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Research Article

SALMONELLA TYPHIMURIUM INFECTION: ANTIOXIDANT STATUS OF INFECTED POULTRY AND EFFICACY OF TREATMENT WITH *KHAYA GRANDIFOLIOLA* EXTRACT

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ABSTRACT

Salmonella infections remain one of the major health problems in both poultry farming and human medicine. In addition, resistance to *Salmonella* has emerged as a global health problem in both sectors. The present study aimed at evaluating the *in vivo* antisalmonellal and antioxidant activities of 95° ethanol extract of *Khaya grandifoliola* using broiler chickens as animal model. Animals were divided into the normal control group, negative control group, positive control group and three test groups treated with the extract at 10, 20 and 40 mg/kg respectively. The antioxidant status of broiler chickens was also evaluated by measuring the concentration of enzymatic antioxidants (catalase, glutathione peroxidase) and biochemical markers (malondialdehyde and nitric oxide). The results showed that infected animals treated with the *Khaya grandifoliola* extract at 40 mg/kg recovered on day 11 after the beginning of the treatment and on day 13 for those treated at the 10 and 20 mg/kg. The antioxidant assay showed that the infection led to the reduction of enzymatic markers in the body of infected animals, while the treatment increases such makers. The infection resulted in a significant increase in serum and pulmonary malondialdehyde. It also caused a significant decrease in cardiac and pulmonary nitric oxide whereas the treatment depending on the doses of the extract tends to normalize these biochemical markers. The overall results showed that *Khaya grandifoliola* extract can be successfully used in the treatment of avian salmonellosis as well as the management of the oxidative stress caused by the infection.

Keywords: *Salmonella* Typhimurium, *Khaya grandifoliola*, antisalmonellal potential, antioxidant status, broiler chickens.

INTRODUCTION

Salmonellosis is a major cause of bacterial enteric illness in both human and animals[1]. *Salmonella* infections remain one of the major health problems in both poultry farming and human medicine. The prevalence of *Salmonella* varies geographically. Numerous studies report the persistence of *Salmonella* infections in poultry farms in Cameroon[2,3], with a prevalence of 66.66% in the Menoua subdivision[3]. Chaiba and Rhazi Filali[4] showed that 24% of broiler farms in Morocco are infected with *Salmonella* spp. In other developing countries, available studies reveal 100% infection in Thailand[5]; 28.6% in Senegal[6] and 21.7% in Brazil[7]. In the countries of the European Union, the prevalence of *Salmonella* has been estimated at an average frequency of 29.7% and 23.7% of the flocks of laying hens and broilers respectively, with large variations between countries and regions[8]. The level of contamination of poultry by *Salmonella* in UK, Malaysia, Greece, Belgium, Spain and Netherlands ranged from 20% to 89%[9]. The *Salmonella* serovars frequently isolated from poultry include *Salmonella* Enteritidis, *Salmonella* Hadar, *Salmonella* Virchow and *Salmonella* Typhimurium. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most prevalent serovars and constitute a health hazard for humans leading to gastroenteritis.

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[10]. *Salmonella* colonizes the gastrointestinal system of poultry, mainly the cecum, where it can persist for a relatively long time, attached to the intestinal epithelium[11]. In poultry, these infections lead to 20% mortality[12], but exceptionally up to 100%. Like infectious diseases, *Salmonella* infections are usually accompanied by oxidative stress[10, 13] and the exploration of some enzymatic and non-enzymatic biomarkers could help to evaluate the oxidative profile during the treatment of birds.

To overcome the mortality problems and poor growth associated with *Salmonella* infections, farmers resort to antibiotics. 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 has been studied[14,15,16] and is increasing in the veterinary sector as well as public health sectors and this has emerged as a global problem. In view of these problems, many governments prohibit the use of antibiotics and advocate other therapeutic strategies (including probiotics, barrier flora and plants). Thus, the use of plants can be considered especially as they have the advantage of being eco-protective. The ethnoveterinary use of plants in the control of avian salmonellosis has also been reported in several studies[10,17]. To our knowledge, no herbal medicine has yet been formulated to control avian salmonellosis. *Khaya grandifoliola* is used in traditional medicine against malaria[18], liver diseases[19] and many infectious diseases. Thus, do *Khaya grandifoliola* extracts contain substances with antisalmonellal and antioxidant activities? The objective of this study was to evaluate the *in vivo* therapeutic

effect of the 95° ethanolic extract of *Khaya grandifoliola* using broilers infected with the *Salmonella* Typhimurium serotype, the serotype responsible for gastroenteritis in humans.

