*Original Research Article*

# *In Vivo* Antisalmonellal Antibacterial Efficacy and Antioxidant Activity of 95% Hydroethanolic Extract of *Bauhiniarufescens* Leaves in Wistar Albino Rats Infected with *Salmonella* Typhi


## 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 need 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 antibacterial efficacy and antioxidant activity of the 95% hydroethanolic extract of *Bauhiniarufescens* (Fabaceae) leaves in rats infected with *Salmonella* Typhi ATCC6539, as an alternative treatment.**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

*Bauhiniarufescens* leaves has mixed antisalmonellal and antioxidant activity *invivo* and could be developed for the treatment of typhoid fever.

**Key words:***Bauhiniarufescens, Salmonella* Typhi, Salmonellosis, Oxidative stress

## 1. INTRODUCTION

Typhoid fever is a foodborne 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 *Bauhiniarufescens*, 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 *Bauhiniarufescens* leaves against typhoid fever and considering the interesting scientific results obtained *invitro* with *Bauhiniarufescens* extracts against multi-resistant bacteria of the genus *Salmonella*[10].For this reason, this extract has been used for antisalmonellal and antioxidant activity *invivo*. Hence the objective of this work which aims

to provide concrete scientific evidence on the *invivo* therapeutic efficacy of *Bauhiniarufescens* 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 *Bauhiniarufescens* 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.

The leaves of *Bauhiniarufescens* were harvested and dried at room temperature 30±2°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 *Bauhiniarufescens* 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 Nº1 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°C for 12 h until complete evaporation of the solvent.The extract obtained was stored in the refrigerator at 4°C until use.

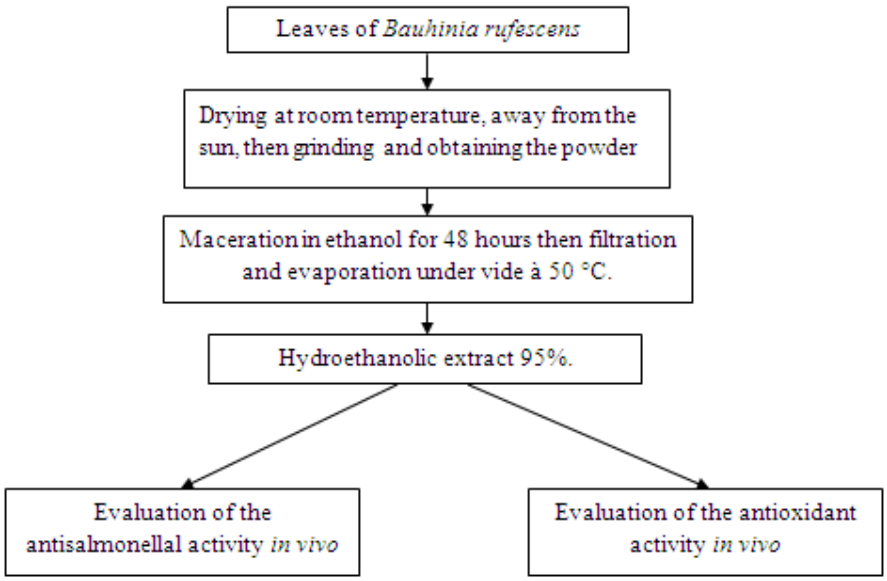


**Fig.1. General Scheme**

### 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°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±1°C on a 12-hour light/dark cycle. Animals were fed a standard diet and received water *adlibitum.*

### 2. 1. 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.

### 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 x 108 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 the animals was performed by blood culture with colony counting on Salmonella-Shigellaagar and converted to *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 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.

**2.4. 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].

*Determination of weight gain or loss*

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

$$W=Wf - W0$$

Where: W=weight gain or loss on day x;

Wf = Body weight of the animal on day x;

W0 = Initial body weight of the animal (before the start of the trial).

**2.5. Determination of enzymatic and non-enzymatic antioxidant markers**

**2.5.1. 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 H2O2 (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.

