



# ***In vivo* Antisalmonellal and Antioxidant Activity of Hydroethanolic Extract of *Bauhinia rufescens* Leaves in Wistar Albino Rats Infected with *Salmonella* Typhi**

**J. Djenguemtar <sup>a,b</sup>, M. S. Chekem Goka <sup>c</sup>, M. Noubom <sup>d</sup>,  
E. Yamako Konack <sup>a,e</sup>, G. T. Kamsu <sup>a</sup>, J. B. Sokoudjou <sup>a,e</sup>,  
H. B. L. Feudjio <sup>a</sup>, N. Kodjio <sup>a</sup> and D. Gatsing <sup>a\*</sup>**

<sup>a</sup> *Research Unit of Microbiology and Antimicrobial Substances, Faculty of Science, University of Dschang, P.O. Box 67: Dschang, Cameroon.*

<sup>b</sup> *Département des Sciences Biomédicales et Pharmaceutiques, Faculté de Sciences de la Santé, Université Adam Barka d'Abéché, BP : 1173, Tchad, Central Africa.*

<sup>c</sup> *Department of Biochemistry, Faculty of Science, Research Unit of Medicinal Plants Biochemistry, Food Sciences and Nutrition, University of Dschang, Cameroon.*

<sup>d</sup> *Département de Microbiologie-Hématologie et Immunologie, Faculté de Médecine et des Sciences Pharmaceutiques, Université de Dschang, Cameroon.*

<sup>e</sup> *Département des Sciences Appliquées à la Santé, Institut Universitaire et Stratégique de l'Estuaire (IUES/INSAM), BP : 4100 Douala, Cameroon.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author DG supervised the study. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/MRJI/2023/v33i21366

### **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/97764>

**Original Research Article**

**Received: 08/02/2023**

**Accepted: 15/04/2023**

**Published: 18/04/2023**

\*Corresponding author: E-mail: [gatsingd@yahoo.com](mailto:gatsingd@yahoo.com);

## ABSTRACT

Infectious diseases such as typhoid fever lead to the formation of free radicals which can have a detrimental effect on the body. Typhoid fever is caused by poor sanitation, lack of clean water and resistance of germs to antibiotics and reactive oxygen species (ROS). Hence an urgent needs to find alternative treatments with little or no toxicity for the treatment of this disease.

**Objective:** This work aims to evaluate the *in vivo* antisalmonellal and antioxidant activity of the 95% hydroethanolic extract of the leaves of *Bauhinia rufescens* (Fabaceae) in rats experimentally infected with *Salmonella* Typhi ATCC6539, as an alternative therapy.

**Methods:** The rats were randomly divided into twelve groups (six per sex) of animals. Thus 3 control groups: (T0) uninfected and untreated; (T-) infected and untreated; (T+) infected and treated with ciprofloxacin (14 mg/kg) and 3 test groups: T1, T2 and T3 infected and treated with different doses of the extract (40, 80 and 117.71 mg/kg respectively). The evolution of the infection and the effectiveness of the treatment were monitored by blood culture, food consumption and weight growth were assessed during the trial; at the end of which the animals were sacrificed and the different parameters were evaluated.

**Results:** Infected animals treated with different doses of the extract showed zero bacterial loads from the twelfth day post infection in both sexes. Regardless of sex, animals treated with the extract at the dose of 117.71mg/Kg were cured by the seventh day after the start of treatment while those treated with the doses of 40 mg/Kg and 80 mg/kg were cured by the ninth day after the start of treatment. Infection induced a significant ( $p < 0.05$ ) decrease in food consumption and weight growth, while treatment induced, at all doses, an increase in food consumption and weight growth. Infection also caused a significant ( $p < 0.05$ ) increase in NO and MDA levels, as well as a significant decrease in catalase and peroxidase activities in animal tissue homogenates. However, treatment resulted in a significant decrease in NO and MDA levels, and a significant ( $p < 0.05$ ) increase in catalase and peroxidase activities.

**Conclusion:** These results showed that the 95% hydroethanolic extract of *Bauhinia rufescens* leaves has mixed antisalmonellal and antioxidant activity *in vivo* and could be developed for the treatment of typhoid fever.

**Keywords:** *Bauhinia rufescens*; *Salmonella Typhi*; salmonellosis; oxidative stress.

## 1. INTRODUCTION

“Typhoid fever is a food borne illness caused by a gram-negative bacterium called *Salmonella* Typhi. It occurs one to two weeks after infection and the most common clinical signs are fever, malaise, abdominal pain, headache, myalgia, nausea, anorexia, constipation and/or diarrhea” [1]. “The World Health Organization estimates 21 million cases per year worldwide and approximately 161000 deaths” [2]. The annual incidence in Africa is 13-845 cases per 100000 populations [3]. Lack of sanitation, inadequate drinking water, inappropriate use of antibiotics and the development of resistance to available antibiotics [4] have made this disease a real public health problem in Chad.

“During microbial infections, macrophages produce free radicals to destroy microorganisms within phagosomes. However, some enterobacteria such as *Salmonella* express the oxyR and oxyS genes that code for several

proteins that allow them to resist these free radicals” [5]. This results in an expansion of free radicals in the host organism causing an imbalance between oxidants and antioxidants [6]. This imbalance leads to oxidative stress which is one of the major complications of *Salmonella* Typhi infections [7]. Thus, the search for new sources of available alternative drugs becomes an issue. Due to their richness in bioactive principles, plants appear as an alternative that can offer a new possibility of effective, inexpensive and accessible control for patients [8]. Especially since the constant search for new plant-based antisalmonellal treatments is one of the priority areas of health policies and is part of the strategies for effective control of typhoid fever [9].

Chad has a very large diversity of plants that can be offered as an alternative to solve this problem or for the discovery of new antimicrobial/antibiotic agents [10]. They are part of a poorly documented and highly exploited field, as more

than 20,000 plant species are used in traditional medicine without having been the subject of chemical and pharmacological studies for many [11]. This is the case of *Bauhinia rufescens*, whose numerous secondary metabolites could justify its traditional use in West and Central Africa for the treatment of diabetes, diarrhea, dysentery, mycosis, fibrosis, jaundice and inflammation [12,13]. In Chad, given the traditional use of *Bauhinia rufescens* leaves against typhoid fever and considering the interesting scientific results obtained *in vitro* with *Bauhinia rufescens* extracts against multi-resistant bacteria of the genus *Salmonella* [10]. For this reason, this extract has been used for antisalmonellal and antioxidant activity *in vivo*. Hence the objective of this work which aims to provide concrete scientific evidence on the *in vivo* therapeutic efficacy of *Bauhinia rufescens* against simulated typhoid fever in rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Preparation of the Extract

The plant material used for this work consisted of *Bauhinia rufescens* leaves, collected in February 2019 in Abeche, eastern Chad (13° 49' 0" North, 20° 49' 0" East.) and identified at the Botanical Unit of the Livestock Research Institute for Development (UBIRED) in N'djamena, Chad, under the reference IRED/LRVZ 1325.

Fig. 1, the leaves of *Bauhinia rufescens* were harvested and dried at room temperature  $30\pm 2^{\circ}\text{C}$  away from the sun and ground using a Moulinex brand Zaiba (Super Blender, China). The powder obtained was stored in a cardboard box at room temperature, in a dry place and protected from humidity and light until their use. The obtained powder was used for the preparation of hydroethanolic extract 95% as described by Kamsu et al, [14]. Briefly, 250 g of *Bauhinia rufescens* leaf powder was macerated with constant stirring for 48 hours in 2500 ml of solvent. The mixture (solvent and extract) was then filtered through Whatman N°1 paper. The filtrate obtained was evaporated with a rotary evaporator (Büchi R200, Switzerland) under vacuum; then placed in a ventilated oven (Memmert) set at  $40^{\circ}\text{C}$  for 12 h until complete evaporation of the solvent. The extract obtained was stored in the refrigerator at  $4^{\circ}\text{C}$  until use.

### 2.2 Microorganism

The microorganism used for the induction of Salmonellosis is a Gram-negative bacterium. A strain of *Salmonella enterica* serovar Typhi ATCC6539, obtained at the Centre Pasteur of Cameroon. Their pure cultures were maintained in Muller-Hinton agar and stored at  $4^{\circ}\text{C}$ .

### 2.3 Experimental Animals

Healthy young Wistar rats (150 - 200 g) of each sex were raised at the University of Dschang animal facility. Animal housing and *in vivo* experiments were conducted in accordance with the European Union guidelines on animal protection (Council EEC 86/609) [15] which were adopted by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon. Rats were housed individually in polypropylene cages at  $25\pm 1^{\circ}\text{C}$  on a 12-hour light/dark cycle. Animals were fed a standard diet and received water *ad libitum*.

