Journal of Advances in Medical and Pharmaceutical Sciences
7(3): 1-13, 2016, Article no.JAMPS.24949
ISSN: 2394-1111

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Antioxidant Effect of Aqueous Extract of *Curcuma longa* Rhizomes (Zingiberaceae) in the Typhoid Fever Induced in Wistar Rats Model

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Authors' contributions

This work was carried out in collaboration between all authors. Author NK was the field investigator and drafted the manuscript. Authors SSA and JBS contributed to the evaluation of antioxidant properties. Author GSSN contributed to the assessment of antityphoid property. Author JRK revised the manuscript. Author DG designed the study and supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/24949
Editor(s):
(1) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) Christopher Larbie, Kwame Nkrumah University of Science and Technology, Ghana.
(2) James Adams, University of Southern California, USA.
(3) S. Sarkiyayi, Modibbo Adama University of Technology,Yola, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/13996

Original Research Article

Received 10th February 2016
Accepted 7th March 2016
Published 4th April 2016

ABSTRACT

Background: The pathogenesis of reactive oxygen species (ROS) linked to chronic diseases such as typhoid fever has warranted the intensive search for plants with antioxidant properties.
Objective: The aim of the present study was to investigate the *in vivo* antioxidant activities of the aqueous extract from *Curcuma longa* rhizomes (Zingiberaceae) in the typhoid fever induced in rats.
Place and Duration of Study: Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon, between March and July 2015.
Methodology: The *Salmonella typhi* infected rats ($1.5x10^8$ CFU) regularly received different doses of plant extract (10, 30 and 60 mg/kg or oxyteracyclin 5 mg/kg) daily for 11 days. The levels of parameters such as catalase, alkaline phosphatase (ALP), total peroxidase, malondialdehyde

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(MDA) and nitric oxide (NO) were determined.
Results: The present study revealed that *Curcuma longa* has significant *in vivo* antityphoid and antioxidant activities and can then be used to protect tissue from oxidative stress. The result showed a significant decrease in tissues and serum catalase and peroxidase, and a significant increase in NO, MDA and ALP levels in negative control animals. The treatment resulted in a significant normalization of the levels of the above markers when compared with neutral control group.
Conclusion: Based on this study, we conclude that the aqueous extract of *Curcuma longa* possesses *in vivo* antioxidant activity and can be used in protecting tissue from oxidative stress in case of typhoid fever.

Keywords: Typhoid fever; Curcuma longa; oxidative stress; antioxidant; Salmonella typhi.

1. INTRODUCTION

Typhoid fever is a systemic infection caused by *Salmonella enterica* serotype (*S. typhi*, *S. paratyphi* A, and *S. paratyphi* B). Worldwide, there is an estimated 22 million episodes of typhoid fever causing 216 500 deaths each year, the overwhelming majority of infections and deaths occurring in developing countries where typhoid fever is endemic [1]. Conventional antimicrobial drugs are becoming more and more unavailable to the common man in Africa due to increased costs. In addition, there is a greater resistance to the common antimicrobials (chloramphenicol, ampicillin and co-trimoxazol) [2]. Moreover, chloramphenicol which had been for long the drug of choice for the treatment of typhoid fever, is being withdrawn from the market due to its medullary toxicity (medullary aplasia) [3,4]. *Salmonella* infection cause the production of superoxide and nitric oxide radical which react together to form peroxynitrite, a strong biological oxidant [5]. Consequently, pathological conditions characterized by oxidative stress can greatly result in typhoid fever or other bacterial infections. Reactive oxygen species (ROS) are a class of highly reactive molecules derived from the metabolism of oxygen. Rapid production of free radicals may lead to oxidative damage of biomolecules and results in disorders such as degenerative diseases, cancer, diabetes, neural disorders and ageing [6,7]. These free radicals occur in the body during an imbalance between ROS and antioxidants. Many medicinal plants have large amount of antimicrobial and antioxidants compounds such as saponins, tannins and triterpenoids, polyphenols and others. The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. The enzymatic antioxidants include catalase, superoxide dismutase and peroxidase which catalyze neutralization of many types of free radicals while the non-enzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases like cancer [8].

Curcuma longa L. (Turmeric), a perennial herb, is a member of Zingiberaceae family widely distributed in the tropical region. In China, *C. longa* is used against diseases associated with abdominal pains. Turmeric has a long tradition of use in the Chinese and Indian medicine, particularly as an anti-inflammatory agent or in the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage and colic [9]. Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia [9]. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent years, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, hepatic disorders, rheumatism and sinusitis [10]. It is traditionally used in the West region of Cameroon for the treatment of malaria, fever, bacterial infections like typhoid fever, diarrhea and symptoms like stomach-ache. The plant is cultivated extensively in India, China, and other countries with a tropical climate [11]. It has oblong, pointed leaves and bears funnel-shaped yellow flowers. The commonly used portion of this plant is its rhizome. Previous investigations focused on turmeric hepatoprotective, anti-inflammatory and anti-carcinogenic properties of the plant. In addition, it is used in gastric ulcer, cardiovascular disease and gastrointestinal disorders [10]. In agreement with the results obtained in previous investigations [12], this work therefore aimed at evaluating the *in vivo* antityphoid and antioxidant activities of the aqueous extract of *Curcuma longa* L. rhizomes.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Extract preparation

The rhizomes of *Curcuma longa* L. were collected from Santchou Division, West Region of Cameroon and were identified by Mr. NGANSOP Eric, a botanist at the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the reference No 42173/HNC. The plant was shade dried and crushed into fine powder. The aqueous extract (decoction) was used in the treatment of infected animals. It was prepared according to Duke [13].