**2.5.2. Determination of peroxidase activity (POD)**

Peroxidase activity was determined in tissue or plasma 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 H2O2 (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.

**2.5.3. 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 (reading at 532 nm). Five hundred microliters of 1% orthophosphoric acid and 500 µL of precipitation mixture (1% thiobarbituric acid in 1% acetic acid) were added to 100 µL 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.

**2.5.4. Determination of nitric oxide (NO) level**

The content of nitric oxide in plasma or tissue homogenates was measured by the reagent of Griess [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 µL of plasma or homogenates.The resulting mixture was homogenized and left in the dark for 5 minutes at room temperature. Then, 340 µL 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; r2=0.9835).

### 2.6. Statistical analysis

Results were expressed as mean ± 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. *Invivo* anti-typhoid activity of the 95% hydroethanolic extract of *Bauhiniarufescens* leaves in infected rats

The *invitro* antisalmonellal activity revealed that the 95% hydroethanolic extract of *Bauhiniarufescens* leaves is the most active on *Salmonella* Typhi strain ATCC6539 [10].For this reason, this extract was used for *invivo* 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 figures 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 *Bauhiniarufescens* 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

Figures 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.

**3.1.3.Effect of treatment on weight growth of rats during the experiment**

Figure4 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 nonsignificant ($p \geq 0.05$) increase in weight growth in both sexes compared with the negative control.

**3.2.*Invivo* 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 *Bauhiniarufescens* 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 *Bauhiniarufescens* 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.

