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Research Article

Antioxidant Effect of Absolute Ethanolic Extract of *Enantia chlorantha* Stem Bark on Typhoid Fever-Induced Wistar Rats

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ABSTRACT

Antioxidants are substances which are capable to protect the body from damages caused by free radical. A variety of free radical scavenging antioxidants exists in the body and many of them are derived from plant sources. The aim of the present study was to investigate the antioxidant activity of the absolute ethanolic extract of *Enantia chlorantha* stem bark on typhoid fever induced rats. *Salmonella* typhi infected rats were treated every day between 7 to 9 AM with different doses of plant extract (9.16, 36.5 and 73 mg/ kg or oxytetracyclin 20 mg/ kg). After 09 days of treatment, the results show that the different doses of this extract have all contributed to the cure of the infected rats, healing marked by the absence of salmonella in animal's blood cultured on *ss* agar. In the other hand, treatment with different doses of plant extract result in a relative and significant *($p < 0.05$)* increase in the level of some blood and tissues antioxidant parameters such as CAT, SOD and POD while the level of alkaline phosphatase, malondialdehyde and nitric oxide as compared to untreated control group animal as compared to the untreated control group animals. Whereas, the level of ALP, MDA and NO were significantly decreased *($p < 0.05$)*. These results showed that the absolute ethanolic extract of *E. chlorantha* stem bark possess antioxidant and antityphoid properties necessary to eradicate the free radical produced during typhoid fever infection.

Keywords: *Enantia chlorantha*; Typhoid Fever; Antioxidant Activity; Oxidative Stress

INTRODUCTION

Typhoid fever is a global health problem and a systemic infection caused by *Salmonella* typhi or *Salmonella enterica* serotype typhi, with more than 22 million cases and 216500 deaths estimated annually [1]. This human pathogen bacterium is transmitted indirectly by ingestion of faecally contaminated food, milk, or water or directly from person to person, with the strongest burden in developing countries due to poor sanitary conditions [2]. From 10 to 14 days after human infection, the bacterium causes low grade fever, headache, white patches in the center of tongue, inflammed bones, abdominal pain, agitation, bloody stool, malaise, loss of appetite, constipation or diarrhea, bradycardia and myalgia. The fever rises to a high plateau, the spleen and liver become enlarged, and rose spots on the skin of the abdomen or chest are seen in rare cases [3]. In order to reduce the prevalence and the complications of this disease, antibiotics such as ampicillin, trimethoprim, chloramphenicol, streptomycin and sulfamethoxazole are used in many countries. Because of the greater resistance to all the three first line antimicrobials (i.e. chloramphenicol, ampicillin and co-trimoxazol) [4] and the medullary toxicity of chloramphenicol which had been for long the drug of choice for the treatment of typhoid fever [5], it is important to look for the new therapeutic options. Natural substances in general and particularly medicinal plants are mostly used worldwide and principally in developing countries [6]. Since bacterial infection like others produces an acute inflammation, it's make the lymphoid tissues stand out from the surrounding mucosa. Moreover, the entrance of *Salmonella* into the body causes the production of superoxide and nitric oxide which react together to form peroxynitrite, a strong biological oxidant [7] indeed, during aerobic respiration in most cells, including prokaryotic cells, reactive oxygen intermediates are produced at low rates. To cope with oxidative stress, bacteria have evolved protective responses that enable them to withstand the survive [8]. In addition, large quantities of reactive oxygen intermediates are produced by phagocytes during uptake of microorganisms when bacteria interact with an eukaryotic host [9]. These reactive oxygen species can lead to serious health problems including sickle cell diseases, atherosclerosis, heart failure, chronic fatigue syndrome [10]. Thus, if the bacteria are prevented from producing reactive oxygen compounds, it can contribute to efficiently fight against the microorganisms. On the other hand, antioxidant compounds such as polyphenols, phenolic acids, flavonoids, and carotenoids [11] are thought to prevent chronic complications in part through their ability to scavenge free radicals [12]. Thus, it may be interesting to find a medicinal plant with dual antimicrobial and antioxidant properties [13]. *Enantia chlorantha*, the subject of our study, is a plant whose demonstrated efficacy in the treatment of diseases such as tuberculosis, malaria [14], urinary tract infections, jaundice [15], diarrhea [16], cough and wounds [17], gastric ulcers [18], infective hepatitis, rickettsia fever, typhoid fever [19]. This plant is also known as the African yellow wood. It's commonly found in the central and Southern forest zones of Cameroon [13]. Its stem bark is used in Cameroon for the management of typhoid fever. This study was designed to assess the antioxidant effect of absolute ethanolic extract of *Enantia chlorantha* stem bark in the typhoid fever-Induced Wistar Rats.