MATERIALS AND METHODS

Collection and identification of plants

The *Khaya grandifoliola* stem barks were collected in Foumban, located in the Noun division, West region of Cameroon, in May 2017. This plant was identified at the National Herbarium Cameroon, Yaoundé, where a voucher specimen was deposited under the reference Number 52661HN. The collected stem barks were air dried at room temperature (23 ± 2 °C) and crushed into fine particles.

Preparation of plant extract of *Khaya grandifoliola*

For the preparation of hydro-ethanolic extracts, 100 g of powdered stem bark were macerated three times at room temperature in 1000 ml of ethanolic solvent (95° EtOH; 70% EtOH; 50% EtOH; 30% EtOH) for 48 hours, then filtered with Whatman No. 1 filter paper. The filtrate was concentrated at 60 °C using a rotary evaporator (Büchi R200) and the resulting solutions were collected in flasks and then left in an oven at 45 °C until complete evaporation of the solvent. All extracts were stored at 4 °C until further use. In our previous study the hydroethanolic extract (95° EtOH) of *Khaya grandifoliola* showed the best antimicrobial activity against *Salmonella* Typhimurium[20], that is why this extract was used to assess its *in vivo* therapeutic effect.

In vitro antisalmonellal assay

Animal model

A total of 48 16-day-old broiler chicks were obtained from the suppliers with their certificates of origin and good health, and randomly allocated into several isolation units.

The chickens used for antisalmonellal testing were housed in pairs in metallic cages (50cm long x 50cm high x 50cm wide) at the Animal Facility of the Department of Biochemistry at University of Dschang, and then they were acclimated for 4 days prior to the actual experiments. Food and water were provided to them at will. 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[21]. The study was also conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals as described in "the European Community guidelines, EEC Directive 86/609/EEC'' (EEC, 1986), on the use of animals for scientific research.

Bacteria and culture media

The clinical bacterial isolates named *Salmonella* Typhimurium (STM) was obtained from the Medical Laboratory of Bacteriology of the Centre Pasteur of Cameroon. The culture media used included Mueller Hinton agar (Italy Liofilchem) and Salmonella Shigella Agar (Italy Liofilchem) for the activation or maintenance of the *Salmonella* isolates, and SSA was used for the cultivation, identification and counting of *Salmonella* Typhimurium colonies in faecal matter from the animals during *in vivo* therapeutic studies.

Preparation of inocula

Bacterial cell suspensions were prepared at 1.5×10^8 CFU/ml corresponding to 0.5 McFarland turbidity. For this purpose, 18 hours old bacterial cultures were prepared on Salmonella Shigella Agar. From this culture, a few colonies of bacteria were aseptically collected with a sterile wire loop and introduced into 10 ml of sterile saline water (0.9%). These suspensions were diluted 100 times with MHB to yield approximately 1.5×10^6 CFU/mL before use.

Applied sanitary prophylaxis

An antistress was given before and 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.

Induction of salmonellosis with Salmonella Typhimurium

Except for the chicks of group 0 which each received 1 ml of physiological water, each of the 40 animals of the other batches (deprived of food overnight) received orally 1 ml of a solution containing 1.5×10^6 CFU/mL of *Salmonella* Typhimurium. The beak was kept closed for a few seconds to avoid the rejection of inoculum[22]. Infected animals were selected on the basis of their faecal bacterial load and clinical signs. Five days after infection, the plants extracts and standard antibiotic were administered orally between 8 and 9 o'clock am to the birds, at a rate of 1 ml of the therapeutic dose for a 750 g animal. 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.