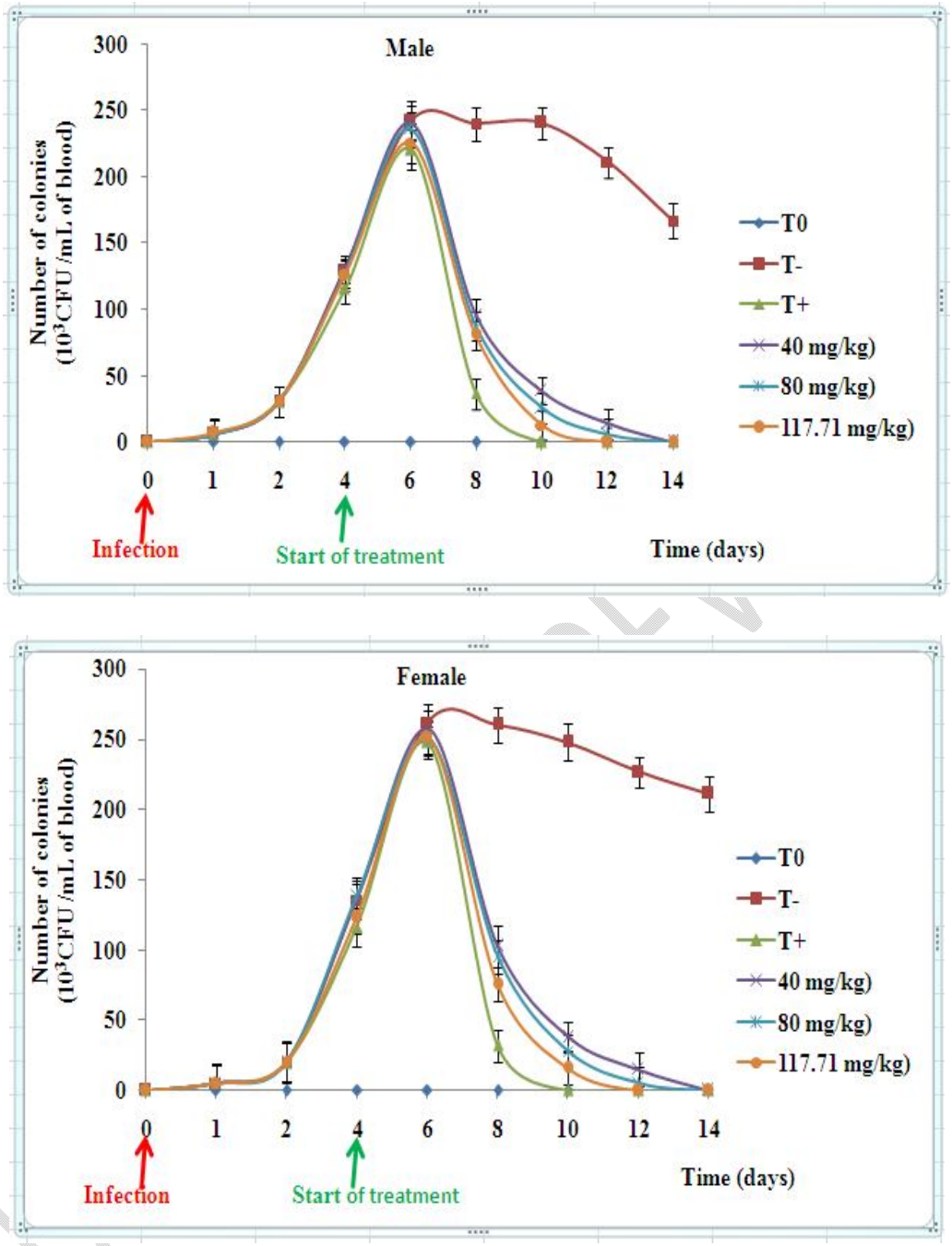



Fig. 2 Evolution of bacterial load after infection and during treatment with different doses of the 95% hydroethanolic extract of *Bauhiniarufescens* leaves in male and female rats as a function of time. The values in the figure are presented as means ± standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated.