MATERIALS AND METHODS

Plant Material: Collection and Identification

The stem bark of *Enantia chlorantha* was collected in Lekie, Central region of Cameroon, in March 2014. Identification of the plant was done at the National Herbarium, in Yaoundé-Cameroon, using a voucher specimen registered under the reference N°25918/ SRFCAM. The stem bark were air-dried at room temperature (23 ± 2°C) away from sunlight and milled to coarse particle at the Biotechnology Centre, University of Yaounde I.

Extract preparation

(100g) of *Enantia chlorantha* Stem bark powdered were macerated three times at room temperature (23 ± 2°C) in 1000ml absolute ethanolic solvent (95% EtOH) for 48 hours, and then filtered with Whatman paper (N°1). The filtrate was concentrated at 45°C using a rotary evaporator (Buchi R200) and the obtained volume was later dried at 40°C. The plant extracts were stored in sterilized bottles at room temperature until usage.

Animals

Adult males and females Wistar (RjHan: WI) rats of 120 to 140 g and 8 to 9 weeks old used in this study were obtained from the animal house of the Department of Biochemistry, University of Dschang, Cameroon for the study. They were kept in rat cages at room temperature (23 ± 2°C) with free access to rat food and water. The experiments were performed in accordance with the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration # 173/CPCSEA, of January 28, 2000), India, on the use of laboratory animals.

Experimental design

Animals were randomly grouped into six groups of eight animals each (males and females) after one week of acclimatization. Except

the animals from group I, the rest were infected by receiving oral administration of 1 mL of the $1.5x10^8$ CFU of *S.* typhi preparation. During the experiment, animals of group I were not infected and were treated only with DMSO (%), and thus serve as neutral control. Animal of group II were infected and non-threated (control group) while those of group VI were treated with oxytetracyclin (positive control). The remaining group animals were treated after infection with different doses of *E. chlorantha* that was 9.16 mg/ kg, 36.5 and 73 mg/ kg respectively for group III, IV and V.

Confirmation after treatment

One milliliter of blood from the treated animals was drawn from the codal vein every two days of treatment and inoculated on already prepared *SS* Agar on petri dishes. The inoculated plates were incubated at 37°C for 24 h. The counts of emerged colonies (black color presentation) were used to evaluate the efficacy of treatment.

Dissection and Blood collection

At the end of the experiment, animals were fasted overnight on the 8th day of treatment. They were then anaesthetized using chloroform vapors, dissected and the blood samples were collected by cardiac puncture in the pain tubes. Serum was obtained by centrifugation of these tubes at 3000 rpm for 10 minutes. Fifteen percent (15%) homogenate of organs (Liver, kidney, lung, heart and spleen) were prepared in normal saline solution, and then centrifuged at 3000 rpm for 15 minutes. The supernatant and sera were used for the determination of biochemical parameters related to oxidative stress such as catalase (CAT), Peroxidase (POD), Malondialdehyde (MDA), nitric oxide (NO), Superoxide Dismutase (SOD), and Alkaline Phosphatase (AP).

BIOCHEMICAL ANTIOXIDANT PARAMETERS

Enzymatic parameters

Catalase assay: Catalase level was evaluated in serum and tissue as described by [20]. Ten microliters of the serum or tissues homogenate were added into 150 µL of phosphate buffer pH 7.4. Then 40 µl of H_2O_2 (50 mM) were also introduced. After 1 minute, 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 cooled immediately. The absorbance was recorded at 570 nm using spectrophotometer "Schimadzu 1501, Japan". Enzymatic activity of catalase was inferred by the Beer-Lambert law [21] in mmol/ min per milliliter of serum or gram of tissue.

Peroxidase assay: Peroxidase level was determined in tissues as described by [22] with slight modifications. Two hundred and fifty microliters of serum or organs homogenates were was taken, and to this were added 500 µl of 10 mM KI solution and 500 µl of 40 mM sodium acetate. The absorbance of potassium per iodide was read at 353 nm, which indicates the amount of peroxidase. Then 10 µl of 15 mM H_2O_2 was added, and the change in the absorbance in 5 min was recorded. Enzymatic activity of peroxidase activity was expressed in µmole/ min per milliliter of serum or gram of tissue by the Beer-Lambert law [21].

Superoxide Dismutase (SOD) assay: SOD activity was determined in tissue by [23] method with some modifications. To 150 µL of homogenates 1 650 µL of phosphate buffer (pH 7.2) and 200 µL of 0.3 mmol/ L epinephrine was also added. The self-oxidation of epinephrine was recorded at 480 nm 30 seconds and then, one minute after its addition by spectrophotometer (Schimadzu 1501, Japan). The SOD activity expressed as percentage of inhibition was calculated taking into account that 50% inhibition correspond to one unit of activity.

Alkaline phosphatase assay: Serum markers namely alkaline phosphatase level was estimated based on colorimetric method using commercial kits (IMNESCOWiedtalstr, Germany respectively) and spectrophotometer "Schimadzu 1501, Japan".

