by *Salmonella* Typhimurium.

2. Materials and methods

2.1. Plant collection and identification

C. schweinfurthii stem barks were harvested in Bamougoum, situated in Mifi division, West region of Cameroon, in July 2016.

This plant was identified at the National Herbarium Cameroon, Yaoundé, where a voucher specimen was deposited under the reference Number 16929/SRF/Cam.

2.2. Preparation of plant extract

The hydroethanolic extract was obtained by macerating the dried powder of the stem bark of *C. schweinfurthii* at room temperature in the mixture of solvent (*i.e.* water/ethanol 50/50, v/v) at rate of 1/3 (w/v). After 48 h, the mixture was filtered using Whatman NO.1 filter paper. The filtrate was concentrated at 60 ℃ in a rotary evaporator under reduced pressure. The residues which constitute the crude extract were recovered in flasks and then left in an oven at 40 ℃ until complete evaporation of the solvent. In our previous study[23], the hydroethanolic extract (HEE50/50) of *C. schweinfurthii* showed the best antimicrobial activity against *Salmonella* Typhimurium, that was why this extract was used to assess its *in vivo* therapeutic effect.

2.3. Animal model

A total of 48 one-day-old female and male Ross 308 broilers were obtained from a local hatchery and randomly allocated into several isolation units. At 12 days post hatch, chickens were housed in pairs in metallic cages with 50 cm of length × 50 cm of height × 50 cm of breadth, and then they were acclimated for 4 d. The protocol approval was not required at the time of the study as the ethics committee was established at the university after the experiment was completed. The experiment was conducted according to the principles and specific guidelines presented in Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd edition, 2010[24].

2.4. Test bacteria and culture media

The clinical bacterial isolates *Salmonella* Typhimurium was collected from Pasteur Centre, Yaoundé, Cameroon. The culture media used included: Mueller Hinton agar (MHA) and Salmonella-Shigella Agar (SSA). MHA was used for the activation of the bacterial isolate; SSA was used for the cultivation, identification and counting of *Salmonella* Typhimurium colonies in faecal matter from the animals during *in vivo* therapeutic studies.

2.5. In vivo therapeutic trial

2.5.1. Applied sanitary prophylaxis

Animals were vaccinated against infectious bronchitis (H120®) and Newcastle disease (Hitchner B®) on day 4 with a booster on day 18 and against Gumboro's disease (IBA Gumboro®) on day 11. At the beginning of the second week, the anticoccidial (Vitacox®) was administered for 3 d in a row each week in the drinking water until the end of the test. An antistress was given not only before and after each vaccination, but also after each handling of the animals (weighing, transfer of chicks from the chicken to the experimental building). The drinking troughs were cleaned daily with the help of jellied water. The footbath placed at the entrance of the livestock building was changed every other day.

2.5.2. Challenge with Salmonella Typhimurium

For salmonellosis induction, 1 mL of *Salmonella* Typhimurium suspension prepared at 10^5 CFU/mL[25] was orally administrated to each animal. The beak was kept closed for a few seconds to avoid the rejection of the inoculum. Infected animals were selected on the basis of their faecal bacterial load and clinical signs. Five days post infection, the plant extract and reference antibiotic were administered (between 8 and 9 o'clock a.m) by oral gavages to the birds at a rate of 1 mL of the therapeutic dose for a 750 g animal bw. The monitoring of the evolution of the bacterial load in chicken feces was carried out from the second day post infection until the end of the treatment.

2.5.3. Grouping of animal and treatment

Animals were grouped into six groups (G1, G2, G3, G4, G5 and G6), each containing eight animals. The animals were treated as follows:

G1 was not infected and received tap water during the treatment period (normal control group) whereas the rest of groups (G2 to G6) were infected.

G2 receive tap water during the treatment (negative control group),

G3 received oxytetracycline at 20 mg/kg bw during treatment (positive control group),

G4, G5 and G6 received the *C. schweinfurthii* extract at different concentrations respectively: 5 mg/kg bw corresponding to the therapeutic dose derived from the minimum inhibitory concentration of hydroethanolic extract of *C. schweinfurthii* against *Salmonella* Typhimurium[23], 19 mg/kg bw corresponding to a quarter of the dose of the traditional practitioner and 75 mg/kg bw corresponding to the daily dose given by traditional practitioner.