### 2.4 Induction and Treatment of Typhoid Fever

"Throughout the experiment, in order to reduce the level of commensal anaerobic bacteria that normally colonize the gut of rats, the animals were given an azithromycin solution (5 mg/mL)" [16]. "A suspension of *Salmonella* Typhi was prepared at 0.5 on the Mc Farland turbidity scale. A saline solution (1 mL 0.9% NaCl), containing approximately  $1.5 \times 10^8$  colony forming units, was administered orally to each animal" [17]. Forty-eight male and female albino rats of the Wistar strain aged 8 to 10 weeks were divided into 12 groups of 4 animals each, including 6 groups of males and 6 groups of females. The selected animals were acclimatized for one week. With the exception of animals in group 1 (uninfected and untreated), all other groups (2-6) were infected. They received a single oral dose (1 mL) of a  $1.5 \times 10^8$  CFU suspension of *Salmonella* Typhi (ATCC6539). Monitoring of infection in animals was performed by blood culture with colony counting on Salmonella/Shigella agar (BK 022-500G) and converted into *Salmonella* CFU per milliliter of blood.

"Efficiency of infection was achieved when the blood bacterial concentration was greater than  $4 \times 10^5$  CFU/ml blood, followed by excretion of watery stool, presence of mucus in the stool,

reduction of activity, and exponential increase in systemic *Salmonella* Typhi load in rats” [18]. “Each animal in each group was housed in its own cage and these animals were treated as follows: Group 1 (uninfected and untreated = T0) received distilled water; Group 2 (typhoid control group, infected and untreated) received distilled water; Group 3 (positive control group) received ciprofloxacin (14 mg/kg); Groups 4, 5, and 6 (test groups) received *Bauhinia rufescens* 95% hydroethanolic extract (40, 80, and 117.71 mg/kg, respectively). The dose of 117.71 mg/kg body weight was obtained from the traditional practitioner's dose. The dose of 40 mg/kg body weight was obtained from the extract MIC and 80 mg/kg body weight is a multiple of 2 of the extract MIC” [10].

During treatment, blood was collected in heparinized tubes and cultured immediately on *Salmonella*/*Shigella* agar (BK 022-500G) once every two days. The decrease of the bacterial load in the blood indicated the effectiveness of the treatment. The test was completed when no *Salmonella* colonies were found in the blood of the animals after the blood was cultured.

At the end of the experiment, the animals were fasted for 12 h and then anesthetized with the combination of diazepam and ketamine (0.2 and 0.1 ml for a 100-gram animal). A blood sample was collected by cardiac puncture, centrifuged at 3000 g for 15 min, and the plasma obtained was used for biochemical analyses. The animals were then dissected and the liver, kidneys, heart, lungs, and spleen were removed. A sample (0.20 g) of each organ was ground in a mortar containing ice and centrifuged. Homogenates of these organs were prepared in phosphate buffer pH 7.2 (20% organ in 80% phosphate buffer) and then centrifuged at 3000g for 15 min. The supernatant (organ homogenate) was stored at -20°C for analyses of oxidative stress parameters.

## 2.5 Determination of Food Consumption

“Food consumption was determined daily by taking the difference between the amounts of food (in g) deposited the day before and the amounts remaining the next day” [14].

### 2.5.1 Determination of weight gain or loss

Weight gain or loss was assessed daily and determined by the following formula [14].

$$W=W_f - W_0$$

Where:

W= weight gain or loss on day x;  
W<sub>f</sub> = Body weight of the animal on day x;  
W<sub>0</sub> = Initial body weight of the animal (before the start of the trial).

## 2.6 Determination of Enzymatic and Non-Enzymatic Antioxidant Markers

### 2.6.1 Determination of catalase activity

Catalase activity was determined by the method of Dimo et al, [19] “Phosphate buffer pH 7.4 (375 μL) was added to 25 μL of tissue homogenate, followed by 100 μL of H<sub>2</sub>O<sub>2</sub> (50 mM). One minute later, 1 mL of potassium dichromate (5%) prepared in 1% acetic acid was introduced into the reaction medium. The resulting mixture was incubated for 10 min in a boiling water bath and then cooled in an ice bath. The optical density was obtained at 570 nm (BIOBASE BK-D590 spectrophotometer, China) against the blank (the extract was replaced by distilled water in the blank tubes). The enzymatic activity of catalase was deduced by the Beer-Lambert law”.

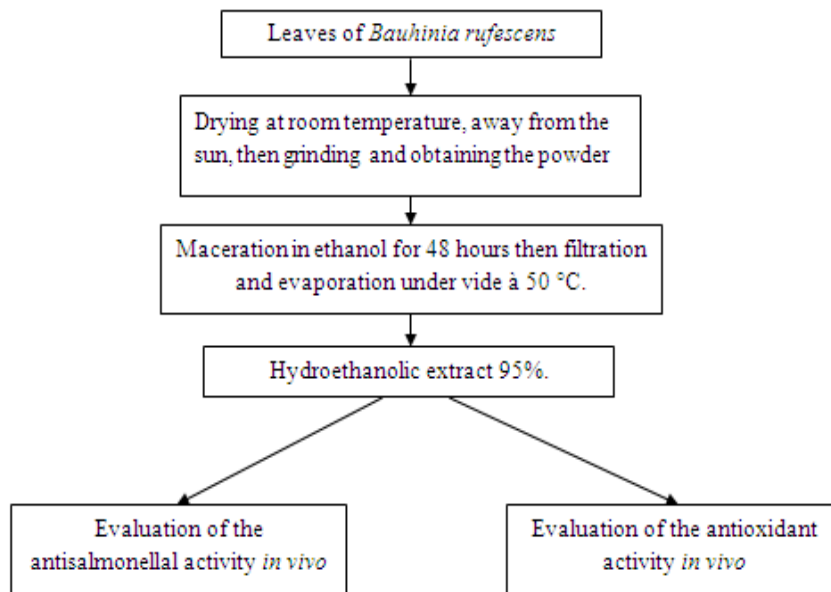
### 2.6.2 Determination of peroxidase activity (POD)

“Peroxidase activity was determined in tissues by the method of Habbu et al.” [20]. The test sample (0.5 ml) was added to 1 ml of 10 mM KI solution and 1 ml of sodium acetate (40 mM). The absorbance of potassium iodide was read at 353 nm (BIOBASE BK-D590 spectrophotometer, China), which indicates the amount of peroxidase. Then 20 μL of H<sub>2</sub>O<sub>2</sub> (15 mM) was added, and the change in absorbance over 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. Specific activity was expressed in terms of units per mg of protein.

### 2.6.3 Determination of malondialdehyde (MDA) level

Lipid peroxidation was assessed by determining the level of malondialdehyde (MDA) using the method of Oyedemi et al, [21]. Malondialdehyde is one of the end products of polyunsaturated fatty acids (PUFAs) decomposition under the effect of free radicals released during stress. In hot acidic medium (pH 2 to 3; 100°C), an MDA molecule condenses with two thiobarbituric molecules (TBA) to form a pink-colored complex



**Fig. 1. General scheme**

(reading at 532 nm). Five hundred microliters of 1% orthophosphoric acid and 500  $\mu\text{L}$  of precipitation mixture (1% thiobarbituric acid in 1% acetic acid) were added to 100  $\mu\text{L}$  of homogenate. The resulting reaction mixture was homogenized and incubated for 15 min in a boiling water bath. After cooling in an ice bath, the mixture was centrifuged at 3500 rpm for 10 min. The absorbance of the supernatant was read at 532 nm (BIOBASE BK-D590 spectrophotometer, China) against the blank. Lipid peroxidation was calculated based on the molar extinction coefficient of MDA and expressed as micromoles of MDA per gram of tissue using the Beer-Lambert law.

#### 2.6.4 Determination of nitric oxide (NO) level

The nitric oxide content in the tissue or tissue homogenates was measured by the reagent of Griess. Greiss, [22]. The absorption of the chromophore during ionization of nitrite with sulfanilamide coupled to naphthylethylene diamine (NED) was read at 520 nm. Three hundred and forty microliters of 1% sulfanilamide (prepared in 5% orthophosphoric acid) was introduced into 340  $\mu\text{L}$  of plasma or homogenates. The resulting mixture was homogenized and left in the dark for 5 minutes at room temperature. Then, 340  $\mu\text{L}$  of 0.1% naphthylethylene diamine (NED) was added to the reaction medium and the mixture was again

left in the dark for 5 minutes. NO concentration was estimated by measuring absorbance at 520 nm (BIOBASE BK-D590 spectrophotometer, China), and the results were expressed in terms of micromoles of NO per gram of tissue or per milliliter of blood using a sodium nitrite calibration curve ( $y=0.0563x +0.1077$ ;  $r^2=0.9835$ ).