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 distilled water during the treatment period (normal control group) whereas the rest of groups (G2 to G6) were infected.

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

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

G4, G5 and G6 received the *Khaya grandifoliola* extract at different concentrations respectively: 10 mg/kg bw corresponding to the therapeutic dose derived from the minimum inhibitory concentration of 95° hydroethanolic extract of *Khaya grandifoliola* against *Salmonella* Typhimurium[20], 20 mg/kg corresponding to a quarter of the dose of the traditional practitioner and 40 mg/kg corresponding to the daily dose given by traditional practitioner.

Bacterial evaluation

Twenty-four hours after inoculation, fresh faeces from each animal were collected and grown on SSA medium. 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 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 were spread on the surface of solidified SSA in the 55 mm type Petri dishes. After incubation for 24 h at 37 ºC, 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 *Khaya grandifoliola* extract (95° EtOH). The chickens

were monitored daily for clinical sign. No mortality was recorded in any of the treatment groups throughout this study.

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 ºC for 3 h and then centrifuged at 3 000 × g for 15 min. Subsequently, the serum (supernatant) was isolated and stored at -18 ºC 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)[23]. 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, POD, Superoxide dismutase, MDA, NO.

Evaluation of the enzymatic parameters

Superoxide dismutase assay

Superoxide dismutase activity was estimated by Kakkar *et al*.[24] method. The reaction mixture which contained 0.1 mL of phenazine methosulfate (186 µL), 1.2 mL of sodium pyrophosphate buffer (0.052 mL; pH 7.0), 0.3 mL of the supernatant after centrifugation (1500 × g for 10 min followed by 10000 × g for 15 min) of homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 mL of NADH (780 µM) and stopped after 1 min by adding 1 mL of glacial acetic acid. The amount of chromogen formed was measured by recording color intensity at 560 nm. Results were expressed in units/mg protein.

CAT (catalase, EC 1.11.1.6) activity

Catalase activity was determined in tissues and serum by kodjio *et al*.[13]. A total of 50 µL of the homogenates was added to tubes containing 750 µL of phosphate buffer (pH 7.4) and 200 µL of 50 mmol/L H_2O_2. After one minute incubation at room temperature, 2 mL of dichromate was added. The mixture was homogenized and incubated at 100 °C for 10 min then cooled in ice bath and the absorbance was recorded at 570 nm using a spectrophotometer (MultiSpec-1501 Schimadzu, Japan). One unit of activity is equal to one mmol/L of H_2O_2 degraded per minute and is expressed as units per milligram of protein.

POD (Peroxidase EC 1.11.1.7) assay

Peroxidase activity was determined in tissues and serum by Habbu *et al*.[25] method. To 0.5 mL tested sample were added 1 mL of 10 mM KI solution and 1 mL of 40 mM sodium acetate. The absorbance of potassium iodide was read at 353 nm, which indicates the amount of peroxidase. Then 20 µL of H_2O_2 (15 mM) was added, and the change in the absorbance in 5 min was recorded. Units of Peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity was expressed in terms of units per mg of proteins.

Non enzymatic parameters

Estimation of lipid peroxidation

The extent of peroxidation in tissues and serum was assessed by measuring the level of malondialdehyde (MDA) according to the method of Kodjio *et al*.[13] with some modifications. A total of 0.5 mL of 1% orthophosphoric acid and 0.5 mL of precipitating mixture (1% thiobarbituric acid, 1% acetic acid) were added to 0.1 mL of tested sample. The mixture was homogenized and heated in boiling water for 15 min and cooled immediately. It was then centrifuged at 5000×g for 10 min and the absorbance of the supernatant was recorded at 532 nm using a spectrophotometer (MultiSpec-1501 Schimadzu, Japan). The peroxidation in the tissues was calculated based on the molar extinction coefficient of malondialdehyde (MDA) (153 $mM^{-1}cm^{-1}$), and expressed in terms of micromoles of MDA/g of tissue.