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 [24] 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 rpm for 10 min and the absorbance of the supernatant was recorded at 532 nm using Schimadzu 1501 spectrophotometer, 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.

Determination of nitrite oxide concentration (NO)

This assay relies on a diazotization reaction that was described by Griess [25]. With some modifications. The Griess Reagent is made up of a freshly prepared sulfanilamide and N-1-Napthyl Ethylenediamine Dihydrochloride (NED) under acidic conditions and protect from light. To 340µL of the experimental sample, 340 µL of freshly prepared 1% Sulfanilamide in 5% orthophosphoric acid were added and after 5 min of incubation in the dark at room temperature (23 ± 2°C), 340µL of the NED Solution (0.1% NED in water) were also added. The resulting solution was well mixed and then incubated away from light 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, and 12.5µM). The results were expressed as Micro molar of Nitrite Equivalents (µMNE) per gram (g) of tissue or per milliliter (mL) of blood.

Statistical analysis

Data obtained were expressed as mean ± SEM (standard error of mean) and were statistically analyzed using one-way ANOVA with the Statistical Package for Social Sciences (SPSS) version 16.0 software. Post hoc analysis using Waller Duncan test was used to compare means of different groups. A *p*-value of 0.05 was considered statistically significant.

RESULTS

In vivo therapeutic test

Infection and treatment: Infected rats presented erect hairs (a sign of fever) and diarrhea. They looked drowsiness, weak and were less active. The slender body became more bulky. Trace of blood and mucus were noted in stool. These symptoms traduced the establishment of the infection in these animals which was clearly revealed by the growth of *Salmonella* colonies on *SS* agar Petri dishes after blood culture. The evolution of the bacterial load in the blood of test animals during the experiment is summarized in (Figure 1). It can

be noted from this figure that the bacteria load increase exponentially during the first three day following the infection. During the first two days following the treatment there were a slightly increased in blood bacteria load. The bacteria load in negative control was significantly high *($p < 0.05$)* at the end of the treatment as compared to that of rats receiving different doses of extract, despite of the slight decrease observed on the 6th day of the experiment. The healing effect of *E. chlorantha* extract was observed from the seventh day, as there was a significant and dose-dependent decrease of bacterial load in infected animals under treatment. Animal receiving oxytetracyclin and 73mg of *E. chlorantha* extract were the first recovery in both males and females groups.

ANTIOXIDANT PARAMETERS

Effect of the treatment on Enzymatic Antioxidant Parameters

Tissues and serum catalase activities: (Table 1) shows the evolution of the activity of the heart, lung, liver, spleen, kidney and serum catalase (CAT) with different treatments. It can be noted that infection has resulted in a significant *($p < 0.05$)* decrease in the activity of heart and spleen catalase both in males and females rats. In the serum and other organs the difference was not Significant *($p < 0.05$)* when compared to the neutral control. Animals receiving the extract of *E. chlorantha* stem bark showed relative and significant increase of Catalase activity as compared to the negative control group animals.

Tissues and serum peroxidase activities and serum alkaline phosphatase (ALP) activities: The evolution of the activity of the heart, lung, liver, spleen, kidney and serum peroxidase as well as serum ALP activity is presented in (Table 2). It can be observed from this that there is a significant *($p < 0.05$)* decrease in liver's peroxidase activity of infected females rats as compare to neutral control. However, there was a relative and slight increase in peroxidase activity of those receiving different doses of extract/ oxytetracyclin as well as neutral control when compared with the negative control animals. The increase was significant *($p < 0.05$)* in the female's heart receiving 73mg/ kg of extract and the male's serum receiving 9.16 mg/ kg of extract as compared to negative control. This table also showed that infection resulted in a significant increase of male and female ALP activity as compared with neutral control. Nevertheless, treatment

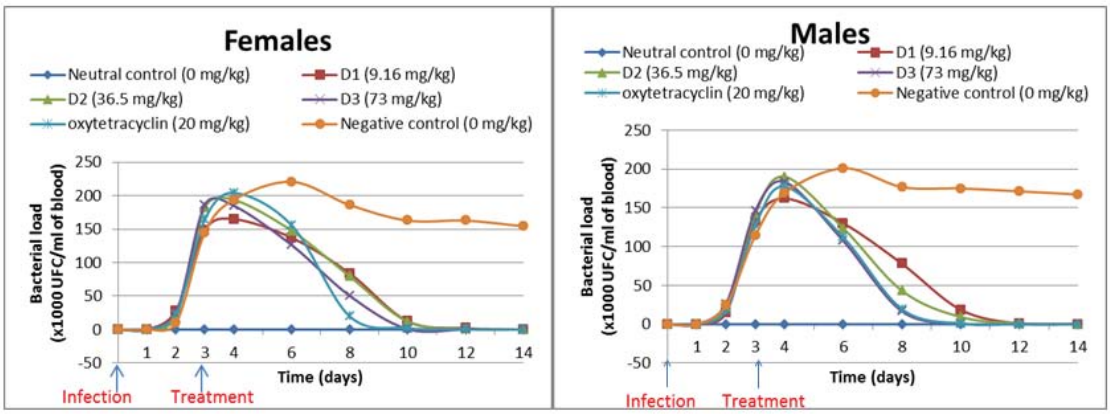

Figure 1: Effect of *E. chlorantha* extract on males and females blood *salmonella* load

Table 1: Evolution of the tissues and serum catalase activity with different treatment.