2.5.4. Bacterial evaluation

Twenty-four hours after inoculation, fresh faeces from each animal were collected and grown on SSA medium; at the same time, blood samples from each animal were collected and grown on SSA every two days. In the Petri dish which had been seeded with blood, the bacterial colonies appeared intermittently, which did not allow a good monitoring of the evolution of the bacterial load. The establishment of the infection was confirmed by the appearance of clinical signs such as diarrhea and the faecal excretion of *Salmonella*. To follow the effect of the plant extract as well as the efficacy of the treatment, the amount of bacterial colonies in the faecal samples was evaluated using the following protocol:

The fresh faecal matter of the each chicken was collected in sterile bottles every day before the treatment. Faecal suspensions were made (10 mg for 0.5 mL sterilized physiological water) and diluted appropriately. Fifty microliters (50 μL) of the resulting solution was spread on the surface of solidified SSA in the 55 mm type Petri dishes. After incubation for 24 h at 37 ℃, the number of colonies

following growth of *Salmonella* Typhimurium in each Petri dish was determined and recorded. The results were converted into the number of colonies per gram of faecal matter per animal. The decrease in faecal bacterial load during treatment was indicative of the *in vivo* antisalmonellal activity of *C. schweinfurthii* hydroethanolic extract (50:50; v/v). The chickens were monitored daily for clinical sign. No mortality was recorded in any of the treatment groups throughout this study.

2.6. Growth performances and relative organ weight determination

2.6.1. Food intake and body weight

The animals were provided with standard food and tap water was *ad libitum*. The amounts of food consumed (FC) were measured daily from the quantity of food supplied and the amount remaining after 24 h as follows:

FC (in g) =Total amount of food given – Amount of food remaining

Life body weight (BW) of each animal was recorded daily. Body weight gain (BWG) of each group of animals was the difference of average body weights during five consecutive days. Feed conversion efficiency was obtained by dividing FC during five day by BWG during that period.

2.6.2. Relative organ weight

The relative organ weight (ROW) of each animal was calculated as follows:

ROW(%)= [Absolute organ weight (g)× 100]/[Body weight of chicken on day of sacrifice (g)]

2.7. Sample collection

At the end of the treatment, the chickens were fasted for 12 h and then weighed, bled, plucked and eviscerated. The blood was allowed to clot by standing at 4 ℃ for 3 h and then centrifuged at 3 000 *g* for 15 min. Subsequently, the serum (supernatant) was isolated and stored at -18 ℃ before the analysis. The homogenate of each organ was prepared in phosphate buffer saline solution at the concentration of 15% (*i.e.* 15 g organ in 100 mL of solution)[26]. The homogenate samples were centrifuged at 3 000 *g* for 15 min, and the supernatants were then collected. Then sera and supernatants were further used for the analysis of biochemical antioxidant markers including CAT, GSH-PX, MDA, NO.

2.8. Determination of enzymatic and non enzymatic antioxidant markers

2.8.1. CAT assay

The method of Dimo *et al*.[27] was used to evaluate the CAT activity. Briefly, 10 µL of the serum or tissues homogenate was added into 150 µL of phosphate buffer (pH 7.4). Then 40 µL of H_2O_2 (50 mM) were also added. After 1 min, 400 µL of potassium dichromate (5%) prepared in the 1% of acetic acid was introduced in the reactional 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 "Jenway, model 1605". Enzymatic activity of CAT was inferred by the Beer-Lambert law[28] in mmole/min per milliliter of serum or gram of tissue.

2.8.2. GSH–PX assay

GSH-PX level was determined in tissue by Habbu *et al*.[29] method with slight modifications. Indeed, 250 µL of serum or organs homogenate was taken, and to this were added 500 µL of potassium iodide solution (10 mM) and 500 µL of sodium acetate (40 mM). The absorbance of potassium per iodide was recorded at 353 nm, which indicates the amount of peroxidase. Then 10 µL of H_2O_2 (15 mM) was added, and the change in the absorbance in 5 min was recorded. Enzymatic activity of peroxidase was expressed in term of µmole/min per milliliters of serum or gram of tissue by the Beer-Lambert law[28].

2.8.3. NO assay

The level of nitrite as a reflection of NO production was estimated using the Griess reagent. In brief, equal volumes of 170 µL of samples or nitrite (0.1 M sodium nitrite in water for standard curve) and 170 µL of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) were mixed and incubated in the dark at room temperature for 5 min. After the incubation, 170 µL of N-1-napthylethylenediamine dihydrochloride solution (0.1% N-1 napthylethylenediamine dihydrochloride in water) was added and incubated as in the previous condition. The concentration of NO was estimated by measuring the absorbance at 520 nm (spectrophotometer Jenway, model 1605) and the results were expressed in terms of micromoles of NO per gram of tissue or per milliliter of blood using a calibration curve of sodium nitrite (y=0.056 3x +0.107 7; r^2=0.983 5).