Nitric oxide (NO) assay: Determination of nitrite concentration

This assay relies on a diazotization reaction was carried out by the modified method of Kodjio *et al.*[13]. The Griess Reagent System is based on the chemical reaction which uses sulfanilamide and N-1-napthylethylenediamine dihydrochloride (NED) under acidic conditions, freshly prepared, and protected from light. To 340 µL of the experimental sample, 340 µL of freshly prepared 1% Sulfanilamide in 5% orthophosphoric acid were added after 5 min of incubation in the dark at room temperature, 340 µL of the NED Solution (0.1% NED in water) were added. The resulting solution was well mixed and then incubated at room temperature for 5 min, protected from light. The absorbance of the colored azo compound formed was measured at 520 nm within 30 minutes. A standard curve was plotted using nitrite ($NaNO_2$) (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM). The results were expressed as Micromolar of Nitrite Equivalents (µMNE) per gram (g) of tissue or per millilitre (mL) of blood.

RESULTS

Efficacy of treatment with *Khaya grandifoliola* extract

The figure below presents the evolution of the faecal bacterial load of the infected and treated chickens during the experiment. It appears that the bacterial load of uninfected and untreated animals remained constant during the experiment. The faecal bacterial load of infected and treated animals shows a bell-shaped pattern with three phases: a latency phase lasting one day where the number of colonies hardly changes; a growth phase during which the bacterial load increases and reaches a peak (for treated animals) on the 3rd day after infection: this phase attests to the infection; Finally, a phase of decline during which the bacterial load gradually decreases, reflecting the effectiveness of the treatment. The animals infected and treated with the different doses of the extract were cured on days 11 after the start of treatment for those treated at the 40 mg/kg dose, and on day 13 for those treated at the 10 and 20 mg/kg doses. Animals treated with oxytetracycline recovered 7 days after starting treatment.

Antioxidant status of infected poultry

Effects of the different doses of treatment on the activity of SOD

Table 1 shows the tissue and serum SOD activity of the treated animals. It was found that the infection caused a significant decrease ($p<0.05$) in serum, cardiac and pulmonary SOD. Compared with the negative control, treatment of the animals at different doses of the extract resulted in a significant increase ($p<0.05$) in cardiac SOD activity. Likewise, an increase in the activity of SOD is noted in the other organs except in the lungs, the liver and the serum respectively for the doses of 10 mg/kg, 20 mg/kg and 40 mg/kg of the extract. The 20 mg/kg and 40 mg/g doses of the extract rather normalized the activity of this parameter in the heart compared to the neutral control.

Effects of different doses of treatment on catalase activity

Table 2 shows the tissue and serum catalase activity of the treated animals. It appears that the infection generally resulted in a decrease in catalase activity in all the organs studied compared to the neutral control. Treatment of animals with the 40 mg/kg dose resulted in an insignificant ($p\geq0.05$) increase in catalase activity in serum. This increase was significant ($p<0.05$) in the liver where the treatment normalized ($p\geq0.05$) this activity compared to the neutral control. All doses of the extract resulted in a significant increase ($p<0.05$) in pulmonary catalase activity and normalized this activity compared to the neutral control.

Effects of the different doses of the treatment on the activity of POD

Table 3 shows the tissue and serum POD activity of the treated animals. It appears that compared to the neutral control, the infection caused a significant decrease ($p<0.05$) in serum and cardiac POD. Treatment of animals with the 20 and 40 mg/kg doses resulted in a significant increase ($p<0.05$) in serum and cardiac POD activity compared to the negative control. The 20 mg/kg of the extract caused a significant increase ($p<0.05$) in pulmonary POD activity and the 40 mg/kg concentration resulted in a normalization of this value compared to the neutral control.

Effects of different doses of treatment on the activity of malondialdehyde

Table 4 shows the activity of tissue and serum malondialdehyde in treated animals. It was found that compared to uninfected and untreated chickens, infection resulted in a significant increase ($p<0.05$) in serum and pulmonary malondialdehyde. Treatment of animals with all doses of the extract resulted in a significant decrease ($p<0.05$) in serum malondialdehyde activity compared to the negative control. The 40 mg/kg of the extract resulted in a significant decrease ($p<0.05$) in pulmonary malondialdehyde activity until normalization.