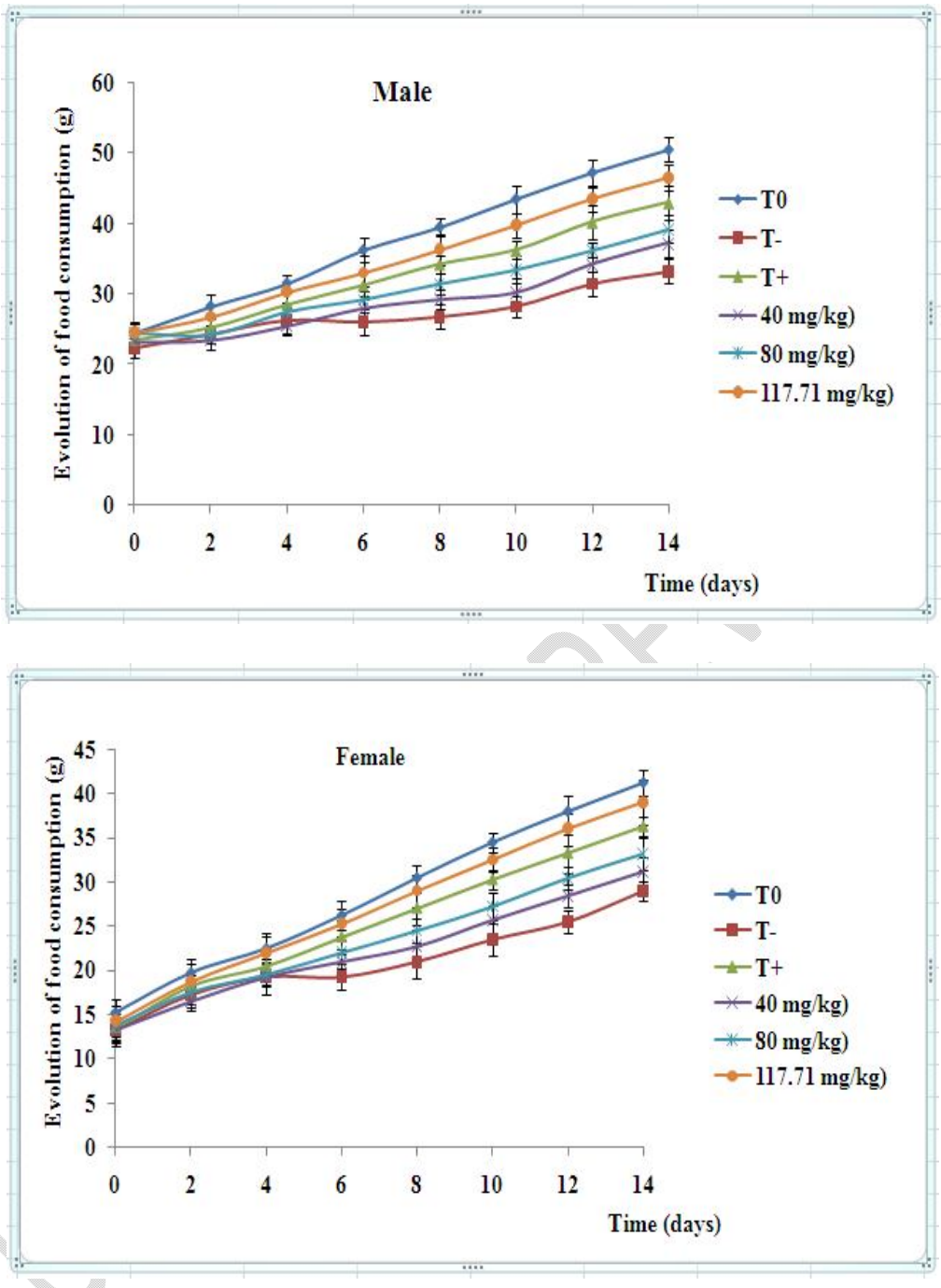
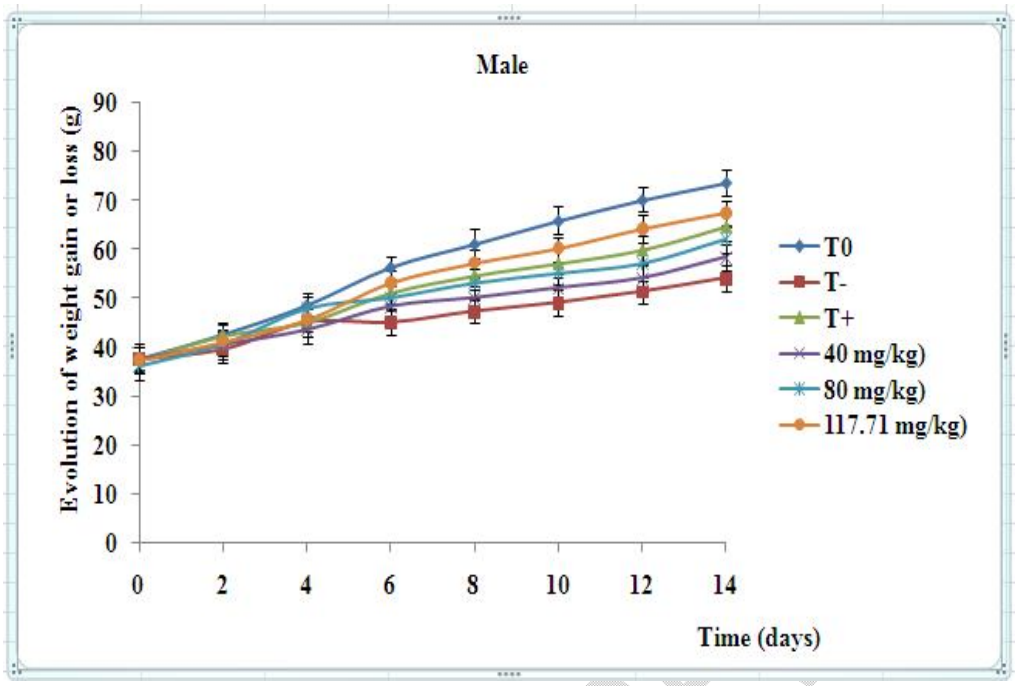



Fig. 3 Evolution of food consumption (g) of infected male and female animals treated with 95% hydroethanolic extract of *Bauhiniarufescens* leaves as a function of time (days). Values in the figure are presented as means ± standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: not infected and untreated.