2.8.4. MDA assessment

MDA is an end product of lipid peroxidation, thus the amounts of MDA can be used to evaluate the degree of lipid peroxidation. The amount of MDA was measured in tissue using thiobarbituric acid test with the modified method of Atsafack *et al*.[30]. Briefly, 50 µL of homogenate, 250 µL of 1% orthophosphoric acid and 250 µL of a precipitating mixture (*i.e* 1% thiobarbituric acid and 1% acetic acid) were mixed. The mixture was homogenated and heated in a water bath for 15 min and immediately cooled. It was then centrifuged at 3 500 *g* for 15 min and the supernatant was recovered and its absorbance was recorded at 532 nm using a spectrophotometer "Jenway, model 1605". The amount of MDA was calculated based on its molar extinction coefficient (153 $mM^{-1}cm^{-1}$), and expressed in terms of micromoles of MDA per gram of tissue or per milliliter of blood.

2.9. Statistical analysis

The results were expressed as mean ± standard deviation. The statistical analysis was carried out using Statistical Package for Social Sciences Software program (release 16.0). Statistical analysis

of data was performed by one way analysis of variance (ANOVA) by General Linear Model's procedure followed by Waller-Duncan test for comparison between the treated and control groups. P values < 0.05 were considered significant.

3. Results

3.1. In vivo antisalmonellal assay

Figure 1 shows the evolution of the bacterial load throughout the experiment. The faecal load of *Salmonella* Typhimurium increased after the infection. The number of colonies was progressively decreased in the infected animals treated with the plant extract or with oxytetracycline. Infected animals treated with oxytetracycline recovered on day 7 after the start of treatment while animals treated with the *C. schweinfurthii* extract at 19 and 75 mg/kg bw on day 9 and those treated at 5 mg/kg bw of extract on day 10.

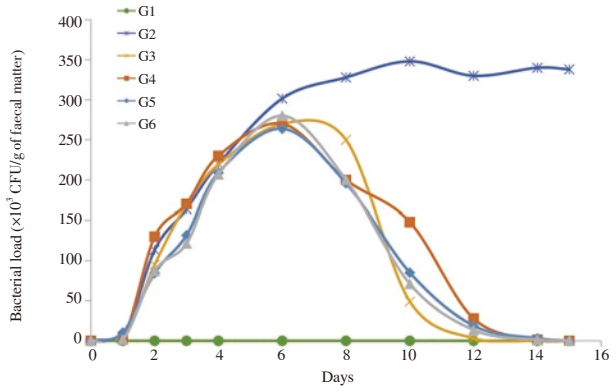

Figure 1. Number of colonies of *Salmonella* Typhimurium in faeces of infected chickens. G1 = normal control received distilled water, G2 = negative control (infected by *Salmonella* Typhimurium and received distilled water), G3= positive control (received oxytetracycline at 20 mg/kg bw), G4, G5 and G6 were treated with 5, 19 and 75 mg/kg bw of extract, respectively. Infection started at 0 day and treatment at day 5 post infection.

3.2. Effect of treatment on growth performances and feed conversion efficiency

Table 1 presents the effect of the different doses of *C. schweinfurthii* extract on the growth performances and feed conversion efficiency of broiler chickens. The plant extract did not change BWG during the treatment. Compared with negative control group, the food intake significantly increased in 19 mg/kg treated group at 5 and 10 days post infection; the same variation was observed in 5 mg/kg treated group at 10 days post infection. However, in 75 mg/kg treated group, a significant reduction of feed intake was observed at 15 days post infection. Compared to normal control, the infection (*i.e.* in the negative control group) did not affect the growth performance during the entire period of experiment. Ten days after (*i.e.* 15 days post infection) treatment, food conversion ratio for group treated with 19 mg/kg and 75 mg/kg was significantly reduced in comparison to normal and negative control groups.

3.3. Effect of the treatment on ROW

Table 2 shows the relative weight of organs at the end of treatment. Compared to the normal control, the infection did not cause any significant variation in the relative weight of the organs. However, there is a decrease in the relative weight of the liver, kidney, spleen and lungs. Compared with negative control group, the infected animals treated with different doses of the extract showed no significant variation in their relative organs weight.

3.4. Effect of treatment on enzymatic and non enzymatic antioxidant parameters

3.4.1. Effect of treatment on CAT in serum and tissues

The activity of CAT in serum and organs is presented in Table 3. In kidney, heart and lungs, the activity of CAT was significantly reduced with the infection whereas in the serum, liver and spleen, the

Table 1. Growth performance and feed conversion efficiency.