Doses (mg/kg)	Catalase (µmol/min/g of tissue or µmol/min/ml of serum)					
	Heart	Lungs	Liver	Spleen	Kidney	Serum
Females						
N	0.008 ± 0.001^{ab}	0.039 ± 0.010^{ab}	0.010 ± 0.003^{a}	0.079 ± 0.0165^{abc}	0.050 ± 0.0232^{a}	0.365 ± 0.017^{a}
9.16	0.005 ± 0.001^{ab}	0.044 ± 0.008^{ab}	0.009 ± 0.002^{a}	0.112 ± 0.004^{c}	0.037 ± 0.001^{a}	0.415 ± 0.021^{a}
36.5	0.004 ± 0.001^{a}	0.048 ± 0.006^{ab}	0.011 ± 0.005^{a}	0.094 ± 0.015^{bc}	0.024 ± 0.005^{a}	0.377 ± 0.039^{a}
73	0.014 ± 0.005^{c}	0.084 ± 0.032^{b}	0.028 ± 0.004^{a}	0.044 ± 0.018^{a}	0.030 ± 0.005^{a}	0.408 ± 0.017^{a}
Oxy	0.010 ± 0.005^{bc}	0.038 ± 0.006^{ab}	0.017 ± 0.009^{a}	0.060 ± 0.011^{ab}	0.040 ± 0.003^{a}	0.378 ± 0.041^{a}
Neg	0.002 ± 0.000^{a}	0.023 ± 0.005^{ab}	0.007 ± 0.002^{a}	0.041 ± 0.006^{ab}	0.054 ± 0.016^{a}	0.328 ± 0.049^{a}
Males						
N	0.007 ± 0.002^{ab}	0.032 ± 0.003^{a}	0.023 ± 0.009^{b}	0.034 ± 0.005^{abc}	0.027 ± 0.005^{a}	0.411 ± 0.040^{a}
9.16	0.004 ± 0.001^{a}	0.032 ± 0.004^{a}	0.003 ± 0.001^{a}	0.044 ± 0.003^{bc}	0.019 ± 0.005^{a}	0.405 ± 0.019^{a}
36.5	0.011 ± 0.005^{b}	0.032 ± 0.009^{a}	0.005 ± 0.001^{a}	0.045 ± 0.004^{c}	0.025 ± 0.003^{a}	0.358 ± 0.016^{a}
73	0.008 ± 0.004^{ab}	0.036 ± 0.001^{a}	0.007 ± 0.003^{a}	0.026 ± 0.004^{a}	0.021 ± 0.002^{a}	0.371 ± 0.014^{a}
Oxy	0.005 ± 0.003^{a}	0.027 ± 0.008^{a}	0.004 ± 0.002^{a}	0.031 ± 0.005^{abc}	0.017 ± 0.004^{a}	0.411 ± 0.025^{a}
Neg	0.003 ± 0.001^{a}	0.029 ± 0.004^{a}	0.007 ± 0.004^{a}	0.026 ± 0.005^{ab}	0.020 ± 0.003^{a}	0.317 ± 0.037^{a}

N: neutral control; Neg: negative control; Oxy: Oxytetracyclin; values of this table are expressed as Means ± SD of four determinations. Values with different letters in the same column and the same sex are significantly different *($p < 0.05$).*

resulted in a significant *(p < 0.05)* and dose-dependent decrease in female rats as compare to the neutral control. In the other hand, there is a slight difference between neutral and negative control in males rats.

Tissues and serum SOD activities: The effect of the treatment on Blood and tissues SOD activity is summarized in (Table 3). It reveals that infection resulted in significant *(p < 0.05)* decrease of heart and lungs SOD activity in male and female rats. However, concerning lungs, this decrease was noted only in male rats. Apart from these variations, there was a relative but not significant change in the animals receiving different doses of extract.

Effect of treatment on Non Enzymatic Parameters

Tissues and serum MDA activities: It can be noted from the (Table 4) that, apart from the significant increases of male serum malondialdehyde activity, no other significant variation was noted, either in male or female rats. Although not significant, treatment has resulted decreases of tissues malondialdehyde level in experimental animals.