Effects of different doses of treatment on the activity of nitric oxide (NO)

Table 5 shows the tissue and serum nitric oxide activity of the treated animals. It was found that the infection caused a significant decrease ($p<0.05$) in cardiac and pulmonary nitric oxide, and a significant increase ($p<0.05$) in serum. Treatment of animals with all doses resulted in a significant decrease ($p<0.05$) in serum nitric oxide level compared to the negative control and normalized this level at 10 mg/kg and 40 mg/kg. Except for the 10 mg/kg, all other doses of the extract normalized this parameter in the lungs compared to the neutral control.

Table 1: Effects of the different doses of the treatment on the activity of SOD

Doses/ Samples	10 mg/kg	20 mg/kg	40 mg/kg	Neutral control	Neutral control	Negative control
	Quantity of Super Oxyde Dismutase (UI/mg of proteins)					
Serum	74.28 ± 3.53^{d}	72.70 ± 3.42^{cd}	57.62 ± 3.10^{ab}	67.36 ± 7.74^{cd}	63.40 ± 8.44^{bc}	48.76 ± 6.98^{a}
Heart	27.57 ± 0.77^{c}	22.13 ± 1.83^{b}	21.76 ± 1.61^{b}	19.63 ± 0.72^{b}	19.45 ± 3.07^{b}	11.08 ± 0.93^{a}
Liver	5.20 ± 0.72^{b}	4.03 ± 0.36^{a}	6.48 ± 0.49^{c}	3.39 ± 0.32^{a}	3.73 ± 0.44^{a}	3.92 ± 0.47^{a}
Lungs	1.95 ± 0.29^{a}	3.75 ± 0.26^{b}	5.67 ± 0.37^{c}	4.10 ± 0.22^{b}	4.22 ± 0.46^{b}	1.96 ± 0.25^{a}

Table 2: Effects of different doses of treatment on catalase activity

Doses/ Samples	10 mg/kg	20 mg/kg	40 mg/kg	Positive control	Neutral control	Negative control
	Catalase quantity (mmol/min/g of organ and mmol/min/ml of blood)					
Serum	0.12 ± 0.024^{ab}	0.099 ± 0.03^{a}	0.15 ± 0.027^{bc}	0.19 ± 0.02^{d}	0.18 ± 0.02^{cd}	0.14 ± 0.03^{bc}
Heart	19.53 ± 1.77^{a}	36.34 ± 5.94^{bc}	31.58 ± 3.16^{ab}	61.68 ± 10.68^{d}	50.83 ± 19.97^{cd}	35.85 ± 4.11^{bc}
Liver	72.75 ± 3.17^{c}	66.49 ± 3.33^{bc}	58.39 ± 7.57^{b}	76.92 ± 5.90^{c}	58.28 ± 10.27^{b}	43.65 ± 4.20^{a}
Lungs	73.44 ± 6.28^{bc}	67.01 ± 6.45^{b}	75.82 ± 3.21^{bc}	83.14 ± 4.21^{c}	71.48 ± 3.25^{bc}	39.99 ± 5.64^{a}

Table 3: Effects of the different doses of the treatment on the activity of POD

Doses/ Samples	10 mg/kg	20 mg/kg	40 mg/kg	Positive control	Neutral control	Negative control
	Quantity of POD (mmol/min/g of organ and mmol/min/ml of blood)					
Serum	3.01 ± 0.677^{ab}	3.61 ± 0.33^{b}	3.744 ± 0.32^{b}	3.36 ± 0.45^{ab}	4.90 ± 0.55^{c}	2.83 ± 0.44^{a}
Heart	1.79 ± 0.26^{ab}	3.47 ± 0.86^{d}	2.41 ± 0.39^{bc}	2.65 ± 0.22^{c}	2.11 ± 0.48^{bc}	1.23 ± 0.47^{a}
Liver	2.00 ± 0.40^{b}	1.75 ± 0.36^{ab}	1.85 ± 0.39^{b}	1.70 ± 0.44^{ab}	1.22 ± 0.32^{a}	1.39 ± 0.38^{ab}
Lungs	4.16 ± 0.53^{a}	5.33 ± 0.37^{b}	3.87 ± 0.48^{a}	4.06 ± 0.16^{a}	3.65 ± 0.26^{a}	3.65 ± 0.30^{a}