Groups	Body weight gain (g)			Feed intake (g)			Feed conversion efficiency (g/g)		
	5 DPI	10 DPI	15 DPI	5 DPI	10 DPI	15 DPI	5 DPI	10 DPI	15 DPI
Negative control	322.25±15.43^{b}	352.00±21.52^{a}	328.25±102.74^{a}	501.39±3.56^{b}	593.00±24.90^{b}	470.06±34.46^{c}	1.55±0.02^{ab}	1.68±0.12^{a}	1.43±0.10^{d}
Oxytetracycline 20 mg/kg	255.00±40.66^{a}	328.50±63.76^{a}	324.25±43.29^{a}	448.25±1.25^{a}	526.00±34.00^{a}	333.25±10.75^{a}	1.76±0.11^{b}	1.60±0.03^{a}	1.03±0.06^{ab}
Normal control	323.00±56.76^{b}	344.75±39.85^{a}	287.25±30.73^{a}	505.44±29.59^{b}	582.92±19.82^{ab}	386.14±36.67^{abc}	1.58±0.24^{ab}	1.69±0.11^{a}	1.34±0.13^{cd}
Extract 5 mg/kg	341.50±28.40^{b}	306.50±26.80^{a}	353.75±33.12^{a}	506.33±18.33^{b}	772.08±4.41^{c}	444.00±69.00^{bc}	1.48±0.07^{a}	2.51±0.04^{c}	1.25±0.16b^{cd}
Extract 19 mg/kg	335.50±17.71^{b}	357.00±13.39^{a}	360.75±13.54^{a}	573.75±0.25^{c}	764.00±26.00^{c}	392.75±68.75^{abc}	1.71±0.01^{ab}	2.14±0.12^{b}	1.09±0.20^{abc}
Extract 75 mg/kg	318.25±39.44^{ab}	368.00±79.68^{a}	377.75±57.01^{a}	500.00±0.50^{b}	592.75±45.25^{b}	348.50±47.00^{ab}	1.57±0.02^{ab}	1.62±0.18^{a}	0.92±0.16^{a}

In each column, values with different letters are significantly different (P<0.05; Waller-Duncan test). DPI: days post infection.

Table 2. Relative weight of organs (g of organ/g of body weight).

Groups	Liver	Kidney	Heart	Lungs	Spleen
Negative control	2.11±0.36^{a}	0.68±0.17^{a}	0.65±0.14^{a}	0.53±0.09^{a}	0.08±0.01^{a}
Oxytetracycline 20 mg/kg	2.55±0.29^{a}	0.81±0.04^{a}	0.65±0.02^{a}	0.50±0.04^{a}	0.10±0.04^{a}
Normal control	2.32±0.24^{a}	0.78±0.06^{a}	0.65±0.06^{a}	0.56±0.04^{a}	0.10±0.02^{a}
Extract 5 mg/kg	2.42±0.24^{a}	0.75±0.09^{a}	0.66±0.06^{a}	0.59±0.07^{a}	0.08±0.02^{a}
Extract 19 mg/kg	2.57±0.11^{a}	0.82±0.11^{a}	0.61±0.05^{a}	0.51±0.04^{a}	0.09±0.02^{a}
Extract 75 mg/kg	2.57±0.38^{a}	0.74±0.06^{a}	0.65±0.05^{a}	0.61±0.09^{a}	0.13±0.04^{a}

In each column, values with different letters are significantly different (P<0.05; Waller-Duncan test).

difference was not significant. As compared to the negative control group, animals receiving the extract at 5 mg/kg showed a significant increase in serum, heart and lungs. The same significant variation was observed at 75 mg/kg of extract treatment in lungs.

3.4.2. Effect of treatment on total peroxidase in serum and tissues

The activity of the total peroxidises in serum, liver, kidney, heart, lungs and spleen is presented in Table 4. The infection (negative control group) generally induced a significant decrease in peroxidase activity ($P<0.05$) compared to normal control group. In comparison to negative control, treatment of the extract at three doses significantly increased peroxidase activity in the serum, liver and spleen. This significant increase ($P<0.05$) was also noted in the kidneys and lungs only at doses 5 and 19 mg/kg. Moreover, compared to the normal control, the treatment of the extract at 5 and 19 mg/kg normalized the activity of peroxidase especially in most organs of the animals.

3.4.3. Effect of treatment on NO

Table 5 presents the tissues and serum level of NO. It can be noted that compared to normal control group, the infection led to the increase of NO levels in the liver, kidneys and lungs. The treatment with different doses of *C. schweinfurthii* extract decreased the NO concentration. Compared to the negative control, the treatment with 5, 19 and 75 mg/kg of extract significantly reduced the level of NO in the kidneys and spleen; treatment with 75 mg/kg of extract significantly reduced NO concentration in the heart and lungs.

3.4.4. Effect of treatment on MDA

The effect of treatment on MDA level in the serum and tissues is presented in the Table 6. As compared to the normal control group, *Salmonella* infection caused a significant increase of MDA concentration in the serum, liver and lungs. The treatment with the plant extract reduced and normalized the MDA level in the serum at all the doses. Significant decreases of MDA level were also found in the liver, heart and lungs of treatment groups.

Table 3. Serum and tissue catalase activities.