Tissues and serum NO activities: (Table 5) present the concentration of NO in the tissues and serum of experimental rats. It showed that infection caused an increase in the tissues and serum NO of all the animals when compared with neutral control. This increase is significant *(p < 0.05)* in negative control male's spleen rats as compared to other group. The administration of different doses of *E. chlorantha* stem bark extract in those animals result in a significant *(p < 0.05)* decrease in the level of heart female's and male NO receiving 73mg/ kg and oxytetracyclin. As far as lung and liver NO is concern, this decrease was observed in animal receiving *E. chlorantha* extract at 9.16 (lung) and 9.16 and 72 mg/ kg (liver). The change in the other tissue and serum are not Significant *(p > 0.05)*.

***In vivo* therapeutic test:** It is well known that typhoid fever infection is characterized by diarrhea or constipation, abdominal pain, intestinal hemorrhage. The establishment of the infection was revealed by the above symptoms and also the anorexia and asthenia. This suggested that bacteria have challenged the non-specific defense

Table 2: Effect of treatment on ALP activity and total peroxidase in tissues and serum.

Doses (mg/kg)	Total peroxidase (µmoL/min/g of tissue or µmoL/min/mL*10^{-2} of serum)						ALP (UI/L)
	Heart	Lungs	Liver	Spleen	Kidney	Serum	
Females							
N	0.021 ± 0.003^{a}	0.078 ± 0.004^{ab}	0.044 ± 0.011^{b}	0.067 ± 0.017^{ab}	0.121 ± 0.013^{a}	0.115 ± 0.007^{a}	53.42 ± 11.47^{a}
9.16	0.018 ± 0.001^{a}	0.084 ± 0.011^{ab}	0.034 ± 0.009^{ab}	0.090 ± 0.008^{b}	0.127 ± 0.003^{a}	0.117 ± 0.003^{a}	71.34 ± 16.31^{c}
36.5	0.027 ± 0.005^{ab}	0.069 ± 0.013^{ab}	0.030 ± 0.004^{ab}	0.075 ± 0.019^{ab}	0.111 ± 0.014^{a}	0.112 ± 0.013^{a}	66.86 ± 7.51^{bc}
73	0.041 ± 0.008^{b}	0.091 ± 0.012^{ab}	0.033 ± 0.005^{ab}	0.071 ± 0.007^{ab}	0.124 ± 0.005^{a}	0.111 ± 0.012^{a}	51.00 ± 12.87^{a}
Oxy	0.022 ± 0.005^{a}	0.108 ± 0.008^{b}	0.027 ± 0.017^{ab}	0.081 ± 0.023^{b}	0.142 ± 0.005^{a}	0.104 ± 0.008^{a}	36.53 ± 13.25^{ab}
Neg	0.018 ± 0.008^{a}	0.051 ± 0.018^{a}	0.019 ± 0.007^{a}	0.036 ± 0.012^{a}	0.119 ± 0.008^{a}	0.124 ± 0.014^{a}	95.46 ± 19.75^{d}
Males							
N	0.010 ± 0.004^{ab}	0.091 ± 0.016^{a}	0.024 ± 0.006^{a}	0.060 ± 0.008^{a}	0.114 ± 0.012^{a}	0.114 ± 0.006^{ab}	79.95 ± 21.11^{ab}
9.16	0.021 ± 0.003^{b}	0.092 ± 0.007^{a}	0.023 ± 0.010^{a}	0.055 ± 0.005^{a}	0.121 ± 0.004^{a}	0.138 ± 0.012^{b}	80.30 ± 19.94^{ab}
36.5	0.006 ± 0.001^{a}	0.098 ± 0.003^{a}	0.036 ± 0.015^{a}	0.066 ± 0.018^{a}	0.139 ± 0.004^{a}	0.101 ± 0.011^{ab}	86.16 ± 17.78^{ab}
73	0.015 ± 0.004^{ab}	0.101 ± 0.004^{a}	0.029 ± 0.009^{a}	0.091 ± 0.011^{a}	0.141 ± 0.004^{a}	0.122 ± 0.012^{ab}	36.19 ± 12.91^{a}
Oxy	0.015 ± 0.004^{ab}	0.096 ± 0.010^{a}	0.046 ± 0.009^{a}	0.089 ± 0.017^{a}	0.128 ± 0.012^{a}	0.104 ± 0.002^{ab}	43.77 ± 4.821^{a}
Neg	0.008 ± 0.003^{ab}	0.091 ± 0.009^{a}	0.016 ± 0.005^{a}	0.052 ± 0.008^{a}	0.107 ± 0.014^{a}	0.094 ± 0.013^{a}	142.7 ± 13.46^{b}

C: Control; Oxy: Oxytetracyclin; values of this table are expressed as Means ± SD of four determinations. Values with different letters in the same column and the same sex are significantly different ($p < 0.05$).

Table 3: Effect of treatment on serum and tissues superoxide dismutase.