Table 4: Effects of different doses of treatment on the activity of malondialdehyde

Doses/ Samples	10 mg/kg	20 mg/kg	40 mg/kg	Positive control	Neutral control	Negative control
	Quantity of malondialdehyde (µmol/g of organ and µmol/ml of blood)					
Serum	0.013 ± 0.006^{a}	0.009 ± 0.004^{a}	0.010 ± 0.005^{a}	0.013 ± 0.003^{a}	0.016 ± 0.003^{a}	0.055 ± 0.016^{b}
Heart	0.577 ± 0.036^{b}	0.460 ± 0.039^{ab}	0.389 ± 0.211^{a}	0.452 ± 0.041^{ab}	0.480 ± 0.041^{ab}	0.581 ± 0.065^{b}
Liver	1.214 ± 0.378^{c}	0.437 ± 0.074^{a}	0.560 ± 0.059^{ab}	0.529 ± 0.077^{ab}	0.501 ± 0.023^{ab}	0.725 ± 0.076^{b}
Lungs	0.411 ± 0.093^{ab}	0.408 ± 0.090^{ab}	0.334 ± 0.030^{a}	0.472 ± 0.017^{b}	0.358 ± 0.030^{a}	0.457 ± 0.070^{b}

Table 5: Effects of different doses of treatment on the activity of nitric oxide (NO)

Doses/ Samples	10 mg/kg	20 mg/kg	40 mg/kg	Positive control	Neutral control	Negative control
	Quantity of Nitric oxide (NO) (µmol/g of organ and µmol/ml of blood)					
Serum	3.42 ± 0.40^{ab}	3.09 ± 0.51^{a}	3.37 ± 0.38^{ab}	3.76 ± 0.78^{ab}	4.01 ± 0.51^{b}	5.00 ± 0.54^{c}
Heart	0.47 ± 0.05^{a}	0.50 ± 0.07^{a}	0.54 ± 0.05^{ab}	0.69 ± 0.05^{b}	1.51 ± 0.13^{d}	1.27 ± 0.20^{c}
Liver	1.62 ± 0.12^{b}	0.60 ± 0.12^{a}	0.80 ± 0.09^{a}	1.41 ± 0.20^{b}	1.55 ± 0.16^{b}	1.50 ± 0.15^{b}
Lungs	1.37 ± 0.18^{ab}	1.47 ± 0.18^{bc}	1.67 ± 0.11^{cd}	1.75 ± 0.09^{d}	1.70 ± 0.12^{cd}	1.13 ± 0.20^{a}