Groups	Serum (mmol/min/mL)	Liver (mmol/min/g)	Kidney (mmol/min/g)	Heart (mmol/min/g)	Lungs (mmol/min/g)	Spleen (mmol/min/g)
Negative control	0.183±0.123^{a}	0.765±0.019^{bc}	0.613±0.027^{a}	1.047±0.073^{ab}	1.170±0.092^{a}	0.607±0.023^{b}
Oxytetracycline 20 mg/kg	0.231±0.026^{bc}	0.650±0.050^{ab}	0.671±0.022^{a}	1.115±0.074^{bc}	1.603±0.218^{cd}	0.517±0.103^{ab}
Normal control	0.207±0.023^{abc}	0.850±0.083^{c}	0.856±0.060^{b}	1.653±0.074^{d}	1.636±0.141^{cd}	0.539±0.033^{ab}
Extract 5 mg/kg	0.222±0.026^{bc}	0.840±0.139^{c}	0.695±0.098^{a}	1.196±0.141^{c}	1.684±0.156^{d}	0.451±0.044^{a}
Extract 19 mg/kg	0.215±0.021^{abc}	0.692±0.053^{b}	0.622±0.065^{a}	1.070±0.101^{abc}	1.346±0.045^{ab}	0.549±0.101^{ab}
Extract 75 mg/kg	0.193±0.017^{ab}	0.577±0.003^{a}	0.700±0.114^{a}	0.960±0.052^{a}	1.460±0.137^{bc}	0.517±0.062^{ab}

In each column, values with different letters are significantly different ($P<0.05$; Waller-Duncan test).

Table 4. Serum and tissue peroxidase activities.

Groups	Serum (μmol/min/mL)	Liver (μmol/min/g)	Kidney (μmol/min/g)	Heart (μmol/min/g)	Lungs (μmol/min/g)	Spleen (μmol/min/g)
Negative control	0.350±0.021^{a}	1.439±0.041^{a}	1.519±0.016^{a}	1.215±0.011^{b}	1.716±0.009^{a}	1.507±0.050^{a}
Oxytetracycline 20 mg/kg	0.582±0.004^{c}	1.705±0.041^{d}	1.717±0.043^{b}	1.553±0.075^{d}	1.761±0.031^{bc}	1.796±0.017^{b}
Normal control	0.612±0.024^{c}	1.651±0.038^{cd}	1.772±0.028^{c}	1.439±0.020^{c}	1.799±0.021^{cd}	1.754±0.026^{b}
Extract 5 mg/kg	0.506±0.046^{b}	1.673±0.035^{cd}	1.740±0.007^{bc}	1.224±0.072^{b}	1.811±0.022^{d}	1.756±0.049^{b}
Extract 19 mg/kg	0.622±0.054^{c}	1.634±0.039^{c}	1.744±0.027^{bc}	1.239±0.082^{b}	1.831±0.036^{d}	1.743±0.070^{b}
Extract 75 mg/kg	0.508±0.058^{b}	1.564±0.039^{b}	1.536±0.039^{a}	1.020±0.034^{a}	1.736±0.022^{ab}	1.765±0.015^{b}

In each column, values with different letters are significantly different ($P<0.05$; Waller-Duncan test).

Table 5. Serum and tissue nitric oxide contents.

Groups	Serum (μmol/mL)	Liver (μmol/g)	Kidney (μmol/g)	Heart (μmol/g)	Lungs (μmol/g)	Spleen (μmol/g)
Negative control	0.131±0.040^{ab}	2.900±0.149^{b}	3.398±0.101^{c}	1.574±0.101^{bc}	3.503±0.082^{c}	2.636±0.037^{c}
Oxytetracycline 20 mg/kg	0.123±0.029^{a}	2.757±0.060^{ab}	2.855±0.170^{b}	1.439±0.106^{ab}	3.344±0.114^{b}	2.740±0.085^{d}
Normal control	0.155±0.035^{abc}	2.633±0.344^{a}	2.655±0.154^{a}	1.684±0.048^{c}	3.327±0.081^{b}	2.836±0.057^{e}
Extract 5 mg/kg	0.168±0.048^{abc}	2.833±0.059^{ab}	2.881±0.046^{b}	1.469±0.103^{ab}	3.479±0.026^{c}	1.708±0.076^{b}
Extract 19 mg/kg	0.249±0.144^{c}	2.985±0.017^{b}	2.841±0.054^{b}	1.574±0.012^{bc}	3.468±0.004^{c}	1.609±0.014^{a}
Extract 75 mg/kg	0.237±0.053^{bc}	2.743±0.148^{ab}	2.609±0.081^{a}	1.331±0.118^{a}	3.190±0.074^{a}	1.641±0.020^{ab}

In each column, values with different letters are significantly different ($P<0.05$; Waller-Duncan test).

Table 6. Serum and tissue malondialdehyde levels.