Doses (mg/kg)	SOD activity (µmol/g of tissue or µmol/ml of serum)					
	Heart	Lungs	Liver	Spleen	Kidney	Serum
	Females					
N	0.194 ± 0.007^{b}	0.200 ± 0.004^{ab}	0.202 ± 0.004^{a}	0.211 ± 0.035^{a}	0.204 ± 0.001^{a}	0.193 ± 0.010^{a}
9.16	0.199 ± 0.003^{b}	0.200 ± 0.005^{ab}	0.202 ± 0.009^{a}	0.198 ± 0.015^{a}	0.192 ± 0.022^{a}	0.292 ± 0.057^{b}
36.5	0.202 ± 0.001^{b}	0.286 ± 0.171^{b}	0.203 ± 0.031^{a}	0.200 ± 0.004^{a}	0.206 ± 0.008^{a}	0.297± 0.096^{ab}
73	0.208 ± 0.011^{b}	0.230 ± 0.039^{ab}	0.201 ± 0.006^{a}	0.198 ± 0.004^{a}	0.205 ± 0.004^{a}	0.214 ± 0.015^{a}
Oxy	0.203 ± 0.066^{b}	0.199 ± 0.001^{ab}	0.187 ± 0.014^{a}	0.245 ± 0.083^{a}	0.203 ± 0.011^{a}	0.204 ± 0.004^{a}
Neg	0.186 ± 0.011^{a}	0.176 ± 0.022^{a}	0.193 ± 0.009^{a}	0.186 ± 0.029^{a}	0.221 ± 0.045^{a}	0.204 ± 0.001^{a}
	Males					
N	0.205 ± 0.009^{b}	0.205 ± 0.007^{b}	0.195 ± 0.022^{ab}	0.199 ± 0.009^{a}	0.228 ± 0.046^{b}	0.221 ± 0.014^{ab}
9.16	0.204 ± 0.011^{b}	0.202 ± 0.004^{b}	0.200 ± 0.006^{ab}	0.191 ± 0.001^{a}	0.203 ± 0.019^{ab}	0.359 ± 0.091^{b}
36.5	0.200 ± 0.004^{b}	0.211 ± 0.015^{b}	0.190 ± 0.023^{ab}	0.197 ± 0.009^{a}	0.182 ± 0.037^{ab}	0.279 ± 0.079^{ab}
73	0.199 ± 0.009^{b}	0.206 ± 0.001^{b}	0.202 ± 0.003^{ab}	0.211 ± 0.014^{ab}	0.192 ± 0.013^{ab}	0.201 ± 0.005^{a}
Oxy	0.187 ± 0.015^{b}	0.206 ± 0.004^{b}	0.692 ± 1.033^{c}	0.204 ± 0.007^{a}	0.190 ± 0.006^{ab}	0.199 ± 0.002^{a}
Neg	0.081 ± 0.078^{a}	0.170 ± 0.008^{a}	0.186 ± 0.014^{a}	0.195 ± 0.004^{a}	0.125 ± 0.077^{a}	0.190 ± 0.003^{a}

N: neutral control; Neg: negative control; Oxy: Oxytetracyclin; values of this table are expressed as Means ± SD of four determinations. Values with different letters in the same column and the same sex are significantly different *(p < 0.05)*.

Table 4: Effect of treatment on membrane lipid peroxidation (malondialdehyde) in the serum and tissues.

Doses (mg/kg)	Malondialdehyde (µM/g of tissue or µM/ml of serum)					
	Heart	Lungs	Liver	Spleen	Kidney	Serum
Females						
N	2.557 ± 0.494^{a}	3.342 ± 1.895^{a}	2.781 ± 0.351^{a}	5.787 ± 1.947^{a}	5.092 ± 1.958^{a}	3.986 ± 0.106^{a}
9.16	2.983 ± 0.212^{abc}	4.352 ± 1.688^{a}	4.867 ± 0.323^{ab}	7.649 ± 1.525^{a}	6.527 ± 1.640^{a}	4.281 ± 0.125^{abc}
36.5	3.701 ± 0.907^{bc}	4.374 ± 1.721^{a}	3.477 ± 0.741^{ab}	6.146 ± 1.347^{a}	6.976 ± 1.470^{a}	4.967 ± 1.114^{bc}
73	2.849 ± 0.823^{ab}	4.687 ± 2.458^{a}	3.117 ± 1.387^{ab}	6.438 ± 2.313^{a}	6.258 ± 0.970^{a}	5.719 ± 0.469^{ab}
Oxy	2.759 ± 0.134^{ab}	4.127 ± 0.617^{a}	4.038 ± 0.792^{ab}	9.308 ± 2.661^{a}	6.617 ± 1.425^{a}	5.555 ± 0.406^{ab}
Neg	3.880 ± 0.447^{c}	5.293 ± 1.320^{a}	5.720 ± 1.938^{b}	9.914 ± 2.584^{a}	6.101 ± 1.912^{a}	6.241 ± 0.998^{c}
Males						
N	1.772 ± 0.636^{a}	3.387 ± 1.751^{a}	3.095 ± 0.808^{a}	5.810 ± 1.138^{a}	7.155 ± 2.791^{a}	4.444 ± 0.544^{a}
9.16	3.566 ± 1.820^{ab}	4.845 ± 0.572^{a}	4.150 ± 0.420^{a}	7.088 ± 1.232^{a}	8.075 ± 1.407^{a}	5.718 ± 1.038^{b}
36.5	3.095 ± 0.693^{ab}	4.172 ± 0.588^{a}	3.611 ± 1.159^{a}	6.931 ± 1.952^{a}	7.223 ± 1.205^{a}	5.654 ± 1.131^{b}
73	2.848 ± 0.476^{ab}	3.723 ± 0.883^{a}	3.095 ± 0.274^{a}	7.783 ± 2.831^{a}	6.056 ± 2.247^{a}	5.032±0.184^{ab}
Oxy	2.759 ± 0.423^{ab}	5.002 ± 1.590^{a}	4.195 ± 1.865^{a}	7.200 ± 2.005^{a}	7.851 ± 2.299^{a}	6.078 ± 0.289^{b}
Neg	4.418 ± 1.537^{b}	5.653 ± 2.072^{a}	4.531 ± 1.462^{a}	7.873 ± 2.990^{a}	8.88 ± 1.098^{a}	7.254 ± 0.537^{c}