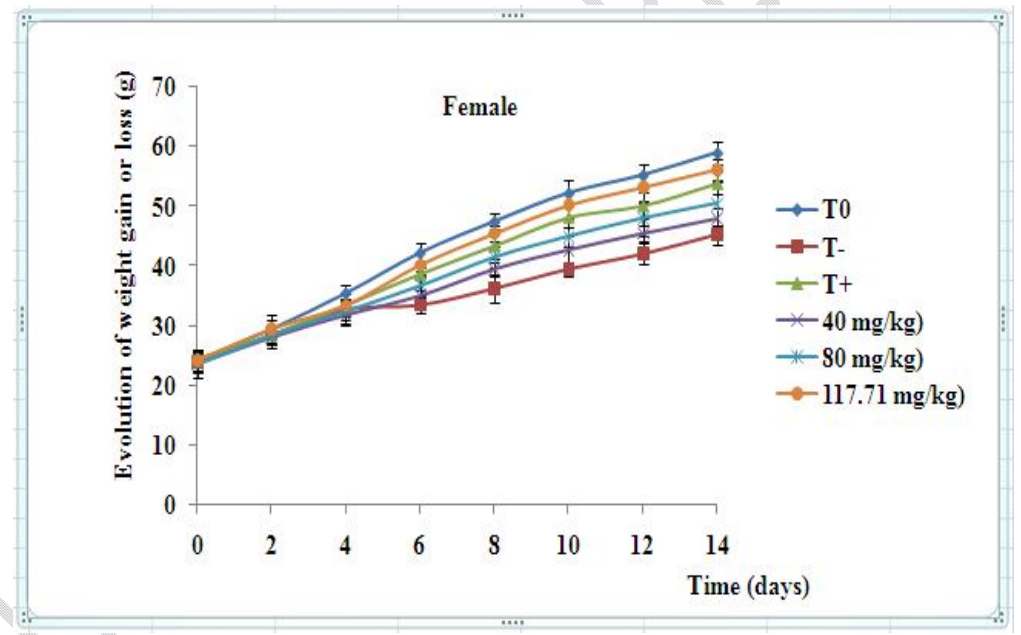




Fig. 4.Evolution in weight of male and female rats after infection and during treatment as a function of time (days). Values in the figure are presented as means ± standard deviation of 4 animals. T+: infected and treated with Ciprofloxacin (14 mg/kg); T-: infected and untreated; T0: uninfected and untreated.

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

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

Data are the mean ± 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 *Bauhiniarufescens* leaves on malondialdehyde (MDA) levels in rat organ tissues.

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

Data are the mean ± 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 *Bauhiniarufescens* leaves on plasma and tissue catalase activity of rat organs.

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

Data are the mean ± 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 *Bauhiniarufescens* leaves on tissue peroxidase activity of rat organs.

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

Data are the mean ± 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.

## 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 *invitro* antisalmonellal activity [10] the 95% hydroethanolic extract of *Bauhiniarufescens* 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 *Bauhiniarufescens* leaves *invivo*. 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 *Bauhiniarufescens* 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 *Bauhiniarufescens* 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 *Bauhiniarufescens* 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 *Bauhiniarufescens* 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.

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 *Bauhiniarufescens* 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 *Bauhiniarufescens* leaves could act by enhancing the expression of genes encoding these enzymes and lead to their catalytic hyperactivity [36]. The anti-salmon, 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 the plant *Bauhiniarufescens* has mixed antisalmonellal and antioxidant activity and can cure *Salmonella* Typhi induced salmonellosis within nine days after the start of treatment from the dose 40 mg/Kg 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 in Chad 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 *invivo*.

**Data Availability**

No additional data are available

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