Groups	Serum (μmol/mL)	Liver (μmol/g)	Kidney (μmol/g)	Heart (μmol/g)	Lungs (μmol/g)	Spleen (μmol/g)
Negative control	0.039±0.000^{b}	0.169±0.003^{c}	0.093±0.003^{b}	0.216±0.009^{d}	0.289±0.003^{e}	0.112±0.004^{a}
Oxytetracycline 20 mg/kg	0.033±0.000^{a}	0.120±0.004^{a}	0.086±0.002^{a}	0.174±0.005^{c}	0.256±0.002^{c}	0.108±0.004^{a}
Normal control	0.034±0.003^{a}	0.129±0.001^{b}	0.093±0.006^{b}	0.223±0.009^{d}	0.226±0.004^{a}	0.111±0.002^{a}
Extract 5 mg/kg	0.032±0.001^{a}	0.113±0.007^{a}	0.096±0.002^{bc}	0.156±0.008^{b}	0.278±0.007^{d}	0.113±0.006^{a}
Extract 19 mg/kg	0.032±0.001^{a}	0.114±0.005^{a}	0.101±0.004^{c}	0.134±0.005^{a}	0.257±0.005^{c}	0.114±0.003^{a}
Extract 75 mg/kg	0.032±0.000^{a}	0.117±0.002^{a}	0.096±0.001^{bc}	0.161±0.010^{bc}	0.248±0.002^{b}	0.109±0.002^{a}

In each column, values with different letters are significantly different ($P<0.05$; Waller-Duncan test).

4. Discussion

Salmonella infects a wide array of animal hosts and is the causative agent of human infection which usually comes from livestock in the form of meat, eggs and similar products. Animal models are essential to providing understanding of pathogenesis as it helps to extrapolate the results to humans[19,31]. In this study; we used broiler chickens not for this purpose but because the large majority of salmonellosis in humans are carried by foodstuffs, mainly those of avian origin. Therefore controlling avian salmonellosis could significantly reduce the prevalence of human gastroenteritis. There are two major forms of diseases that occur in humans namely enteritis and systemic typhoid[31] depending on involved *Salmonella* serotypes. We used *Salmonella* Typhimurium in this experiment as model organisms because *Salmonella* Typhimurium is among the most incriminated pathogens involving in foodborne human salmonellosis (*i.e.* human gastroenteritis). In this study, chickens were kept in metallic cage from the infection to the treatment periods and this model presented many advantages as described by Bjerrum *et al*.[32] in their research work where infected chicken were kept in isolators. In fact, this enabled us to easily control the infection by preventing problem of reinfection, avoiding the spread of bacteria between groups and contamination of the environment. It is important to point out that the nature and severity of the disease in chickens are dependent on the infecting serovar, breed and the genetic background, age and immune status of the bird[33]. In this study, we used broilers chicken of 12 days posthatch because it was previously demonstrated that *Salmonella* Typhimurium infection in chickens may elicit gastroenteritis in young birds; however, adult birds can serve as lifetime hosts for this organism without showing signs of infection[34]. Similarly, severe systemic disease cannot be reproduced in immunocompetent adult birds[3,35].

In this study, the *in vivo* antisalmonellal efficacy of the *C. schweinfurthii* extract has been highlighted. The *in vivo* therapeutic efficacy of several plant extracts[19,20,30,36] against several *Salmonella* serotypes has been already demonstrated using several animal models (rats and broilers). Most of these extracts treated salmonellosis in the same time as conventional drugs. It has been demonstrated that the ability of *Salmonella* to persist and multiply within chicken macrophages is essential for *Salmonella* pathogenesis and the establishment of a systemic infection. The establishment of infection was characterized in the infected broiler chickens by the appearance of clinical signs such as diarrhea, weakness and the increase of *Salmonella* Typhimurium faecal load. An intermittent appearance of *Salmonella* Typhimurium was observed in the blood culture of infected chickens suggesting that bacteria have challenged the non-specific defense mechanism of birds but are momentarily present in the blood stream or tissues like liver and spleen. The intermittent appearance of faecal *Salmonella* was previously observed by Tchoua[37] in an experimental model of chicken infected and treated by various doses of *Erica mannii*. These results suggest that the faecal or blood load of *Salmonella* greatly depends on the age of broiler chickens. The intermittent appearance of *Salmonella* in negative control group can be explained by the fact that the immune system of chickens tried to stop the systemic *Salmonella* expansion but did not promote a sufficient host's defense. One day after the start of treatment, a slight increase in the bacterial load was observed in infected animals treated with the different doses of extract and antibiotic (oxytetracycline), after this phase, a progressive decline in the fecal load of these treated animals was observed up to elimination of this load between 7 and 10 days. This reduction may be due to the combined action of the *C. schweinfurthii* extract and immune system of bird since a slight decrease of bacterial load was also observed in negative control group animal[19,36]. Previous investigation demonstrated that oral administration of ginseng stem-leaf saponins to chickens could enhance the immune response[38,39]. The *in vivo* antisalmonellal efficacy of hydroethanolic extract of *C. schweinfurthii* could be explained by the presence of phenols, flavonoids, saponins, steroids and terpenoids in this extract as previously assessed[23]. Indeed, some compounds belonging to certain classes of these secondary metabolites (terpenoids and flavonoids) are well known for their antisalmonellal activities[40,41]. At the molecular level, compounds such as gallic acid and scopoletin found in plants belonging to *Canarium* genus[42] could act synergistically and could be partly responsible for the anti-infectious activity of *C. schweinfurthii*. Indeed, it has been shown that in addition to its immunomodulatory effect[43], scopoletin reduces the intracellular survival of *Salmonella typhi* within U937 human macrophage cell line[44]. Gallic acid has an antioxidant activity in addition to its *in vitro* and *in vivo* antibacterial effect against *Salmonella* Typhimurium[45]. The ability of the extract of *C. schweinfurthii* to cure salmonellosis in broilers could be explained by its ability to directly kill *Salmonella* and/or boost the immune system of the host.