#### 2.7 Statistical Analysis

Results were expressed as mean  $\pm$  standard deviation. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) by the general linear model procedure followed by the Waller-Duncan test for comparison between the treatment and control groups. P values  $<0.05$  were considered significant.

### 3. RESULTS

#### 3.1 *In vivo* Anti-Typhoid Activity of the 95% Hydroethanolic Extract of *Bauhinia Rufescens* Leaves in Infected Rats

The *in vitro* antisalmonellal activity revealed that the 95% hydroethanolic extract of *Bauhinia rufescens* leaves is the most active on *Salmonella* Typhi strain ATCC6539 [10]. For this reason, this extract was used for *in vivo* antisalmonellal activity.

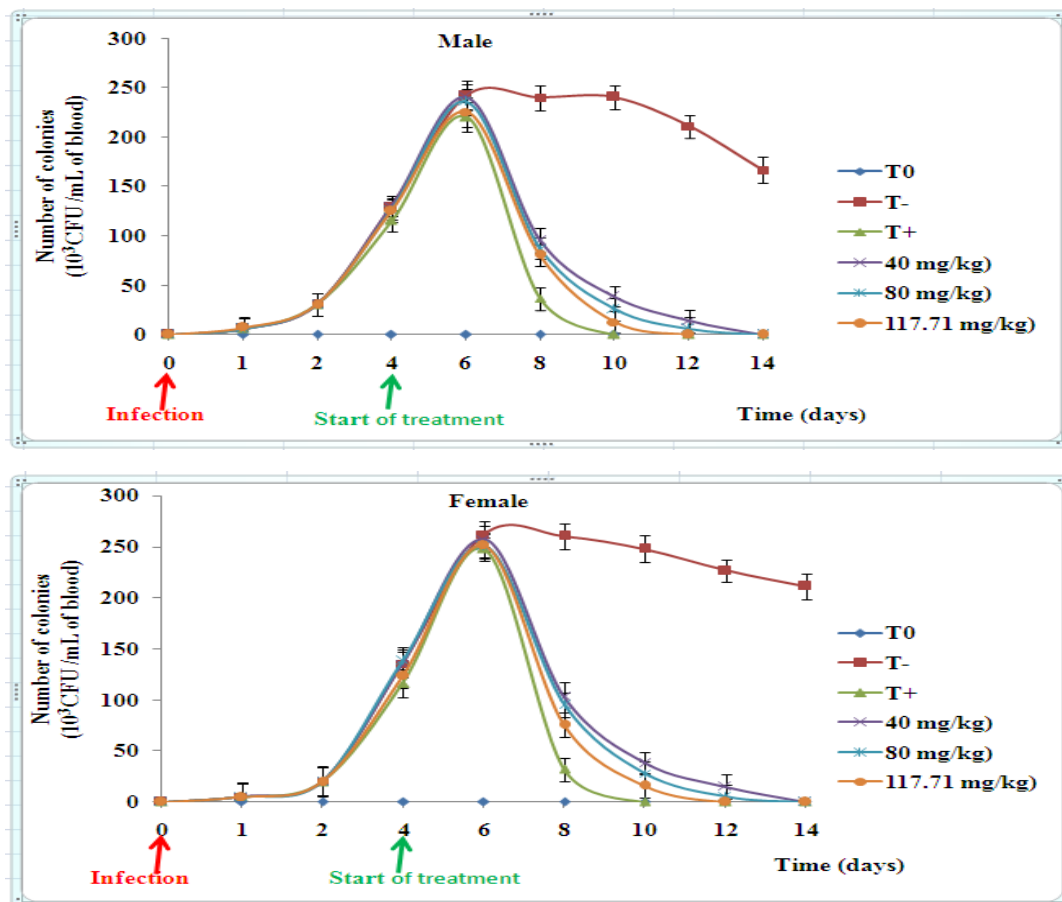
### 3.1.1 Evolution of the bacterial load

The evolution of the bacterial load in the blood of male and female animals is shown in fig 2. The bacterial load remains zero in uninfected and untreated animals and progressive in infected and untreated animals, these figures presented curves in three phases. The first phase is the slow growth phase between day zero and day two, followed by an exponential growth phase between day two and day six. After the administration of the extract, a significant decrease in the number of colonies was observed in animals treated with the 95% hydroethanolic extract of *Bauhinia rufescens* leaves and ciprofloxacin from day seven, marking the effectiveness of the treatment, which corresponds to the decline phase. Infected animals treated with different doses of the extract showed a zero bacterial load from day 12 post-infection in both males and females.

The recovery of the animals was dose-dependent.

### 3.1.2 Effect of treatment on food consumption of rats during the experiment

Fig. 3 present the evolution of food consumption of male and female animals. It can be seen that infection induced a significant decrease ( $p < 0.05$ ) in food consumption in infected and untreated animals (negative control) compared to uninfected and untreated animals (neutral control). At all dose levels, treatment induced an increase in food consumption in animals of both sexes compared to infected and untreated animals throughout the treatment period. In both male and female animals, this food consumption was proportional to the dose of extract administered. There was no significant difference ( $p \geq 0.05$ ) in food consumption between doses.



**Fig. 2 Evolution of bacterial load after infection and during treatment with different doses of the 95% hydroethanolic extract of *Bauhinia rufescens* leaves in male and female rats as a function of time**

The values in the figure are presented as means  $\pm$  standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated.

### 3.1.3 Effect of treatment on weight growth of rats during the experiment

Fig. 4 show the changes in weight growth of male and female rats respectively during the experiment. In general, the infection caused a significant ( $p < 0.05$ ) decrease in the weight growth of male and female rats compared to the neutral control. In contrast, treatment of animals with different doses of extract resulted in a non-significant ( $p \geq 0.05$ ) increase in weight growth in both sexes compared with the negative control.

## 3.2 In vivo Antioxidant Potential of the 95% Hydroethanolic Extract of *Bauhinia rufescens* Leaves

### 3.2.1 Effect of treatment on levels of non-enzymatic markers of oxidative stress

Tables 1 and 2, show the effect of treatment on tissue nitric oxide (NO) and MDA levels in animals of both sexes after treatment. Analysis of these tables reveals that infection generally induced an increase in tissue NO and MDA levels in all negative control animals of both sexes compared with neutral control animals. However, treatment with the 95% hydroethanolic extract of *Bauhinia rufescens* leaves resulted in a dose-dependent decrease in tissue NO and MDA levels in all treated animals compared with negative control animals.

### 3.2.2 Effect of treatment on the activity of enzymatic markers of oxidative stress

The effect of treatment with 95% hydroethanolic extract of *Bauhinia rufescens* leaves on plasma and tissue catalase and tissue peroxidase activity of animals is presented in Tables 3 and 4, respectively. Analysis of these arrays reveals that infection induced a significant ( $P < 0.05$ ) decrease in the activity of these two antioxidant enzymes in negative control animals in both sex compared with neutral control animals. However, treatment resulted in a dose-dependent increase in the activity of these two antioxidant enzymes in all treated animals compared with negative controls.

## 4. DISCUSSION

Typhoid fevers are caused by *Salmonella* adapted to humans, namely: *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella*