2.1.2 Experimental animals

This test was carried out using *Salmonella typhi* induced typhoid in Wistar rat model. Forty eight mature Wistar albino rats weighing between 150 and 200 g (24 males and 24 females, 8- 9 weeks old) were reared in the Animal house of the Department of Biochemistry, University of Dschang-Cameroon. Rats were given free access to pellet and water. Prior to the test, animals were housed under the test conditions for a period of one week. They were handled according to standard protocols for the use of laboratory animals. The studies were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

2.1.3 Bacterial strain and culture media

The strain of *Salmonella typhi* (ATCC 6539) was obtained from the American Type Culture Collection (ATCC) on already prepared *Salmonella shigella* Agar (SSA). It was maintained on Mueller-Hinton agar slant at 4 ℃ and subcultured on a fresh appropriate agar plate for 24hrs. The culture medium used was *Salmonella-Shigella* Agar (SSA) for activation and maintenance of the strain.

2.2 Methods

2.2.1 Phytochemical screening

Curcuma longa rhizomes aqueous extract was screened for different classes of compounds, including alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes, using standard methods [14].

2.2.2 Typhoid induction

The preparation of *Curcuma longa* inoculum was done using 18 h old bacterial cultures prepared in nutrient agar. A few colonies of bacteria were collected aseptically and introduced into sterile 0.90% saline solution. A final suspension was obtained compared to 0.5 Mc Farland turbidity. This solution (1 ml), containing about 1.5 $x10^8$ CFU, was orally administered to each animal [4]. Only infected animals were selected on the basis of their blood colony counts.

2.2.3 Determination of traditional healers dose

Approximately 25 g of plant (*Curcuma longa*) powder, as indicated by the traditional healers, were boiled in 1000 ml of water during 15 minutes. The solution was cooled and filtered using Whatman paper No 1. Two glasses (daily dose of the traditional healers) were taken, filtered and concentrated under reduced pressure at 70℃ using a rotary evaporator (Buchi R-200). This yielded a mass of 2110 mg. By assuming that an adult individual has an average weight of 70 kg, we came to the conclusion that each patient receives a dose of 2110 mg/70 kg bw corresponding to 30 mg/kg bw approximately.

2.2.4 Experimental design

The animals were randomly distributed into six groups of four animals each (six female groups F_0, F_1,F_2, F_3, F_4 and F_5 and six male groups M_0, M_1, M_2, M_3, M_4 and M_5) with similar average body weight on the seventh day after acclimatization. Apart from animals of groups 1, those of all other groups (2-6) were infected. On the fourth day after infection, the animals were treated as follows:

- Groups one (F_0 and M_0) serving as neutral control was not infected and received distilled water during the treatment period;
- Groups two (F_1 and M_1), negative control groups, received only distilled water during the treatment period.
- Groups three (F_2 and M_2), positive control groups, received oxytetracyclin (5 mg/kg body weight) during the treatment.
- Groups four, five and six (F_3, F_4, F_5, M_3, M_4 and M_5) considered as test groups, received the aqueous *Curcuma longa* rhizomes extract at concentrations of

10.00, 30.00 and 60.00 mg/kg body weight, corresponding to traditional healers dose/3, traditional healers dose, and 2 traditional healers dose respectively.

The treatment was done by administering the extract orally, every morning from 7 to 9 am GMT every day for eleven days. Every two days, during the experimental time, the blood was collected into heparinized tubes and assessed immediately for the bacterial load.

2.2.5 Evaluation of the treatment efficiency

Fifty (50) µL of blood were drawn from the caudal vein of treated animals and inoculated on already prepared SSA on Petri dishes. The inoculated plates were incubated at 37 ℃ for 24 hrs. The counts of emerged colonies were used to evaluate the efficacy of treatment. The results were converted into the number of colonies per ml of blood per animal.

2.2.6 Food intake

Food intake was measured every day during the experimental time and recorded as mean weight of food per group.

2.2.7 Blood collection and dissection

At the end of the experiment, animals were subjected to a 12-hour food fasting and their blood samples were collected by cardiac puncture from chloroform vapors anaesthetized rats into sterilized dry tubes. The tubes were allowed to clot and were centrifuged at 3000 rpm for 10 minutes to obtain the serum. Animals were further dissected and different organs (liver, kidney, lung, heart and spleen) were removed. Fifteen percent (15%) homogenate of these organs 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), alkaline phosphatase (ALP).