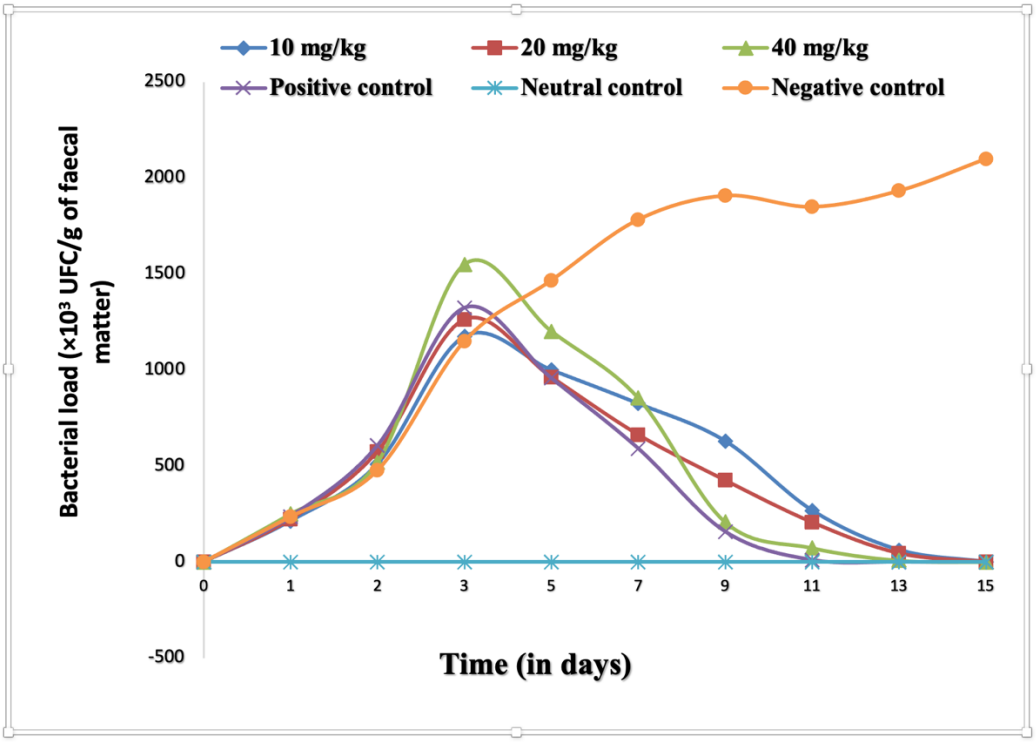

Figure 1. Evolution of the number of colonies of *Salmonella* Typhimurium in faeces of broiler chickens.

DISCUSSION

Antisalmonellal potential of the *Khaya grandifoliola* extract

Poultry can become infected with many different types of *Salmonella*; about 10 percent of all *Salmonella* spp. have been detected in poultry. The most important are *Salmonella* Typhimurium and *Salmonella* Enteritidis[1]. The large majority of salmonellosis in humans is carried by foodstuffs, mainly those of avian origin[10]. Therefore, controlling avian salmonellosis could significantly reduce the prevalence of human gastroenteritis. The therapeutic efficacy of several plant extracts against several *Salmonella* serotypes using several animal models (rats and broilers) has been demonstrated[13,17,26,27]. The establishment of *Salmonella* infection in broilers was manifested by diarrhea, anorexia, somnolence, poor growth and weakness, which corroborates the work of Ali and Sultana[1] which states that in case of fowl typhoid, infected birds will have a yellow or greenish diarrhoea mainly in adults but may also in young birds. The animals infected and treated with the different doses of *Khaya grandifoliola* were cured on day 11 after the start of treatment for those treated at the 40 mg/kg dose, and on day 13 for those treated at the 10 and 20 mg/kg. These results agree with the research work of Sokoudjou *et al.*[10] who showed that the infected animals treated with the *Canarium schweinfurthii* extract at 19 and 75 mg/kg bw recovered on day 9 after the beginning of the treatment. The antisalmonellal activities obtained suggest that the extract has retained its properties because the work of Yamako Konack *et al.*[20] showed that the 95° ethanol extract of *Khaya grandifoliola* was active *in vitro* with a minimum inhibitory concentration of 128 µg/ml against *Salmonella* Typhimurium.

At the chemical level, compounds belonging to polyphenols, tannins, alkaloids, flavonoids, saponins, anthocyanins and anthraquinons[20] could be responsible for the *in vivo* antisalmonellal activity of *Khaya grandifoliola*. This antisalmonellal properties can be due to the action of one or more specific active components of the extract; this explanation corroborates the finding of Sokoudjou *et al.*[28,29] who showed that some compounds including scopoletin and canarimoic acid isolated from the stem barks of *Canarium schweinfurthii* were active against *Salmonella* Enteritidis and *Salmonella* Typhimurium. In addition, some compounds belonging to the classes of terpenoids, flavonoids, polyphenols and alkaloids are well known for their antisalmonellal activities[28,30-33]. Finally, this antisalmonellal potential in broilers may be linked to the ability of these secondary metabolites to directly kill *Salmonella* or boost the immune system of the host.