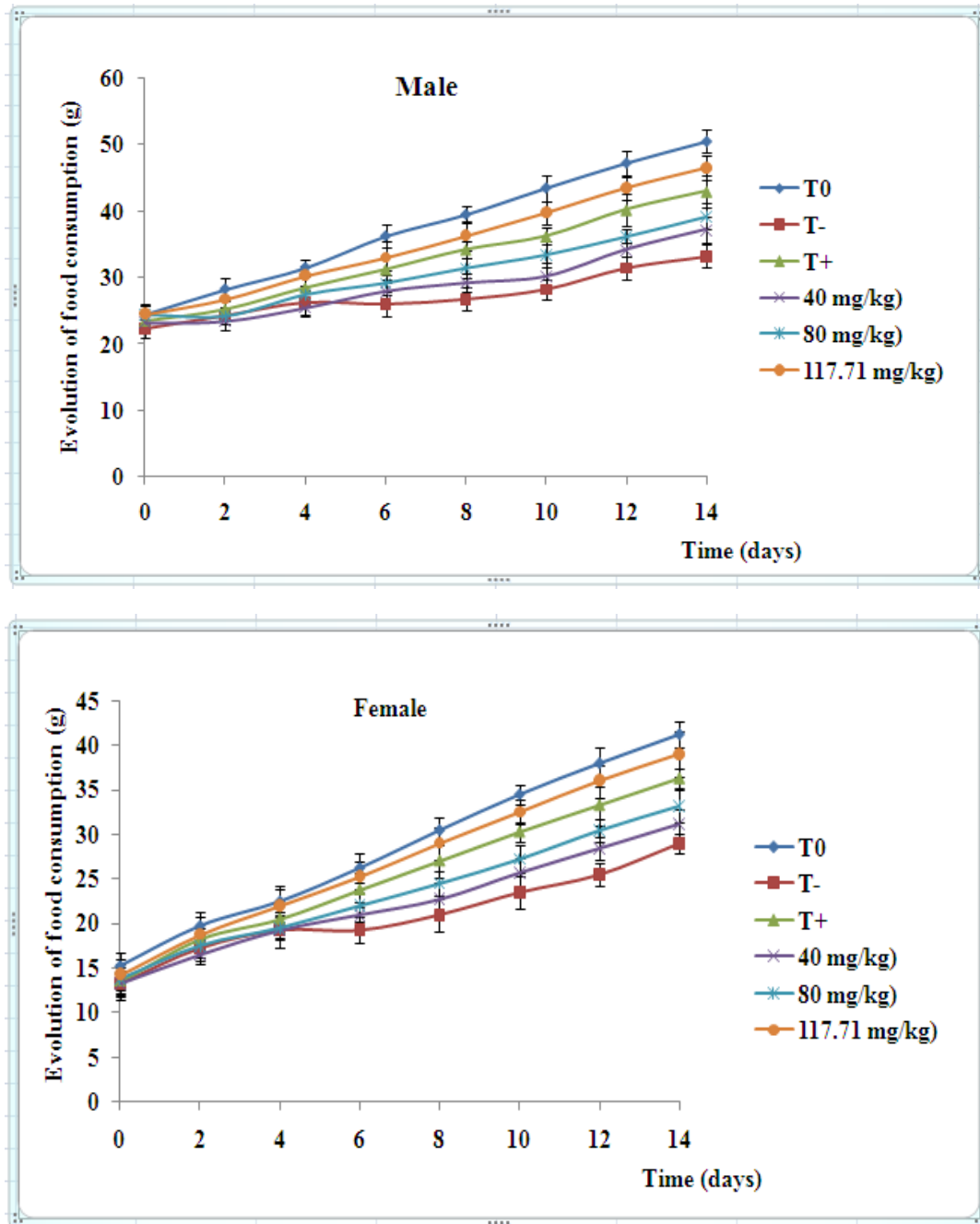
Paratyphi B [23]. "Thus, *Salmonella* Typhi induces systemic infection in humans and rats [24] while *Salmonella* Typhimurium induces systemic infection in mice and rats, but only localized gastroenteritis in humans. Due to its *in vitro* antisalmonellal activity [10] the 95% hydroethanolic extract of *Bauhinia rufescens* leaves was used for the *in vivo* test". For this reason, we chose *Salmonella* Typhi to infect rats while studying the activity of the 95% hydroethanolic extract of *Bauhinia rufescens* leaves *in vivo*. Albino rats of Wistar strain were used in this study. After infection, we observed a latent period of 24 hours, during which time there was no significant increase in *Salmonella* Typhi colonies in the blood of the animals. During this latent period the bacteria acclimatize to the environmental conditions and synthesize the enzymes necessary to metabolize the substrate present [25]. The number of *Salmonella* Typhi colonies in the blood of rats after the latent period is explained by the fact that the infection has taken place. The decrease in bacterial load observed during treatment would be due to the antibacterial action of the extract, a continuous increase in load was observed in infected and untreated animals.

Animals treated with different doses of 95% hydroethanolic extract of *Bauhinia rufescens* leaves were kept free from *Salmonella* between the tenth and twelfth day of the test, both in males and females. Animals treated with 95% hydroethanolic extract of *Bauhinia rufescens* leaves at dose 117.71 were cured on the seventh day after the start of treatment while those treated at 40 mg/Kg and 80 mg/Kg were cured on the ninth day after the start of treatment; thus, the duration of treatment is dose dependent. The healing of animals at different doses of the 95% hydroethanolic extract of *Bauhinia rufescens* leaves is thought to be related to the presence of different secondary metabolites revealed by phytochemical analysis of the extract [10]. Large varieties of phenols including flavonoids from medicinal plants are known to have antibacterial properties [26].

Infection of the animals resulted in a decrease in food consumption compared to the neutral control. However, daily administration of the 95% hydroethanolic extract of *Bauhinia rufescens* leaves resulted in increased food consumption in both male and female animals compared to the negative control. In addition, in both males and females, intake was proportional to the treatment

dose. Infection resulted in weight loss, whereas treatment resulted in a slight increase over the negative control. Weight gain in both males and females was dose-dependent. This result

corroborates that of Kodjio, et al. [7] who showed that plant extracts could increase food intake and weight gain in rats during induced salmonellosis.



**Fig. 3. Evolution of food consumption (g) of infected male and female animals treated with 95% hydroethanolic extract of *Bauhinia rufescens* leaves as a function of time (days)**

Values in the figure are presented as means  $\pm$  standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated



**Table 1. Effect of 95% hydroethanolic extract of *Bauhinia rufescens* leaves on nitric oxide (NO) levels in rat organ tissues**

Sex	Dose	Heart	Liver	Lungs	Spleen	Kidneys
Male	T0	0.729 $\pm$ 0.030 <sup>a</sup>	0.680 $\pm$ 0.056 <sup>a</sup>	2.101 $\pm$ 0.050 <sup>a</sup>	2.705 $\pm$ 0.369 <sup>a</sup>	0.614 $\pm$ 0.065 <sup>a</sup>
	T-	2.257 $\pm$ 0.173 <sup>d</sup>	0.942 $\pm$ 0.039 <sup>d</sup>	2.963 $\pm$ 0.034 <sup>e</sup>	5.956 $\pm$ 0.386 <sup>b</sup>	3.660 $\pm$ 0.069 <sup>d</sup>
	T+	1.084 $\pm$ 0.030 <sup>c</sup>	0.858 $\pm$ 0.056 <sup>cd</sup>	2.283 $\pm$ 0.042 <sup>d</sup>	2.750 $\pm$ 0.393 <sup>a</sup>	0.969 $\pm$ 0.065 <sup>c</sup>
	40mg/kg	1.022 $\pm$ 0.030 <sup>bc</sup>	0.822 $\pm$ 0.056 <sup>bc</sup>	2.248 $\pm$ 0.042 <sup>cd</sup>	2.714 $\pm$ 0.393 <sup>a</sup>	0.933 $\pm$ 0.065 <sup>c</sup>
	80mg/kg	0.907 $\pm$ 0.030 <sup>b</sup>	0.751 $\pm$ 0.056 <sup>ab</sup>	2.186 $\pm$ 0.027 <sup>bc</sup>	2.643 $\pm$ 0.393 <sup>a</sup>	0.854 $\pm$ 0.069 <sup>bc</sup>
	117,71 mg/kg	0.765 $\pm$ 0.030 <sup>a</sup>	0.716 $\pm$ 0.056 <sup>a</sup>	2.150 $\pm$ 0.027 <sup>ab</sup>	2.608 $\pm$ 0.393 <sup>a</sup>	0.809 $\pm$ 0.070 <sup>b</sup>
Female	T0	2.976 $\pm$ 0.017 <sup>a</sup>	2.585 $\pm$ 0.195 <sup>a</sup>	1.755 $\pm$ 0.115 <sup>a</sup>	0.663 $\pm$ 0.038 <sup>a</sup>	1.404 $\pm$ 0.267 <sup>a</sup>
	T-	3.860 $\pm$ 0.052 <sup>e</sup>	5.583 $\pm$ 0.500 <sup>b</sup>	2.155 $\pm$ 0.081 <sup>c</sup>	0.840 $\pm$ 0.032 <sup>c</sup>	2.612 $\pm$ 0.174 <sup>b</sup>
	T+	3.331 $\pm$ 0.017 <sup>d</sup>	2.941 $\pm$ 0.195 <sup>a</sup>	1.977 $\pm$ 0.156 <sup>ab</sup>	0.840 $\pm$ 0.038 <sup>c</sup>	1.462 $\pm$ 0.048 <sup>a</sup>
	40mg/kg	3.260 $\pm$ 0.017 <sup>c</sup>	2.869 $\pm$ 0.195 <sup>a</sup>	1.941 $\pm$ 0.156 <sup>ab</sup>	0.805 $\pm$ 0.038 <sup>c</sup>	1.377 $\pm$ 0.158 <sup>a</sup>
	80mg/kg	3.154 $\pm$ 0.017 <sup>b</sup>	2.763 $\pm$ 0.195 <sup>a</sup>	1.870 $\pm$ 0.156 <sup>a</sup>	0.734 $\pm$ 0.038 <sup>b</sup>	1.302 $\pm$ 0.154 <sup>a</sup>
	117,71 mg/kg	3.012 $\pm$ 0.017 <sup>a</sup>	2.621 $\pm$ 0.195 <sup>a</sup>	1.835 $\pm$ 0.156 <sup>a</sup>	0.698 $\pm$ 0.038 <sup>ab</sup>	1.266 $\pm$ 0.154 <sup>a</sup>