During the treatment of salmonellosis, it was also important to evaluate the effect of *C. schweinfurthii* extract on the growth performance of broiler chickens. The food conversion ratio is a parameter that helps to better evaluate the conversion rate of input in term of food intake into output *i.e.* body weight gain. At end of the treatment (15 DPI), food conversion ratio for groups treated with 19 mg/kg and 75 mg/kg of *C. schweinfurthii* extract was significantly reduced compared to normal and negative control groups, suggesting that the extracts at these concentrations have positive effect on food conversion efficiency. This effect could be due to the increase in the BWG which is directly linked to the food intake. The decrease in feed conversion ratio indicates that the administration of these doses (19 mg/kg and 75 mg/kg) could improve the digestibility of food and promote better absorption of the nutrients. The present results corroborate the findings of Kana *et al*.[22] which suggest that supplementing broilers diet with *Afrostyrax lepidophyllus* bark indicated favorable influences on growth performance, blood serum components and reduced the cost of production.

As compared to all controls, the infected animals treated at different doses of the extracts showed no significant variation in their relative organs weight. This preliminary result suggests that actives compounds present in this extract are not toxic to chickens.

The present results corroborate the findings of Sokoudjou[46] who revealed that different doses of the stem bark extracts of *Ceiba pentandra* have no significant effect on the relative weight of the organs of rats. These results are also comparable to those of Onu[47] who revealed that feeding 0.25% of the gingember, garlic and the gingember-garlic mixture to broiler chickens has no significant effect on the relative weight of the liver and the heart.

It is well known that the *Salmonella* infection is accompanied by the oxidative stress[19,30,36,37]. In the living body, free radicals can generate ROS in cells, which can contribute to cell and tissue damage. Therefore, the ability of a cell to maintain functional homeostasis depends on either the fast induction of protective antioxidant enzymes or abilities of non enzymatic antioxidants to scavenge oxidant species. Antioxidant enzymes are considered to be a primary defense against cell oxidant damage. The enzymatic antioxidants like CAT and peroxidase (GPx) are widely distributed in all animal tissues, and their greatest activity is present in the red blood cells. CAT within the peroxisomes and cytosolic GPx, are implicated in the conversion of H_2O_2, a powerful and potentially harmful oxidizing agent, into H_2O and O_2[48]. Therefore, CAT and GPx, essential components of the endogenous antioxidative defense system, act by maintaining the cellular redox balance[30]. As for bird, they have the ability to convert highly reactive hydrogen peroxide into water.

The effect of treatment on CAT activity of the serum and tissues of broiler chickens was evaluated. CAT is known as the most important cellular antioxidant. It can degrade O_2 and decompose H_2O_2, and result in a decrease in oxidative stress, which is an effective way of protection from ROS attack. In living systems, the oxidant/antioxidant status is kept in balance by CAT. In kidney, heart and lungs, the activity of CAT was significantly reduced with *Salmonella* Typhimurium infection. This reduction of the CAT activity in negative control group may indicate an insufficient elimination of hydrogen peroxide which can lead to oxidative stress. According to Yu *et al*.[16], significant reduction of the activities of enzymatic antioxidant like CAT indicates oxidative stress. As compared to the negative control group, animals receiving the extract at 5 mg/kg showed a significant increase in CAT activity of serum, heart and lungs. The same significant variation was observed at 75 mg/kg for lungs CAT activity. This significant increase of CAT activity indicates the antioxidant effect of *C. schweinfurthii* extract. This plant extract can act either by scavenging ROS or by enhancing the expression of genes that code for CAT involved in antioxidant function[16,49].