Effect of the *Khaya grandifoliola* extract against the stress induced by infection

The role of free radicals and active oxygen is well recognized in the pathogenesis and evolution of many enteric illness such as salmonellosis in both human and animals[10,13,34,35]. In the scientific community, it is well known that salmonellosis is due to the formation of the active metabolite (superoxide radical). This interacts with molecular oxygen to form the hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). Both radicals are capable of binding to proteins and other macromolecules with simultaneous attack on poly-unsaturated fatty acids to produce tissue peroxidation of the cells[13,36].

The antioxidant defense mechanisms include enzymatic and non-enzymatic antioxidants, playing a significant role in sustaining the physiological levels of O_2 and H_2O_2 and eradicating the peroxides generated from bacterial infection and inadvertent exposure to toxic drugs. Any natural medications with antioxidant profiles may help maintaining health when continuously taken as components of dietary food, spices, or remedies. Among the antioxidants are enzymes such as superoxide dismutase, catalase and peroxidase[13,37,38]. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are enzymatic antioxidants widely distributed in all animal tissues, and the highest activity is found in the red blood cells. SOD, CAT and POD decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals[37,39]. Therefore, the reduction in the activity of SOD, CAT and POD in negative control animals may result in several

deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. In our study, treated groups with 40 mg/kg of *Khaya grandifoliola* extract generally showed significant increase in SOD activity, and this could be responsible for the cure effect of extract. In fact, the administration of 90° ethanolic extract to treated chicken enhanced the superoxide dismutase, catalase, and peroxidase profiles, dose-dependently, by acting as a strong free radical quencher and protecting the tissues. Therefore, superoxide dismutase, peroxidase and catalase are essential for the endogenous anti-oxidative defense system to scavenge reactive oxygen species and maintain the cellular redox balance.

Lipid peroxidation (LPO) is an auto-catalytic, free-radical mediated, destructive process, whereby polyunsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides[40]. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde[41]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism[42]. Thiobarbituric acid reactive substance (TBARS) levels were measured as a marker of LPO and malondialdehyde (MDA) production. Malondialdehyde is an endogenous genotoxic product of enzymatic and ROS-induced LPO whose adducts are known to exist in DNA isolated from healthy human being[43]. In our study, the level of TBARS in the extract treated groups decreased when compared to negative control. This decrease in the TBARS levels may indicate increase in the activities of glutathione peroxidase and hence inactivation of LPO reactions[44].

Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals[45]. The results of this study showed that the infection leads to an imbalance of cytosolic redox status in favor of prooxidants, putting organs in a state of oxidative stress[13]. The increased level of NO in the tissues of negative control animals suggests that the macrophages have excessively produced that compound to destroy the *Salmonella* Typhimurium; there was an imbalance between prooxidants and antioxidants. Indeed, high levels of NO favor pathways inducing cell cycle arrest, mitochondria respiration, senescence, or apoptosis[46]. In this study, treatment of animals with all doses of *Khaya grandifoliola* resulted in a significant decrease in serum nitric oxide level compared to the negative control and normalized this level at 10 mg/kg and 40 mg/kg. These results corroborate the findings of Sokoudjou *et al.*[10] who showed that the infection of broiler chickens leads to the increase of NO while the treatment with *Canarium schweinfurthii* extracts reduced the NO concentration suggesting that the active compounds of the extract may interact with the splenic macrophages to reduce NO production.

CONCLUSION

In conclusion, the *in vivo* antisalmonellal potential of the 95° ethanol extract of *Khaya grandifoliola* was evaluated and the results revealed that the doses 10, 20 and 40 mg/kg can be successfully used in the treatment of salmonellosis in broiler chickens. We also evaluated during the experiment the effect of *Khaya grandifoliola* on antioxidant profile of broilers chickens; the results showed that it may contribute to an improvement in the health status of infected birds. Further studies need to be done on the toxicity of *Khaya grandifoliola* as well as its side effects because a plant may be pharmacologically active but remains toxic for the sick organism.

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