Data are the mean  $\pm$  standard error of the mean ( $n = 4$ ). In the same column and for the same sex, values with different letters are significantly different ( $p < 0.05$ ). T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated

**Table 2. Effect of 95% hydroethanolic extract of *Bauhinia rufescens* leaves on malondialdehyde (MDA) levels in rat organ tissues**

Sex	Dose	Heart	Liver	Lungs	Spleen	Kidneys
Male	T0	0.324 $\pm$ 0.013 <sup>a</sup>	0.354 $\pm$ 0.015 <sup>a</sup>	0.191 $\pm$ 0.038 <sup>a</sup>	0.328 $\pm$ 0.160 <sup>a</sup>	0.594 $\pm$ 0.040 <sup>a</sup>
	T-	0.560 $\pm$ 0.022 <sup>c</sup>	0.439 $\pm$ 0.011 <sup>c</sup>	0.648 $\pm$ 0.037 <sup>a</sup>	0.592 $\pm$ 0.298 <sup>d</sup>	0.924 $\pm$ 0.015 <sup>c</sup>
	T+	0.438 $\pm$ 0.017 <sup>b</sup>	0.384 $\pm$ 0.003 <sup>b</sup>	0.496 $\pm$ 0.055 <sup>a</sup>	0.386 $\pm$ 0.088 <sup>c</sup>	0.739 $\pm$ 0.025 <sup>b</sup>
	40mg/kg	0.435 $\pm$ 0.022 <sup>b</sup>	0.367 $\pm$ 0.006 <sup>ab</sup>	0.369 $\pm$ 0.089 <sup>a</sup>	0.356 $\pm$ 0.110 <sup>b</sup>	0.690 $\pm$ 0.010 <sup>b</sup>
	80mg/kg	0.368 $\pm$ 0.047 <sup>a</sup>	0.361 $\pm$ 0.003 <sup>a</sup>	0.275 $\pm$ 0.031 <sup>a</sup>	0.335 $\pm$ 0.076 <sup>a</sup>	0.588 $\pm$ 0.057 <sup>a</sup>
	117,71 mg/kg	0.328 $\pm$ 0.008 <sup>a</sup>	0.358 $\pm$ 0.014 <sup>a</sup>	0.218 $\pm$ 0.022 <sup>a</sup>	0.331 $\pm$ 0.045 <sup>a</sup>	0.572 $\pm$ 0.015 <sup>a</sup>
Female	T0	0.323 $\pm$ 0.010 <sup>a</sup>	0.632 $\pm$ 0.052 <sup>a</sup>	0.316 $\pm$ 0.159 <sup>a</sup>	0.277 $\pm$ 0.095 <sup>a</sup>	0.705 $\pm$ 0.047 <sup>a</sup>
	T-	0.448 $\pm$ 0.029 <sup>c</sup>	0.834 $\pm$ 0.022 <sup>c</sup>	0.387 $\pm$ 0.024 <sup>a</sup>	0.520 $\pm$ 0.013 <sup>b</sup>	0.961 $\pm$ 0.027 <sup>c</sup>
	T+	0.376 $\pm$ 0.007 <sup>b</sup>	0.755 $\pm$ 0.018 <sup>bc</sup>	0.364 $\pm$ 0.119 <sup>a</sup>	0.371 $\pm$ 0.043 <sup>a</sup>	0.868 $\pm$ 0.103 <sup>ab</sup>
	40mg/kg	0.350 $\pm$ 0.016 <sup>ab</sup>	0.720 $\pm$ 0.077 <sup>ab</sup>	0.466 $\pm$ 0.197 <sup>a</sup>	0.321 $\pm$ 0.023 <sup>a</sup>	0.785 $\pm$ 0.080 <sup>ab</sup>
	80mg/kg	0.327 $\pm$ 0.015 <sup>a</sup>	0.685 $\pm$ 0.031 <sup>ab</sup>	0.343 $\pm$ 0.059 <sup>a</sup>	0.308 $\pm$ 0.088 <sup>a</sup>	0.740 $\pm$ 0.191 <sup>a</sup>
	117,71 mg/kg	0.340 $\pm$ 0.020 <sup>a</sup>	0.643 $\pm$ 0.100 <sup>a</sup>	0.340 $\pm$ 0.075 <sup>a</sup>	0.289 $\pm$ 0.059 <sup>a</sup>	0.736 $\pm$ 0.121 <sup>a</sup>

Data are the mean  $\pm$  standard error of the mean ( $n = 4$ ). In the same column and for the same sex, values with different letters are significantly different ( $p < 0.05$ ). T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated

**Table 3. Effect of 95% hydroethanolic extract of *Bauhinia rufescens* leaves on plasma and tissue catalase activity of rat organs**