N: neutral control; Neg: negative control; Oxy: Oxytetracyclin; values of this table are expressed as Means ± SD of four determinations. Values with different letters in the same column and the same sex are significantly different *($p < 0.05$)*.

Table 5: Effect of the treatment on Nitric oxide (µmole/g of tissue or µmole/ml of serum) in the serum and tissues.

Doses (mg/kg)	Nitric oxide (µmole/g of tissue or µmole/ml of serum)					
	Heart	Lungs	Liver	Spleen	Kidney	Serum
Females						
N	0.161 ± 0.100^{ab}	1.874 ± 0.385^{a}	0.750 ± 0.224^{a}	1.699 ± 0.623^{a}	5.114 ± 0.762^{a}	2.506 ± 0.287^{a}
9.16	0.342 ± 0.146^{ab}	2.503 ± 0.329^{a}	1.096 ± 0.433^{a}	1.251 ± 0.205^{a}	5.732 ± 1.003^{a}	3.834 ± 0.265^{a}
36.5	0.184 ± 0.075^{ab}	1.623 ± 0.580^{a}	0.609 ± 0.171^{a}	1.399 ± 0.278^{a}	3.922 ± 0.653^{a}	2.944 ± 0.761^{a}
73	0.036 ± 0.003^{a}	2.879 ± 0.985^{a}	0.586 ± 0.120^{a}	0.905 ± 0.164^{a}	5.281 ± 0.476^{a}	2.498 ± 0.731^{a}
OXY	0.039 ± 0.005^{a}	3.337 ± 0.677^{a}	0.869 ± 0.126^{a}	1.229 ± 0.682^{a}	5.662 ± 1.104^{a}	2.401 ± 0.450^{a}
Neg	0.840 ± 0.466^{b}	2.952 ± 0.527^{a}	0.945 ± 0.071^{a}	2.576 ± 1.643^{a}	6.879 ± 1.282^{a}	3.981 ± 1.078^{a}
Males						
N	0.355 ± 0.148^{ab}	2.191 ± 0.656^{a}	0.355 ± 0.141^{a}	0.6477 ± 0.135^{a}	4.622 ± 1.133^{a}	2.566 ± 0.158^{a}
9.16	0.441 ± 0.081^{ab}	2.352 ± 0.546^{a}	0.283 ± 0.157^{a}	2.074 ± 0.173^{b}	5.566 ± 0.261^{a}	4.792 ± 0.893^{a}
36.5	0.263 ± 0.088^{ab}	3.206 ± 0.277^{ab}	0.392 ± 0.064^{a}	2.096 ± 1.700^{b}	4.369 ± 1.025^{a}	2.428 ± 0.718^{a}
73	0.168 ± 0.056^{a}	2.694 ± 0.133^{ab}	0.500 ± 0.143^{a}	1.030 ± 0.269^{b}	3.049 ± 1.501^{a}	3.958 ± 0.648^{a}
Oxy	0.095 ± 0.039^{a}	2.982 ± 0.525^{ab}	0.662 ± 0.323^{a}	2.280 ± 1.201^{b}	6.288 ± 0.655^{a}	2.678 ± 0.198^{a}
Neg	0.467 ± 0.255^{b}	4.522 ± 0.690^{b}	1.295 ± 0.520^{b}	2.487 ± 0.561^{b}	6.305 ± 0.910^{a}	5.171 ± 1.407^{a}

N: neutral control; Neg: negative control; Oxy: Oxytetracyclin; values of this table are expressed as Means ± SD of four determinations. Values with different letters in the same column and the same sex are significantly different *($p < 0.05$)*.

mechanism of rats, and have proliferated in animals organs after having invaded the blood system [26]. The marked reduction of bacteria load in culture media after administration of various dose of *E. chlorantha* stem bark extract confirmed the antimicrobial potency of the plant and therefore suggests its efficacy in the treatment of typhoid fever. This reduction may be due to the combined action of the extract and immune system since slight decreases of bacteria load was also noted in negative control group animal [26]. Rats treated at dose 73 mg/ kg bw of extract recovered almost at the same period as those treated with oxytetracyclin. The results demonstrate that this plant possess compounds endow with antibacterial activity. In fact, *E. chlorantha* stem bark was report to possess flavonoids, phenol and alkaloids, which have already shown several pharmacological properties including antibacterial properties [27].