2.3 Evaluation of Biochemical Antioxidant Parameters

2.3.1 Enzymatic parameters

2.3.1.1 CAT (catalase, EC 1.11.1.6) activity

Catalase activity was determined in tissue and serum by Fodouop et al. [15] method with some modifications. 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 ℃ for 10 min then cooled in ice bath and the absorbance was recorded at 570 nm using Schimadzu 1501 spectrophotometer, 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.

2.3.1.2 POD (Peroxidase EC 1.11.1.7) assay

Peroxidase activity was determined in tissues and serum by Habbu et al. [16] 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.

2.3.1.3 Alkaline phosphatase (ALP) essay

The serum alkaline phosphatase (ALP) was determined using commercial kit (Teco Diagnostics, USA).

2.3.2 Non enzymatic parameters

2.3.2.1 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 Fodouop et al. [15] 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 5 000×g 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.

2.3.2.2 NITRIC oxide (NO) essay: Determination of nitrite concentration

This assay relies on a diazotization reaction that was described by Griess [17] with some modifications. 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.

2.4 Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Within group, comparisons were performed by the analysis of variance using ANOVA test. Significant difference ($p<0.5$) between control and experimental groups were assessed using Waller Duncan test.

3. RESULTS

3.1 Phytochemical Composition

The results of qualitative analysis showed that aqueous extract of *Curcuma longa* rhizome contains alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins, and triterpenes (Table 1).

Table 1. Phytochemical composition of decocted extract

Phytochemical groups	Extract
Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	+
Anthocyanin	-
Triterpenes and steroids	+
Anthraquinone	+
Phenols	+

+: present; - : absent

3.2 Antityphoid Properties

The aqueous extract (decoction) was tested *in vivo* on a *Salmonella typhi*-induced typhoid fever in Wistar rats. The evolution of the bacterial load in the blood of experimental rats throughout the experiment is summarized in Fig. 1. From the first to the fourth day after infection, the bacterial load continuously increased in the blood of infected animals. The healing effect of *Curcuma longa* extract was observed from the tenth day, as there was a significant and dose-dependent decrease of bacterial load in infected animals under treatment. Similarly, there was a slight decrease in bacterial load in negative control group animals (infected untreated groups), but the load remained relatively high compared to those of animals receiving different doses of plant extract after 11 days of treatment. In the Females, the extract at 60 mg/kg was more effective compared to the reference drug (oxytetracyclin).

3.3 Food Consumption and Organ Indices Estimation

3.3.1 Food consumption

The infection was negatively affected by food consumption. But, during the treatment, food intake increased in a dose-dependent manner (Fig. 2).

3.3.2 Organ indices estimation

The effects of treatment on relative organ indices of both male and female rats are summarized in Table 2. There were no significant changes ($p>0.05$) in the liver, kidney, heart and lung to body weight ratios in both groups. However, the treatment significantly ($p<0.05$) increased the spleen relative weight of the negative control in both male and female. In the female groups, the relative weight of studied organs of negative control animals increased compared to other groups (Table 2).

3.4 Enzymatic Antioxidant Parameters

3.4.1 Effect of treatment on catalase (CAT) level

The evolution of the tissues and serum CAT activity as a function of extract doses were shown in Table 3. From this table it appears that the infection resulted in a significant ($p<0.05$) decrease in the CAT activity in all the groups compared to neutral control. However, the

treatment with the various doses of extract resulted in significantly higher ($p < 0.05$) tissues and serum catalase level when compared to the negative control rats. The only exception was the spleen in which the catalase activity remained as increase, but not significantly ($p>0.05$) different when compared to the negative control.

3.4.2 Effect of treatment on total peroxidase (POD)

Table 4 presents the effect of the treatment on POD activity in the tissues and serum. The result show significantly decrease ($P<0.05$) POD level, in the tissues and serum of the negative control animals compared to the neutral control groups. This decreased was not significant in the spleen (males and females). However, the treatment with the various doses of extract increased the tissues and serum peroxidase level when compared with the negative control rats. In contrast, the POD levels in the spleen were normal (not significantly different ($p>0.05$)) in both females and males groups.

3.4.3 Effect of treatment on alkaline phosphatase (ALP)

The effect of infection on serum ALP in *Salmonella typhi*-induced typhoid fever in rats is presented in Fig. 3. From this figure, it can be noted that the significantly ($p< 0.05$) increased activity of serum ALP was observed in negative control animals compared to neutral control animals. However, the alkaline phosphatase serum level is not significantly ($p > 0.05$) different between the infected and treated rats and neutral control, suggesting that the treatment decreased the level (activity) of the ALP in all the groups compared to the negative control groups.