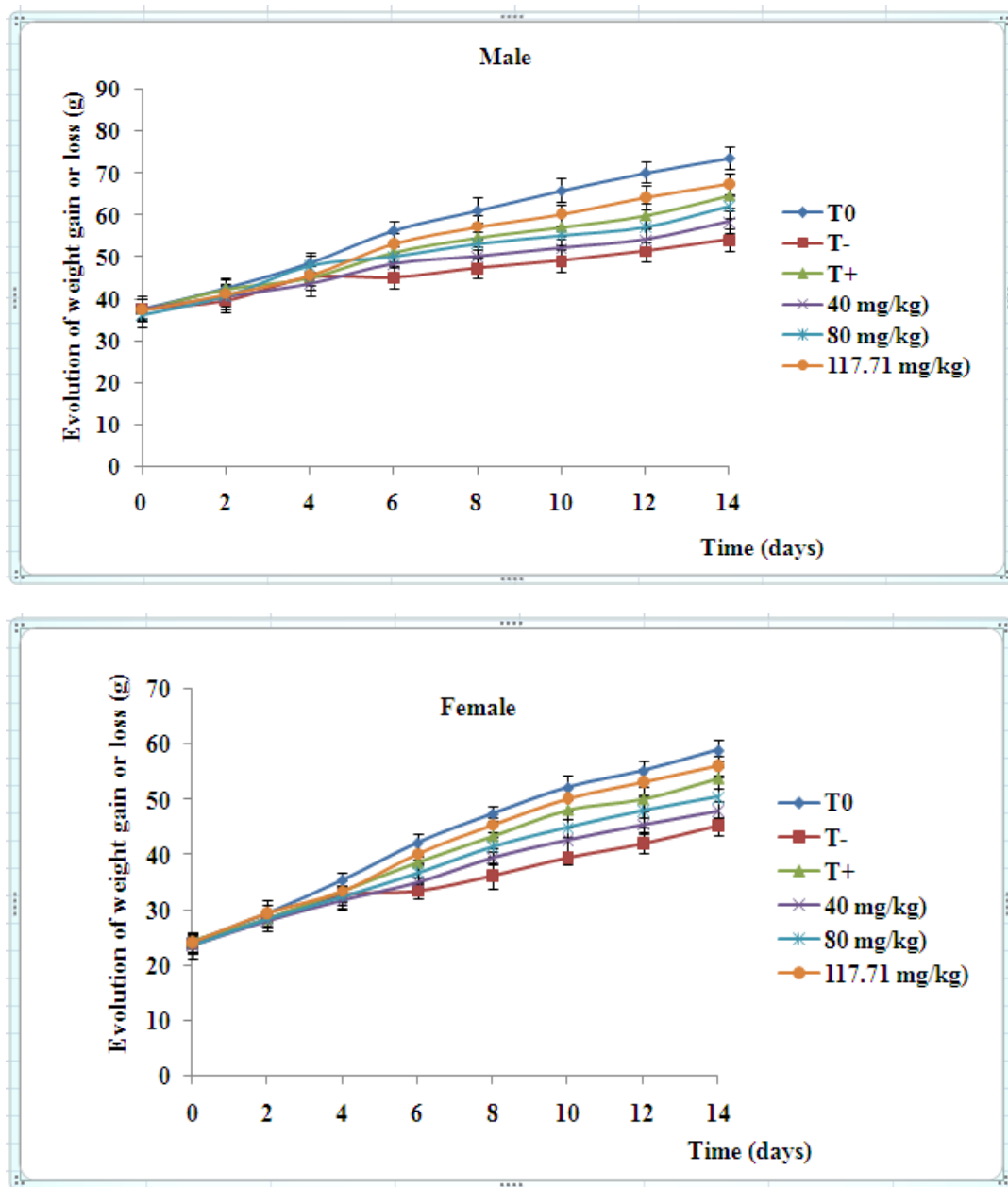
Sex	Doses	plasma	Heart	Liver	Lungs	Spleen	Kidneys
		Catalase activity ( $\mu\text{mol}/\text{min}/\text{mg}$ of tissues and $\mu\text{mol}/\text{min}/\text{mL}$ of plasma)					
	T0	0.158 $\pm$ 0.001 <sup>f</sup>	15.519 $\pm$ 0.190 <sup>f</sup>	12.406 $\pm$ 0.199 <sup>e</sup>	24.715 $\pm$ 0.470 <sup>f</sup>	15.642 $\pm$ 0.160 <sup>f</sup>	19.658 $\pm$ 0.163 <sup>e</sup>
	T-	0.086 $\pm$ 0.001 <sup>a</sup>	9.694 $\pm$ 0.330 <sup>a</sup>	8.357 $\pm$ 0.260 <sup>a</sup>	17.532 $\pm$ 0.239 <sup>a</sup>	8.437 $\pm$ 0.300 <sup>a</sup>	12.308 $\pm$ 0.044 <sup>a</sup>
	T+	0.101 $\pm$ 0.001 <sup>b</sup>	10.621 $\pm$ 0.277 <sup>b</sup>	9.355 $\pm$ 0.179 <sup>b</sup>	19.116 $\pm$ 0.056 <sup>b</sup>	10.535 $\pm$ 0.227 <sup>b</sup>	14.445 $\pm$ 0.209 <sup>b</sup>
Male	40mg/kg	0.116 $\pm$ 0.001 <sup>c</sup>	12.520 $\pm$ 0.285 <sup>c</sup>	10.649 $\pm$ 0.219 <sup>c</sup>	21.080 $\pm$ 0.046 <sup>c</sup>	12.624 $\pm$ 0.122 <sup>c</sup>	15.420 $\pm$ 0.257 <sup>c</sup>
	80mg/kg	0.138 $\pm$ 0.001 <sup>d</sup>	13.199 $\pm$ 0.126 <sup>d</sup>	11.296 $\pm$ 0.031 <sup>d</sup>	22.327 $\pm$ 0.173 <sup>d</sup>	14.518 $\pm$ 0.018 <sup>d</sup>	18.359 $\pm$ 0.359 <sup>d</sup>
	117,71 mg/kg	0.146 $\pm$ 0.001 <sup>e</sup>	14.651 $\pm$ 0.072 <sup>e</sup>	12.202 $\pm$ 0.082 <sup>e</sup>	23.572 $\pm$ 0.261 <sup>e</sup>	15.102 $\pm$ 0.096 <sup>e</sup>	19.390 $\pm$ 0.254 <sup>e</sup>
	T0	0.086 $\pm$ 0.001 <sup>f</sup>	8.206 $\pm$ 0.141 <sup>d</sup>	14.476 $\pm$ 0.349 <sup>f</sup>	18.522 $\pm$ 0.172 <sup>e</sup>	11.301 $\pm$ 0.346 <sup>d</sup>	14.655 $\pm$ 0.350 <sup>e</sup>
	T-	0.041 $\pm$ 0.001 <sup>a</sup>	5.455 $\pm$ 0.101 <sup>a</sup>	9.184 $\pm$ 0.081 <sup>a</sup>	13.476 $\pm$ 0.292 <sup>a</sup>	8.276 $\pm$ 0.073 <sup>a</sup>	8.489 $\pm$ 0.311 <sup>a</sup>
	T+	0.056 $\pm$ 0.001 <sup>b</sup>	6.677 $\pm$ 0.410 <sup>b</sup>	11.243 $\pm$ 0.211 <sup>b</sup>	15.557 $\pm$ 0.332 <sup>b</sup>	8.581 $\pm$ 0.266 <sup>a</sup>	10.372 $\pm$ 0.287 <sup>b</sup>
Female	40 mg/kg	0.062 $\pm$ 0.001 <sup>c</sup>	7.414 $\pm$ 0.341 <sup>c</sup>	12.341 $\pm$ 0.188 <sup>c</sup>	16.494 $\pm$ 0.116 <sup>c</sup>	9.786 $\pm$ 0.168 <sup>b</sup>	12.460 $\pm$ 0.176 <sup>c</sup>
	80 mg/kg	0.069 $\pm$ 0.001 <sup>d</sup>	7.430 $\pm$ 0.083 <sup>c</sup>	13.448 $\pm$ 0.288 <sup>d</sup>	17.441 $\pm$ 0.379 <sup>d</sup>	10.545 $\pm$ 0.201 <sup>c</sup>	13.676 $\pm$ 0.278 <sup>d</sup>
	117,71 mg/kg	0.078 $\pm$ 0.001 <sup>e</sup>	8.192 $\pm$ 0.138 <sup>d</sup>	14.079 $\pm$ 0.078 <sup>e</sup>	18.161 $\pm$ 0.156 <sup>e</sup>	10.688 $\pm$ 0.164 <sup>c</sup>	14.274 $\pm$ 0.167 <sup>e</sup>

Data are the mean  $\pm$  standard error of the mean ( $n = 4$ ). In the same column and for the same sex, values with different letters are significantly different ( $p < 0.05$ ). T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated

**Table 4. Effect of 95% hydroethanolic extract of *Bauhinia rufescens* leaves on tissue peroxidase activity of rat organs**

Sex	Doses	Heart	Liver	Lungs	Spleen	Kidneys
		Peroxidase activity ( $\mu\text{mol}/\text{min}/\text{g}$ of tissues)				
	T0	0.759 $\pm$ 0.022 <sup>c</sup>	2.021 $\pm$ 0.249 <sup>c</sup>	0.934 $\pm$ 0.047 <sup>d</sup>	2.108 $\pm$ 0.093 <sup>d</sup>	1.337 $\pm$ 0.247 <sup>c</sup>
	T-	0.545 $\pm$ 0.032 <sup>a</sup>	1.338 $\pm$ 0.044 <sup>a</sup>	0.622 $\pm$ 0.010 <sup>a</sup>	1.248 $\pm$ 0.014 <sup>a</sup>	0.616 $\pm$ 0.007 <sup>a</sup>
	T+	0.643 $\pm$ 0.028 <sup>b</sup>	1.717 $\pm$ 0.020 <sup>b</sup>	0.714 $\pm$ 0.016 <sup>b</sup>	1.486 $\pm$ 0.012 <sup>b</sup>	0.849 $\pm$ 0.033 <sup>b</sup>
Male	40mg/kg	0.656 $\pm$ 0.008 <sup>b</sup>	1.734 $\pm$ 0.011 <sup>b</sup>	0.742 $\pm$ 0.009 <sup>bc</sup>	1.541 $\pm$ 0.019 <sup>bc</sup>	0.854 $\pm$ 0.009 <sup>b</sup>
	80mg/kg	0.662 $\pm$ 0.004 <sup>b</sup>	1.775 $\pm$ 0.024 <sup>b</sup>	0.774 $\pm$ 0.004 <sup>c</sup>	1.588 $\pm$ 0.007 <sup>c</sup>	0.936 $\pm$ 0.040 <sup>b</sup>
	117,71 mg/kg	0.743 $\pm$ 0.006 <sup>c</sup>	1.965 $\pm$ 0.040 <sup>c</sup>	0.928 $\pm$ 0.007 <sup>d</sup>	2.037 $\pm$ 0.025 <sup>d</sup>	1.252 $\pm$ 0.026 <sup>c</sup>
	T0	0.609 $\pm$ 0.042 <sup>d</sup>	1.533 $\pm$ 0.007 <sup>d</sup>	1.209 $\pm$ 0.172 <sup>c</sup>	0.832 $\pm$ 0.006 <sup>d</sup>	1.752 $\pm$ 0.018 <sup>d</sup>
	T-	0.357 $\pm$ 0.011 <sup>a</sup>	1.124 $\pm$ 0.019 <sup>a</sup>	0.941 $\pm$ 0.024 <sup>a</sup>	0.604 $\pm$ 0.002 <sup>a</sup>	1.075 $\pm$ 0.007 <sup>a</sup>
	T+	0.485 $\pm$ 0.072 <sup>b</sup>	1.321 $\pm$ 0.014 <sup>b</sup>	1.057 $\pm$ 0.028 <sup>ab</sup>	0.634 $\pm$ 0.001 <sup>b</sup>	1.413 $\pm$ 0.010 <sup>b</sup>
Female	40mg/kg	0.527 $\pm$ 0.024 <sup>bc</sup>	1.334 $\pm$ 0.004 <sup>bc</sup>	1.071 $\pm$ 0.004 <sup>b</sup>	0.635 $\pm$ 0.001 <sup>b</sup>	1.428 $\pm$ 0.000 <sup>bc</sup>
	80mg/kg	0.546 $\pm$ 0.008 <sup>bc</sup>	1.341 $\pm$ 0.007 <sup>c</sup>	1.124 $\pm$ 0.005 <sup>bc</sup>	0.637 $\pm$ 0.002 <sup>b</sup>	1.435 $\pm$ 0.002 <sup>c</sup>
	117,71 mg/kg	0.556 $\pm$ 0.009 <sup>cd</sup>	1.351 $\pm$ 0.005 <sup>c</sup>	1.169 $\pm$ 0.010 <sup>bc</sup>	0.820 $\pm$ 0.003 <sup>c</sup>	1.743 $\pm$ 0.003 <sup>d</sup>