Antioxidant test: Bacterial infections in living cells result in a releasing of toxins whose metabolism may lead to an increased formation of highly reactive molecules that can cause oxidative damage to the entire body [8]. Typhoid fever induced by *Salmonella* typhi is due to the formation of the active metabolite (superoxide radical). The antioxidant defense mechanisms include enzymatic and non-enzymatic antioxidants play a crucial 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. Among the antioxidants are enzymes such as catalase and peroxidase [29,30]. The increases in the level of catalase in animal receiving different doses of plant extract suggest that this plant extract possess catalase-like compounds which have contributed to the rapid break down of hydrogen peroxide produced during

bacterial infection and by negative feedback mechanism contributed to the inhibition of endogenous production of catalase. Indeed, catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production [31,32].The fact that the administration of the extract of *E. chlorantha* has corrected the decrease in the level of heart spleen and liver catalase induced by the infection could demonstrate that it has an *in vivo* antioxidant activity and is capable of ameliorating the effects of ROS in biologic system [33,34]. Peroxidase (POD) is widely distributed in all animal tissues, and the highest activity is found in the red blood cells. It decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [29,35]. So, the reduction in its activity in negative control animals might be due to the number of deleterious effects induced by the assimilation of superoxide radical and hydrogen peroxide [36]. However, the relative and significant increase observed in animals treated with different doses of extract of *E. chlorantha* could explain its healing effect. Superoxide Dismutase (SOD) plays a key role in the detoxification of superoxide radical, thereby protecting cells from damage induced by free radicals [37]. The reduction in SOD activity after infection could be due to the oxidation of catalase enzymes [38]. The increased in serum and tissues activities of SOD as observed in this study reveals the *in vivo* antioxidant activity of the extract. In fact, the rise in SOD activity level is due to the up regulation of SOD gene to increase the expression to enhance the defense activity of SOD required to quench excessively generated superoxide radical [45]. Serum Alkaline Phosphatase (ALP) acts as a marker of hepatic damages. [28] Have shown that oxidative stress is linked to the increase in the level of serum alkaline phosphatase. This elevation indicates cellular leakage and loss of functional integrity of cell membrane in the liver [39]. So, the significant $(P < 0.05)$ increase in ALP activity of the serum of infected and non-treated control might be due to increased synthesis, in presence of increasing biliary pressure [40]. The decrease of this activity in rats treated with *E. chlorantha* stem bark could be due to the healing state of the animals. This result corroborates the fact that serum level of ALP becomes normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [41]. The investigation of the direct effect of the plant extract on lipid peroxidation was done by measuring MDA level. The decrease of the MDA level in the heart, serum and liver of negative control rats after the administration of *E. chlorantha* stem bark extract suggests that the plant extract could improve the pathological condition of the rats by reducing lipid peroxidation. Indeed, an increase in the level of lipid peroxidation products results in increased levels of oxygen free radicals, which attack the polyunsaturated fatty acids in cell membranes and cause lipid peroxidation [42]. Nitrite Oxide (NO) is Known to participate in the regulation of the redox potential of various cells and might be involved in the protection against or the production of oxidative stress within various tissues depending on its concentration. The excess of nitric oxide reacts with oxygen to generate nitrite and peroxynitrite anions, which act as free radicals [43]. The significant $(P < 0.05)$ increased in the level of NO in the spleen of negative control groups of males rats could be explained by its excessive production to destroy the microorganism. In the other hand, the decrease observed in the animals treated with the extract suggests that the extract is able to prevent the alteration of some biomolecules [44]. phytochemicals analysis of the *E. chlorantha* extracts reveals the presence of polyphenols, flavonoids and triterpenes which are known to possess antioxidant activity [32]. The antioxidant activity could then be lead to the anti-Salmonella activity of the ethanolic extract of *E. chlorantha* stem bark.

CONCLUSION

The antioxidant and anti-lipid peroxidation effects of the ethanolic extract of *E. chlorantha* may be the rationale behind some of its folkloric uses and may be responsible for some of its pharmacological effects. These data suggest that extracts from these plant species should be further investigated in order to isolate bioactive components that might function as potential anti-salmonella agents, in order to minimize the damage caused by excessive oxidant production.

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