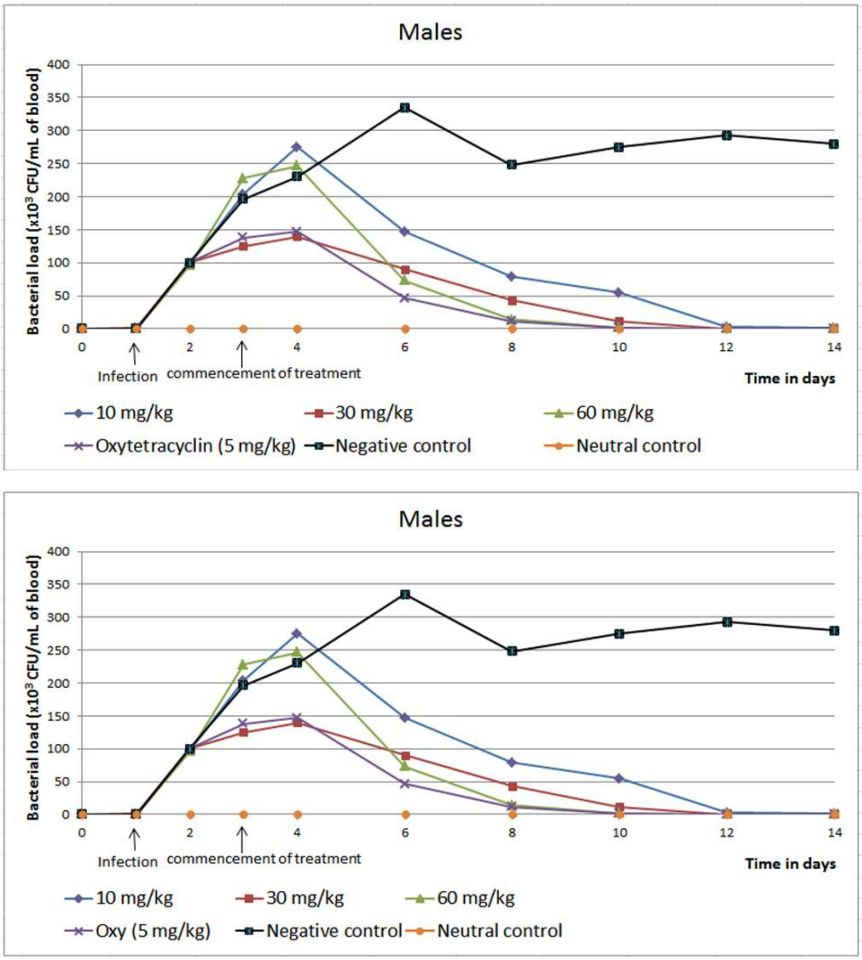

Fig. 1. Evolution of treatment of infected rats as a function of decocted *Curcuma longa* extract dose and sex (males and females)

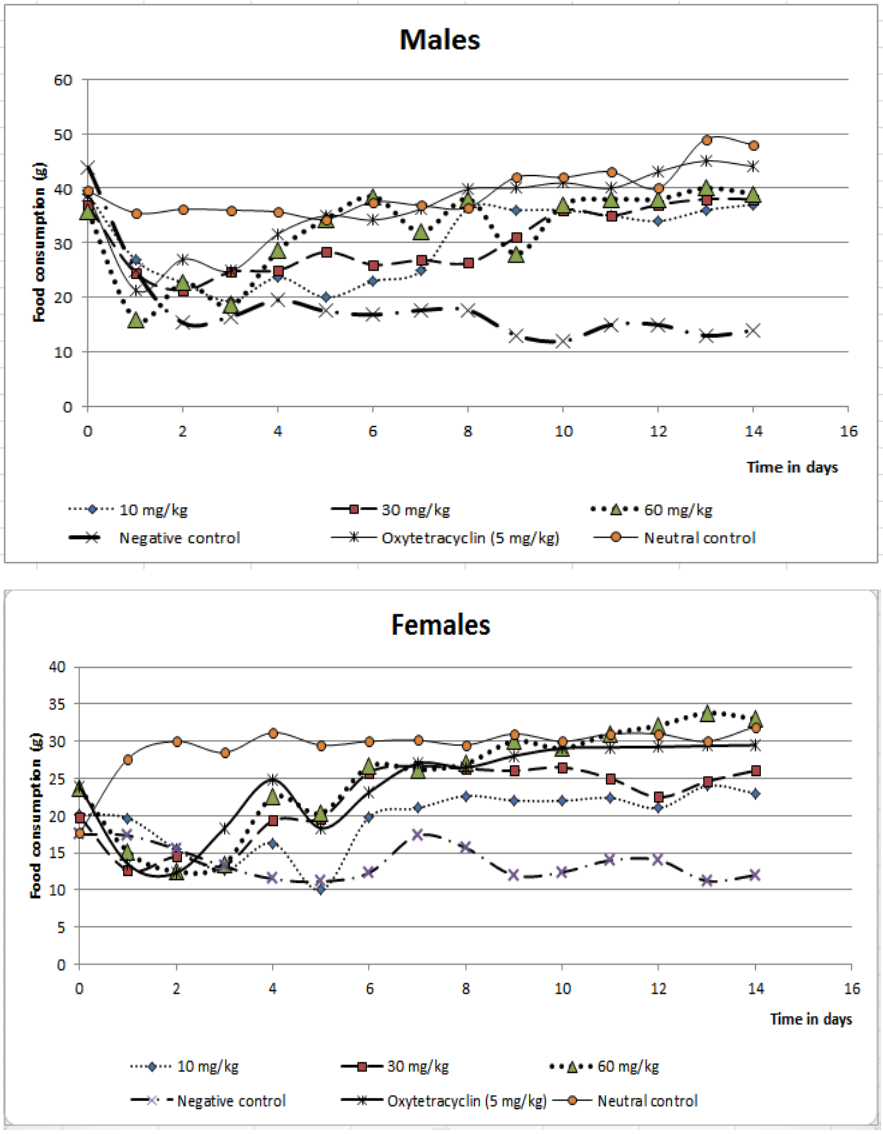

Fig. 2. Evolution of food consumption as a function of treatment and sex (males and females)