Data are the mean  $\pm$  standard error of the mean ( $n = 4$ ). In the same column and for the same sex, values with different letters are significantly different ( $p < 0.05$ ). T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated



**Fig. 4. Evolution in weight of male and female rats after infection and during treatment as a function of time (days)**

Values in the figure are presented as means  $\pm$  standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: uninfected and untreated

“Enterobacteriaceae of the genus *Salmonella* in addition to natural protein factors (Dps) that allow them to resist destruction by host macrophages [27], express the oxyR and oxyS gene that encodes several proteins that allow it to resist free radicals produced by macrophages” [5]. “Macrophages produce nitric oxide (NO) through the action of inducible nitric oxide synthase, which metabolizes arginine to citrulline and NO. Nitric oxide is involved in a dynamic process by

macrophages to destroy *Salmonella*” [28]. The increase in tissue nitric oxide levels and the worsening of the general condition (high bacterial load, emaciation, death, etc.) of negative control animals suggest that *Salmonella* Typhi have developed resistance against the free radicals produced by macrophages to destroy them [7]. However, the significant decrease in nitric oxide levels in the tissues of infected rats treated with different doses of the extract compared to

negative controls, reveals that the 95% hydroethanolic extract of the leaves of *Bauhinia rufescens* acts on the one hand by inhibiting the resistance of the bacteria to free radicals, which is responsible for the antibacterial activity by stimulation of the immune system.

And on the other hand as an antioxidant with as a consequence the decrease of free radicals observed after recovery in a dose-dependent manner in all treated animals compared to infected and untreated animals. Excess free radicals (nitric oxide) react with all biological substances, but with a stronger tropism for polyunsaturated fatty acids that constitute cell membranes [29].

Malondialdehyde (MDA) is a good indicator of the degree of lipid peroxidation related to *Salmonella*-induced tissue damage [30]. It is one of the end products of oxidative degradation of polyunsaturated fatty acids, and is a frequently used marker to assess lipid peroxidation in tissues [31]. In the present study, the infection induced the increase of MDA levels which could not only reflect the high level of free radicals and therefore a significant stress, but also the lipid peroxidation marked by an increase of malondialdehyde (MDA) level. Whereas, treatment resulted in a significant ( $p < 0.05$ ) decrease in MDA levels in all organs in both sexes compared to infected and untreated animals.

Antioxidant enzymes are the most important part of the defense system against damage caused by superoxide anions and hydrogen peroxides. These enzymes play a coordinated and synergistic role in preventing oxidative damage from reactive oxygen species [32]. Catalases and peroxidases are enzymatic antioxidants widely distributed in all animal tissues and act by breaking down hydrogen peroxide to protect tissues from highly reactive hydroxyl radicals [33]. Thus, the artificial induction of salmonellosis in rats resulted in a significant decrease in the activities of measured antioxidant enzymes (catalase and peroxidase) in infected and untreated animals compared to uninfected and untreated animals. This could be due to the overproduction of reactive oxygen species, peroxidation and cell damage. According to Akomalafe et al, [34], *Salmonella* infections cause an imbalance in the redox state of the organism in favor of oxidants. These observed results suggest the inability of the organism and

its natural antioxidants to prevent the excessive formation of free radicals in situations of external aggression.

However, treatment resulted in a dose-dependent increase in catalase and peroxidase activity in all treated animals compared to infected and untreated animals. These observations indicate the ability of this extract to neutralize hydroperoxides and therefore reduce damage due to the accumulation of these substances. According to Oyedemi and Afolaya [35], a failure in these enzymes can lead to cellular damage caused by the accumulation of superoxides and hydroperoxides. Also, the 95% hydroethanolic extract of *Bauhinia rufescens* leaves could act by enhancing the expression of genes encoding these enzymes and lead to their catalytic hyperactivity [36]. The antisalmonellal, antioxidant and histoprotective power of this extract is due to the secondary metabolites (phenolic compounds such as flavonoids) they contain [10] and therefore their effectiveness has been demonstrated [37].

## 5. CONCLUSION

From the results of this work, it can be concluded that the 95% hydroethanolic extract of the leaves of *Bauhinia rufescens* has a dose-dependent mixed antisalmonella and antioxidant activity and can cure salmonellosis after the start of treatment in male and female rats. The extract reduces oxidative stress and physical disorders associated with *Salmonella* Typhi infection. These results can be used as preliminary scientific data in the treatment of *Salmonella* infections and could be good sources of active principles that could be as molecules for the synthesis of new drugs against typhoid fever. However, this would only be possible after extensive therapeutic and toxicological studies *in vivo*.

## ETHICAL APPROVAL

All animal procedures were performed after approval from the Ethics Committee of the University of Dschang-Cameroon (Project No. BCH1202/FS/UDs/2018) and ethical guidelines and procedures for handling laboratory animals were followed.

## ACKNOWLEDGEMENTS

The authors thank the *Centre Pasteur du Cameroun* for providing the *Salmonella* Typhi stock cultures (ATCC6539) for this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Coburn B, Grassl AG, Finlay BB. *Salmonella*, the host and disease. Immunology Cellular Biology. 2007;85: 112–118. DOI: 10.1038/sj.icb.7100007
2. WHO (World Health Organization). Vaccins antityphoïdiques. Relevé épidémiologique hebdomadaire. 2018; 93(13):153-172.
3. Fusheini A, Gyawu SK. Prevalence of typhoid and paratyphoid fever in the hohoe municipality of the volta region, ghana: a five-year retrospective trend analysis. Annals of Global Health. 2020;86(1):111–110. DOI:https://doi.org/10.5334/aogh.2833
4. Gordana M, Bogdanka A, Dragica T, Milena L, Brankica D. Antibiotic susceptibility of *Salmonella* spp.: a comparison of two surveys with a 5 years interval. Journal of IMAB - Annual Proceeding. 2012;18(1):216-219. DOI:10.5272/jimab.2012181.216
5. Madigan M, Martinko J. Diagnostic microbiologique et immunologique. In Brock Biologie des Microorganismes (11<sup>ème</sup> éd), Pearson Education: Paris, France. 2007;1046:ISBN: 978-2-35745-076-9
6. Hazra B, Santanu B, Nripendranath M. Antioxidant and free radicals scavenging activity of *Spondias pinnata*. National library of Medicine. 2008;(8):63. DOI: 10.1186/1472-6882-8-63
7. Kodjio N, Atsafack SS, Njateng SSG, Sokoudjou BJ, Kuate RJ, Gatsing D. Antioxidant effect of aqueous extract of *Curcuma longa* Rhizomes (Zingiberaceae) in the typhoid fever induced in wistar rats Model. Journal of Advances in Medical and Pharmaceutical. 2016;7(3):1-13. DOI: 10.9734/JAMPS/2016/24949
8. Gatsing D, Tchakoute V, Ngamga D, Kuate JR, Tamokou JDD, Nji-Nkah BF, Tchouanguep MF, Fodouop SPC. *In vitro* antibacterial activity of *Crinum purpurascens* Herb. Leaf extract against the *Salmonella* species causing typhoid fever and its toxicological evaluation. Iran Journal of Medical Science. 2009;34(2): 126-136.
9. Famen LCN, Talom BT, Tagne RS, Fodouop SPC, Kamsu GT, Kodjio N, Fowa AB, Gatsing D. *In vivo* Antioxidant Activity of Hydroethanolic Extracts of *Terminalia avicennioides* (Combretaceae) in *Salmonella* typhi-infected wistar rat's model. Tropical Journal Naturel Production Research. 2021;5(7):1185-1191. DOI: 10.26538/tjnpr/v5i7.3
10. Djenguemtar J, Yamako Konack E, Sokoudjou JB, Kamsu GT, Feudjio HBL, Kodji N, Gatsing D. *In vitro* antisalmonellal and antioxidant activity of hydroethanolic and aqueous extracts of *Bauhinia rufescens* leaf and stem bark extracts. Microbiology Research Journal International. 2022;32(1):32-46. DOI:10.9734/MRJI/2022/v32i130366
11. Mabona U, Viljoen A, Shikanga E, Van Vuuren S. Antimicrobial activity of southern African medicinal plants with dermatological screening approach, to combination studies and the isolation of a bioactive compound. Journal of Ethnopharmacology. 2013;148 (1):45-55. DOI : 10.1016/j.jep.2013.03.056
12. Da silva KLD, Filho VC. Plants of the genus *Bauhinia*: chemical composition and pharmacological potential. Química Nova. 2002;25:449-454. DOI: 10.1590/S0100-40422002000300018
13. Abubakar MS, Musa AM, hmed AA, Hussaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the hausa and fulani tribes of northern nigeria. Journal Ethnopharmacol. 2007;111,(3): 625-629. DOI : 10.1016/j.jep.2007.01.011
14. Kamsu GT, Simo Tagne R, Fodouop SPC, Famen NLC, Kodjio N, Ekom ES, Gatsing D. *In vitro* antisalmonellal and antioxidant activities of leaves extracts of *Tectonagrandis*L. F. (Verbenaceae). European Journal of Medicinal Plants. 2019;29(4):1–13. DOI: 10.9734/ejmp/2019/v29i430164
15. Smith JA, Van den Broek FAR, Martorell JC, Hackbarth H, Ruksenas O, Zeller W. Principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA working group on ethical evaluation of animal experiments. Laboratore Animal. 2007;41:143-160.