The infection induces a significant reduction of peroxidase activity in infected chickens. It had previously been shown that *Salmonella typhi* infection in rats leads to a significant decrease in peroxidase activity[19]. The decomposition of hydrogen peroxide outside the mitochondria may cause damage to the outer mitochondrial membrane because the reaction of hydrogen peroxide with superoxide anion leads to the hydroxyl radical which is highly detrimental compound for biomolecules (DNA, lipids and proteins)[15,49]. Indeed, like CAT, GSH-PX participates in the regulation of hydrogen peroxide, a compound that is relatively toxic to the body[15,48]. These enzymes are in the main protective system because they destroy not only H_2O_2, but also toxic organic peroxides formed by oxidation of fatty acids or cholesterol[15]. The administration of the various concentrations of extract of *C. schweinfurthii* increases the activities of peroxidase in chickens, preventing the accumulation of excess free radicals that could lead to oxidative stress.

The effect of treatment on the non enzymatic antioxidant markers as MDA and NO was also evaluated. Intestinal epithelial cells are the first line of defense against enteric infections and the role of macrophages is crucial in *Salmonella* infection and pathogenesis. These macrophages produce NO which is a physiopathological modulator, depending on it levels in biological system. Indeed, the resulting NO, or its products coming from its interactions with oxygen (O_2) or iron and low-molecular weight thiols, are preferentially bacteriostatic for *Salmonella*, whereas reaction of NO and superoxide (O_2-) generates peroxynitrite ($ONOO^-$) which is a bactericidal compound for *Salmonella*[14]. The antisalmonellal activity of reactive nitrogen species emanates from the modification of redox active thiols and metal prosthetic groups of key molecular targets of the electron transport chain, DNA and DNA-associated proteins, transcription factors and central metabolic enzymes[14]. Several studies have indicated that low relative concentrations of NO seem to favor cell proliferation and antiapoptotic responses and higher levels of NO favour pathways inducing cell cycle arrest, mitochondria respiration, senescence, or apoptosis. During infection, *Salmonella* displays a plethora of defenses that modulate the delivery of iNOS-containing vesicles to phagosomes, scavenge and detoxify reactive nitrogen species, and repair biomolecules damaged by these toxic species[14]. In turn, overproduction of NO may lead to oxidative stress. In this study, *Salmonella* infection induced a significant increase of NO mainly in liver, kidneys and lungs. In infected group treated with the extracts, there was a decrease in NO concentration; so tracking of NO could prevent the negative effects of it overproduction (*i.e.* oxidative stress). This antioxidative activity could be attributed to the presence of bioactive compounds in *C. schweinfurthii* extract which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. This result corroborates the *in vitro* finding of Sokoudjou *et al*.[23] who showed that extracts from this plant have a relative NO scavenging activity, suggesting that this plant can be used to control or manage oxidative and nitrosative stresses linked to the *Salmonella* infection. In this study, the infection leads to the increase of NO while *C. schweinfurthii* extracts reduced the NO concentration, which suggests that the active compounds of the extract may interact with the splenic macrophages to reduce NO production.

MAD is formed as an end product of lipid peroxidation[15], therefore the extent of lipid peroxidation by ROS is commonly monitored by MDA levels[16,17]. It can be noted that as compared to the normal control group, *Salmonella* infection leads to a significant increase of MDA concentration in the serum, liver and lungs, indicating the increased lipid oxidation especially oxidation of polyunsaturated fatty acids. This finding corroborates several previous works[19,30]. This could be due to the increase of the free

radical formation and decrease of the antioxidant enzymes. The treatment with the plant extract reduces and normalizes the MDA level in the serum at all the doses. Significant decreases of MDA level were also found in the liver, heart and lungs after treatment. These results suggest that this extract exerts its antioxidant activity by preventing oxidation of tissues by free radicals.

In conclusion, the *in vivo* antisalmonellal evaluation of the hydroethanolic extract of *C. schweinfurthii* revealed that it can be successfully used in the treatment of avian salmonellosis. Infected animals treated with the *C. schweinfurthii* extract at 19 and 75 mg/kg bw recovered on day 9 after the beginning of the treatment. We also evaluated in the present experiment the effect of *C. schweinfurthii* extract on antioxidant profile of broilers chickens; the results showed that it may contribute to an improvement in the health status and growth performance of infected birds. However, a plant may be very active, but also toxic; thus further studies will be done on its toxicological properties as well as side effects associated to the treatment. Further investigations will also help to clarify the mechanism of action of the hydroethanolic extract of *C. schweinfurthii*.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Authors' contributions

DG and JRK designed the study and supervised the work. JBS took part in all the experiments as principal investigator. FGD and ABF contributed to the assessment of antisalmonellal activities. NK contributed to the evaluation of antioxidant properties and data analysis. GTK and SPCF contributed in manuscript writing and editing. All authors discussed results and approved the final manuscript.

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