3.5 Non Enzymatic Parameters

3.5.1 Effect of treatment on malondialdehyde (MDA)

Generally, the MDA tissues and serum concentrations increased in negative control animals compared to the neutral control animals. The treatment decreased the tissues peroxidation when compared to the negative control. But, this was not always significantly ($p>0.05$) different from serum or different organs, or from males and females (Table 5). In the liver and serum from the females, the MDA concentration was not significantly ($p>0.05$) different on any group. However, the concentration of the MDA is inversely proportional to the dose of the extract from every sex and any organ.

3.5.2 Effect of treatment on nitric oxide (NO)

Table 6 presents the tissues and serum concentration of NO in control and experimental groups of rats. Except liver from the Males, the increased of NO levels was observed in infected rats compared to neutral control groups. The administration of *Curcuma longa* aqueous extract and oxytetracyclin tends to normalize the NO levels.

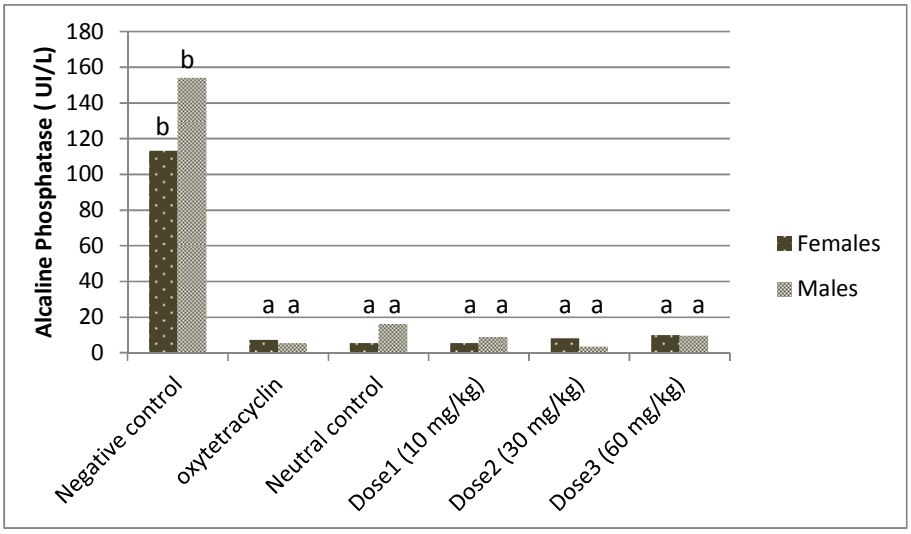

Fig. 3. Effect of treatment on the serum alkaline phosphatase

Values of this figure are expressed as Means ± SD of four determinations. Values with different letters in the same sex are significantly different ($p < 0.05$)

Table 2. Effect of the treatment on the relative weight of the organs (g/kg)

Sex	Groups and doses	Relative weight (%)				
		Liver	Kidney	Heart	Lungs	Spleen
Females	10 mg/kg	3.53±0.30^{a}	0.70±0.03^{a}	0.36±0.05^{a}	0.69±0.01^{a}	0.36±0.14^{a}
	30 mg/kg	3.44±0.11^{a}	0.73±0.14^{a}	0.37±0.02^{a}	0.61±0.03^{a}	0.49±0.03^{a}
	60 mg/kg	3.50±0.68^{a}	0.65±0.14^{a}	0.36±0.05^{a}	0.59±0.06^{a}	0.49±0.14^{a}
	Negative control	4.12±0.01^{a}	0.79±0.00^{a}	0.41±0.14^{ab}	0.93±0.15^{a}	0.66±0.10^{b}
	Oxy. 5 mg/kg	3.65±0.38^{a}	0.77±0.07^{a}	0.36±0.06^{a}	0.83±0.04^{a}	0.45±0.07^{a}
	Neutral control	4.03±0.25^{a}	0.87±0.20^{a}	0.37±0.01^{a}	0.80±0.20^{a}	0.41±0.65a
Males	10 mg/kg	3.77±0.34^{a}	0.82±0.39^{a}	0.38±0.06^{a}	0.72±0.34^{ab}	0.55±0.17^{ab}
	30 mg/kg	3.42±0.13^{a}	0.60±0.04^{a}	0.32±0.00^{a}	0.56±0.11^{a}	0.37±0.05^{a}
	60 mg/kg	2.87±0.38^{a}	0.56±0.04^{a}	0.30±0.02^{a}	0.56±0.15^{a}	0.26±0.03^{a}
	Negative control	4.24±1.99^{ab}	0.76±0.05^{a}	0.44±0.19^{ab}	0.68±0.14^{a}	0.70±0.14^{b}
	Oxy. 5mg/kg	3.55±0.37^{a}	0.74±0.08^{a}	0.31±0.08^{a}	0.68±0.16^{a}	0.58±0.18^{ab}
	Neutral control	4.34±0.93^{a}	0.79±0.18^{a}	0.34±0.08^{a}	0.77±0.08^{ab}	0.44±0.12^{a}