- DOI: 10.1258/002367707780378212
16. Choi JG, Kang OH, Lee YS, Chae HS, Oh YC, Brice OO, Kim MS, Sohn DH, Kim HS, Park H, Shin DW, Rho JR, Kwon DY. In Vitro and In Vivo Antibacterial Activity of Punica granatum Peel Ethanol Extract against *Salmonella*. Evid-Based Complementary Alternative Medecine. 2011;1-8.  
DOI: 10.1093/ecam/nep105
  17. Havelaar AH, Garssen J, Takumi K, Koedam MA, Dufrenne JB, Van Leusden FM, De La Fonteyne L, Bousema JT, Vos JG. A rat model for doseresponse relationships of *Salmonella* enteritidis infection. Journal of Applied Microbiology. 2001;91(3):442-452.  
DOI: 10.1046/j.1365-2672.2001.01399
  18. Tala DS, Gatsing D, Fodouop SPC, Fokunang C, Kengni F, Djimeli MN. In vivo antisalmonella activity of aqueous extract of *Euphorbia prostrata* Aiton (Euphorbiaceae) and its toxicological evaluation. Asian Pacific Journal Tropical Biomedicine. 2015;5(4): 310-318.  
DOI: 10.1016/S2221-1691(15)30350-6
  19. Dimo T, Tsala DE, Dzeufiet DPD, Penlap BV, Njifutie N. Effects of *Alafia multiflora* stape on lipid peroxidation and antioxidant enzyme status in carbon tetrachloride-treated rats. Pharmacologyonline.2006;2:76-89.  
DOI: 10.4236/as.2014.513135
  20. Habbu P, Shastry R, Mahadevan KM, Joshi H, Das S. Hepatoprotective and antioxidant effects of argyreia speciosa in rats. Afr J Trad Compl Altern Med. 2008; 5(2):158-164.  
DOI: 10.4314/ajtcam.v5i2.31268
  21. Oyedemi SO, Bradley G, Afolayan AJ. In vitro and vivo antioxidant activities of aqueous extract of Strychnos henningsii Gilg. African Journal of Pharmacy and Pharmacology. 2010;4(2): 70-78. DOI:10.5897/AJPP.9000176
  22. Griess P. Bemerkungen zu der abhandlung der HH, Weselsky, Benedikt. Ueber einige azoverbindungen. Chem Ber. 1879;12:426-8.  
DOI: 10.47115/bsagriculture.1162523
  23. Elgroud R. Contaminations du poulet de chair par les salmonelles non typhiques en élevages et abattoirs de la wilaya de Constantine: Caractérisations phénotypiques et génotypiques par ERIC-PCR, IS-PCR et PFGE. Thèse de Doctorat en Sciences Vétérinaires. Université Mentouri Constantine, Algérie. 2009:1-28.
  24. Adeyi AO, Jinadu AM, Arojoye OA, Alao OO, Ighodaro OM, Adeyi OE, In vivo and in vitro antibacterial activities of Momordica charantia on *Salmonella* Typhi and its effect on liver function in typhoid-infected rats. Journal of Pharmacognosy and Phytotherapy. 2013;5(11):183-188.  
DOI: 10.5897/JPP2013.0291
  25. Cornu M. Dynamique des populations bactériennes en cultures mixtes : Thèse de Doctorat. Université Claude Bernard-Lyon I, Lyon, France; 2000.
  26. Teponno RB, Tapondjou AL, Gatsing D, Djoukeng JD, Abou-Mansour E, Tabacchi R, Tane P, Stoekli-Evans H, Lontsi D. Bafoudiosbulbins A, and B, two antisalmonella clerodane diterpenoids from *Dioscorea bulbifera* L. var sativa. Phytochemistry.2006;67(17):1957-1963.  
DOI : 10.1016/j.phytochem.2006.06.019
  27. Eriksson S, Lucchini S, Thompson A, Rhen M, Hinton J.C. Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella* enterica. Molecular Microbiology. 2003;47: 103-118.  
DOI: 10.1046/j.1365-2958.2003.03313
  28. Vazquez Torres A, Jones Carson J, Mastroeni P, Ischiropoulos H, Fang FC. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. Journal of Experimental Medical. 2000; 192:227-236.  
DOI:10.1084/jem.192.2.227
  29. Fakurazi S, Sharifudin SA, Arulselvan P. Moringa oleifera hydroethanolic extracts effectively alleviate Acetaminophen-Induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules. 2012;17:8334-8350.  
DOI: 10.3390/molecules17078334
  30. Sharida F, Syazana AS, Palanisamy A. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules. 2012; 17:8334-8350.  
DOI: 10.3390/molecules17078334
  31. Lim CY, Mat JS, Abdulla MA, Abdul AA. In Vivo Biochemical and gene expression analyses of the antioxidant activities and

- hypocholesterolaemic properties of *Tamarindus indica* Fruit Pulp Extract. PLOS ONE. 2013;8(7):e70058. DOI: 10.1371/journal.pone.0070058
32. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a Universal defense regulator. *Antioxidants*. 2020;9:681. DOI: 10.3390/antiox9080681
33. Radhika R, Krishnakumari S, Umashankar V, Sudarsanam D. Effect of enzymatic antioxidants of Rheum emodiin alloxaninduced diabetic rats. *International Journal Biology Chemical Sciences*. 2010; 4(6);1905-1913.
34. Akomolafe RO, Adeoshun IO, Fakunle JB, Iwalewa EO, Ayoka AO, Ajayi OE. Effects of artemether on the plasma and urine concentrations of some electrolytes in rats. *African Journal of Biotechnology*. 2011;10 (20):4226-4233. DOI: 10.5897/AJB07.500
35. Oyedemi SO, Afolayan AJ. In vitro and *in vivo* antioxidant activity of aqueous leaves extract of *Leonotis leonurus*. *International Journal of Pharmacology*. 2011;7(2):248-256. DOI:10.3923/ijp.2011.248.256
36. Yu J, Chen Y, Zhai L, Zhang L, Xu Y, Wang S. Antioxidative effect of ginseng stem-leaf saponins on oxidative stress induced by cyclophosphamide in chickens. *Poultry Science*. 2015;94(5):927-933. DOI:10.3382/ps/pev055
37. Adejuwon SA, Imosemi IO, Ebokaiwe PA, Omirinde JO, Adenipekun AA. Protective role of *Telfairia occidentalis* in irradiation induced oxidative stress in rat brain. *International Journal Biology Chemical Sciences*. 2014;8(3):843-853. DOI:10.4314/ijbcs.v8i3.1

© 2023 Djenguemtar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

The peer review history for this paper can be accessed here:  
<https://www.sdiarticle5.com/review-history/97764>