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 ($p < 0.05$)

4. DISCUSSION

4.1 Antityphoid Effect

The establishment of infection was reflected by some changes in animals physiology including the excretion of watery stool, the presence of blood and mucus in the stool, the reduction of its activity and the exponential increase in the rate of *S. typhi* in the blood of rats after administration of infectious load. This suggested that the bacteria proliferated in the organs after having invaded the system, and challenged the non-specific defense mechanism of rats. The decrease of the bacterial load observed with treatment may be due to the combined action of the extract and immune system given the fact that this decrease was also noted in the negative control group (infected and untreated). Animals treated with therapeutic doses (10 and 30 mg/kg) recovered two (males) and four (females) days later after those treated with 60 mg/kg. The phytochemical screening revealed the presence of several classes of compounds in *Curcuma longa* rhizome extract including alkaloids, anthraquinons, flavonoids, phenols, saponins, tannins, and triterpenes. Some of these secondary metabolites (flavonoids, alkaloids) have already shown several pharmacological properties including antibacterial properties [15,18-21].

Table 3. Effect of treatment on catalase in the serum and tissues

Groups and doses	Liver	Kidney	Spleen	Lungs	Heart	Serum
	Quantities of catalase (µmol/min/g of tissue or µmol/min/ml of serum)					
	Females					
10 mg/kg	10.940±1.645^{a}	16.816±2.351^{a}	13.626±1.189^{a}	16.213±9.764^{a}	11.610±4.016^{a}	0.243±0.011^{a}
30 mg/kg	46.453±4.734^{b}	32.510±2.078^{b}	15.413±5.143^{a}	52.553±15.530^{b}	39.020±0.969^{b}	0.353±0.015^{c}
60 mg/kg	47.200±13.804^{b}	59.630±3.423^{c}	18.260±15.601^{a}	79.786±11.059^{c}	35.850±10.017^{b}	0.333±0.047^{bc}
Negative control	5.726±0.488^{a}	12.340±1.460^{a}	12.660±2.981^{a}	10.236±5.760^{a}	9.850±1.007^{a}	0.226±0.080^{a}
Oxy. 5 mg/kg	15.886±7.788^{a}	12.760±2.609^{a}	14.196±5.274^{a}	12.490±3.245^{a}	10.246±0.900^{a}	0.276±0.023^{ab}
Neutral control	58.640±16.911^{b}	56.390±17.781^{c}	15.186±2.929^{a}	56.570±20.982^{b}	42.243±12.868^{b}	0.346±0.023^{bc}
	Males					
10 mg/kg	13.343±7.445^{a}	13.110±1.292^{a}	25.030±2.871^{a}	14.860±5.855^{a}	9.920±3.193^{ab}	0.253±0.005^{a}
30 mg/kg	44.826±12.081^{b}	61.820±20.552^{b}	15.086±8.541^{a}	35.720±6.950^{ab}	19.783±8.065b^{cd}	0.356±0.032^{b}
60 mg/kg	45.486±2.728^{b}	66.130±17.159^{b}	19.366±2.059^{a}	47.646±23.135^{b}	24.523±4.745^{d}	0.363±0.032^{b}
Negative control	6.900±3.686^{a}	11.080±2.018^{a}	12.263±0.653^{a}	13.420±2.849^{a}	5.640±3.409^{a}	0.220±0.043^{a}
Oxy. 5 mg/kg	6.203±2.152^{a}	23.466±14.039^{a}	18.870±6.580^{a}	14.600±1.622^{a}	11.190±3.021^{abc}	0.230±0.017^{a}
Neutral control	46.056±31.103^{b}	103.683±30.935^{c}	103.686±53.642^{b}	36.163±16.491^{ab}	23.413±12.064^{cd}	0.366±0.020^{b}

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 total peroxidase in the serum and tissues

Groups and doses	Liver	Kidney	Spleen	Lungs	Heart	Serum
	Total peroxidase (µmol/min/g of tissue or µmol/min/ml*10^{-2} of serum)					
	Females					
10 mg/kg	1.230±0.531^{a}	1.670±0.311^{a}	3.010±0.108^{a}	0.876±0.249^{a}	1.753±0.433^{ab}	7.230±0.344^{a}
30 mg/kg	2.063±0.063^{ab}	2.723±1.809^{a}	3.123±0.877^{a}	1.240±0.308^{a}	2.230±0.495^{b}	7.570±0.244^{a}
60 mg/kg	2.736±0.270^{b}	1.970±0.135^{a}	4.156±0.475^{a}	2.620±0.792^{b}	2.386±0.931^{b}	8.100±1.437^{a}
Negative control	1.046±0.548^{a}	1.673±0.525^{a}	2.766±0.370^{a}	0.863±0.321^{a}	0.276±0.087^{a}	6.683±0.669^{a}
Oxy. 5 mg/kg	1.556±0.839^{ab}	1.683±0.136^{a}	5.946±0.905^{a}	1.103±0.325^{a}	1.413±0.407^{ab}	9.073±0.322^{a}
Neutral control	2.596±0.579^{b}	5.410±1.143^{b}	5.663±0.354^{a}	3.533±0.248^{b}	3.220±0.017^{b}	13.370±1.012^{b}
	Males					
10 mg/kg	0.890±0.403^{ab}	1.743±0.061^{a}	4.670±0.080^{a}	0.973±0.020^{a}	0.943±0.029^{a}	7.273±0.197^{b}
30 mg/kg	1.996±0.904^{bc}	2.530±0.084^{ab}	3.403±0.254^{a}	3.693±0.079^{b}	2.763±0.142^{bc}	7.310±0.041^{b}
60 mg/kg	2.350±0.882^{c}	5.396±0.278^{b}	4.986±0.086^{a}	3.670±0.184^{b}	2.476±0.062^{bc}	7.573±0.305^{b}
Negative control	0.513±0.165^{a}	1.653±0.097^{a}	2.836±0.332^{a}	0.783±0.044^{a}	0.563±0.033^{a}	5.076±0.155^{a}
Oxy. 5 mg/kg	1.193±0.634^{abc}	1.923±0.086^{a}	3.843±0.048^{a}	1.106±0.068^{a}	1.480±0.020^{ab}	5.320±0.418^{a}
Neutral control	2.250±0.792^{bc}	4.150±0.204^{ab}	6.950±0.152^{a}	4.406±0.023^{b}	3.040±0.087^{c}	10.300±0.681^{c}

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 treatment on membrane lipid peroxidation (malondialdehyde) in the serum and tissues

Groups and doses	Liver	Kidney	Spleen	Lungs	Heart	Serum
	Quantities of malondialdehyde (µM/g of tissue or µM/ml of serum)					
	Females					
10 mg/kg	0.313±0.150^{a}	0.150±0.026^{b}	0.283±0.102^{de}	0.146±0.023^{ab}	0.173±0.092^{b}	0.0100±0.000^{a}
30 mg/kg	0.283±0.192^{a}	0.140±0.043^{b}	0.143±0.046^{bc}	0.083±0.055^{a}	0.040±0.010^{b}	0.0100±0.000^{a}
60 mg/kg	0.160±0.045^{a}	0.083±0.063^{ab}	0.086±0.040^{ab}	0.063±0.045^{a}	0.020±0.000^{a}	0.0067±0.005^{a}
Negative control	0.326±0.050^{a}	0.183±0.023^{b}	0.343±0.015^{e}	0.610±0.571^{b}	0.160±0.043^{b}	0.0100±0.000^{a}
Oxy. 5 mg/kg	0.173±0.105^{a}	0.136±0.083^{b}	0.226±0.028^{cd}	0.133±0.058^{ab}	0.160±0.017^{b}	0.0167±0.011^{a}
Neutral control	0.116±0.041^{a}	0.053±0.040^{a}	0.053±0.025^{a}	0.163±0.055^{ab}	0.076±0.020^{a}	0.0167±0.005^{a}
	Males					
10 mg/kg	0.446±0.162^{d}	0.276±0.055^{bc}	0.153±0.075^{ab}	0.176±0.064^{a}	0.136±0.037^{b}	0.016±0.005^{abc}
30 mg/kg	0.376±0.173^{cd}	0.140±0.010^{a}	0.146±0.098^{ab}	0.130±0.078^{a}	0.080±0.087^{ab}	0.013±0.011^{abc}
60 mg/kg	0.353±0.133b^{cd}	0.100±0.060^{a}	0.070±0.026^{a}	0.056±0.015^{a}	0.063±0.075^{ab}	0.003±0.005^{a}
Negative control	0.066±0.047^{a}	0.316±0.077^{c}	0.300±0.036^{c}	0.636±0.253^{b}	0.176±0.030^{b}	0.020±0.010^{bc}
Oxy. 5 mg/kg	0.146±0.075^{ab}	0.176±0.092^{ab}	0.280±0.070^{c}	0.230±0.017^{a}	0.020±0.017^{a}	0.010±0.000^{abc}
Neutral control	0.150±0.081^{abc}	0.126±0.037^{a}	0.210±0.043b^{c}	0.240±0.072^{a}	0.090±0.020^{ab}	0.016±0.005^{abc}

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

Groups and doses	Liver	Kidney	Spleen	Lungs	Heart	Serum
	Quantities of nitric oxide (µmole/g of tissue or µmole/ml of serum)					
	Females					
10 mg/kg	0.136±0.008^{abc}	0.433±0.098^{a}	0.273±0.028^{a}	0.323±0.011^{abc}	0.180±0.070^{ab}	5.073±0.785^{a}
30 mg/kg	0.263±0.012^{bc}	0.546±0.057^{a}	0.393±0.188^{a}	0.293±0.087^{ab}	0.196±0.035^{ab}	5.436±0.782^{a}
60 mg/kg	0.2667±0.012^{bc}	0.230±0.072^{a}	0.343±0.244^{a}	0.416±0.051^{bcd}	0.220±0.052^{ab}	4.886±1.065^{a}
Negative control	0.290±0.003^{c}	0.620±0.104^{a}	0.716±0.202^{a}	0.483±0.110^{d}	0.266±0.011^{b}	5.723±1.238^{a}
Oxy. 5 mg/kg	0.083±0.005^{a}	0.506±0.050^{a}	0.550±0.390^{a}	0.453±0.085^{cd}	0.193±0.037^{ab}	3.716±0.208^{a}
Neutral control	0.113±0.003^{ab}	0.416±0.035^{a}	0.393±0.113^{a}	0.256±0.023^{a}	0.166±0.015^{a}	3.970±0.678^{a}
	Males					
10 mg/kg	0.440±0.022^{b}	0.516±0.087^{a}	0.906±0.075^{b}	0.410±0.038^{ab}	0.196±0.041^{a}	4.750±0.934^{a}
30 mg/kg	0.516±0.011^{b}	0.703±0.095^{a}	0.493±0.028^{a}	0.326±0.062^{a}	0.170±0.072^{a}	5.566±0.485^{a}
60 mg/kg	0.366±0.029^{ab}	0.510±0.070^{a}	0.503±0.020^{a}	0.316±0.058^{a}	0.200±0.078^{a}	5.366±0.139^{a}
Negative control	0.033±0.002^{a}	0.700±0.086^{a}	0.970±0.090^{b}	0.956±0.118^{c}	0.240±0.091^{a}	5.900±0.422^{a}
Oxy. 5 mg/kg	0.060±0.005^{a}	0.563±0.080^{a}	0.796±0.091^{b}	0.583±0.047^{b}	0.176±0.080^{a}	3.360±0.710^{a}
Neutral control	0.310±0.014^{ab}	0.486±0.062^{a}	0.363±0.019^{a}	0.176±0.041^{a}	0.096±0.035^{a}	3.210±0.235^{a}

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 ($p < 0.05$)

Infection of rats with *S. typhi* has resulted in the modification of redox cell equilibrium state. The administration of different doses of extract over a period of 11 days does not only protect animals from oxidative stress but also scavenge free radical formed by infection.

4.2 Effect of the Extracts against the Stress Induced by Infection

In the scientific community, it is well known that typhoid fever induced by *Salmonella typhi* 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 [22].

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 catalase and peroxidase [23,24].

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. CAT and POD decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [23,25]. Therefore, the reduction in the activity of CAT and POD in negative control animals may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. In our study, treated groups with 30 mg/kg and 60 mg/kg *Curcuma longa* extract generally showed significant increase in CAT activity, and this could be responsible for the cure effect of extract. In fact, the administration of decocted extract to treated rats enhanced the catalase and peroxidase profiles, dose-dependently, by acting as a strong free radical quencher and protecting the tissues. Therefore peroxidase and catalase are essential for the endogenous antioxidative defense system to scavenge reactive oxygen species and maintain the cellular redox balance.

Serum alkaline phosphatase (ALP) activities on the other hand are related to functioning of hepatocytes. An increase in its activity may be due to increased synthesis in presence of increased biliary pressure [26].

The infection of animals caused a significant ($P<0.05$) elevation of ALP enzyme level when compared to control. There was a significant ($P<0.05$) restoration of this enzyme level upon administration of the rhizome extract. The reversal of increased serum ALP enzyme in *Salmonella*-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzyme. This is in agreement with the commonly accepted view that serum level of ALP returns to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [27]. Effective control of ALP level points towards an early improvement in the secretory mechanism of the hepatic cells.

The increase of the malondialdehyde (MDA) level in organs and serum induced by infection suggests increased membrane peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Hydroxyl radical can react with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause lipid peroxidation and cellular damage [23,28,29]. The treatment with *Curcuma longa* significantly reverses these changes.

Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals [30]. The results of this study showed that the infection lead to an imbalance of cytosolic redox status in favor of prooxidants, putting organs in a state of oxidative stress. 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 typhi*; there was an imbalance between prooxidants and antioxidants.

Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of *Curcuma longa* on *Salmonella* induced typhoid fever and oxidative stress in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. Phytochemical studies reveal the presence of phenolic compounds such as flavonoids and

Anthraquinone in the extract. The antioxidant and the power of these compounds have been previously demonstrated; flavonoids are histoprotectives [29,31,32].

5. CONCLUSION

The results of the present study show that the aqueous extract of *Curcuma longa* is active on *S. typhi* infected rat. Moreover it improves organs function, and reduces the generation of free radicals. This plant may therefore be recommended for the treatment of typhoid fever after the toxicological studies which will constitute our next step.

CONSENT

It is not applicable.

ETHICS

They were handled according to standard protocols for the use of laboratory animals. The studies were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Dr (Mrs) Fonkoua Marie-Christine, Pasteur Centre, Yaoundé, Cameroon, and Mrs Gatsing Lucie for their cooperation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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