

Effect of *Chromolaena odorata* Extracton Hematotoxicity and Spleen Histopathology Induced by *Salmonella typhi* in Wistar Rats

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Abstract

Salmonella typhi is a facultative intracellular pathogen that causes typhoid fever in humans. In the present study, the effect of *Salmonella typhi* infection on hematological indices and spleen histology in Wistar rats was investigated and was followed by an evaluation of the ameliorative potential of the methanol leaf extract of *Chromolaena odorata* (MLECO) compared with ciprofloxacin treatment. The animals were divided into six groups: group 1 was normal control, group 2 was infected with *Salmonella typhi* without treatment (negative control), groups 3, 4 and 5 were *Salmonella typhi* infected and treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively and group 6 was also *Salmonella typhi* infected and treated with 500 mg/70kg of ciprofloxacin. The animals were inoculated with a single infectious dose of *Salmonella typhi* bacteria and thereafter, treated with the extract and ciprofloxacin for a period of seventeen days, after the animals were confirmed infected. The rats were humanely sacrificed and blood samples taken for hematological investigations, and the spleen harvested and processed for histological examinations. *Chromolaena odorata* administration reversed the adverse hematotoxicity and histopathological changes in the spleen induced by *Salmonella typhi* infection.

Keywords

Chromolaena odorata, Hematotoxicity, Spleen Histopathology, *Salmonella typhi*, Wistar Rats

1. Introduction

Blood is one of the major homeostatic systems of the body and therefore sup-

ports normal integrity, viability and adaptive responses [1], with the functionality of the internal state of its systems undergoing dynamic change in harmony with the strength, nature, and duration of exposure to infectious internal (e.g. disease) and external environmental factors (e.g. stress, drugs and toxins etc.). Infection with pathogenic bacteria such as *Salmonella typhi* produce unfavorable adjustment in hematological indices, including the WBC, neutrophil, lymphocytes, monocytes, as well as Platelets, RBC, PCV, Hb, hematocrit, Mean corpuscular Volume, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), [2] and [3]. *Chromolaena odorata* (L.), (Asteraceae, Eupatorieae), is a perennial climbing shrub that forms dense, twisted bushes of about 1.5 - 3.0 m in height, showing brown and fibrous stem at maturity while shoot tips and young stems appear green and herbaceous [4]. According to Younes [5], *Chromolaena odorata* leaf extracts display antibacterial activities against *Pseudomonas aeruginosa*, *Streptococcus faecalis* while [6] reported that the ethanol and expressed extraction of *C. odorata*'s roots, showed a significant antibacterial activity against *Escherichia coli* and *Salmonella typhi*. In still another study to determine the anti-microbial effect of *Chromolaena odorata* extracts, it was reported that the plant exhibits concentration-dependent antibacterial effects in a pattern similar to those of penicillin, cefuroxime ciprofloxacin, ampicillin and ceftriaxone against *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Bacillus anthracis* [7]. Thus, with these properties, this study intends to examine the possibility of reversing the adverse haematological and histological changes in the spleen associated with *Salmonella typhi* infection.

2. Methods

2.1. Collection of Samples

Leaves of *Chromolaena odorata* were fetched from Omuoko-Aluu community and cleaned of soil and dust by washing with tap water. The leaves were identified at the Department of Plant Science and Biotechnology, University of Pot Harcourt.

2.2. Plant Extraction

After collection, the leaves of the plant were shade-dried at room temperature (32°C - 35°C) to constant weight over a period of seven (7) days. The method employed a little modification of the cold maceration extraction method as described by [8]. 50 g of powdered leaves of *Chromolaena odorata* was dissolved in 1000 ml of seventy percent methanol in a 2 litre conical flask and was shaken vigorously at 1 hour intervals, for a period of 12 hours and left to stand over-night at room temperature for effective extraction. After, the extract was taken and filtered by using a 0.45 millipore filter paper. The clear solution obtained was then concentrated with a rotary evaporator at 40°C and 200 rpm and subsequently, on a steam bath at 40°C. The semi-solid extract obtained was stored in sterile pre-weighed screw capped bottles and labeled accordingly. The

extracts were stored in desiccators at room temperature until when needed.

2.3. Isolation and Culturing the Organisms

All media used in this study were prepared following the manufacturer's instruction. *Salmonella typhi* was isolated from typhoid patients in "University of Port Harcourt, Teaching Hospital. Bile salt broth (broth culture) [9] and streptokinase broth (clot culture) [10] were used for enrichment. The enriched samples showing visible turbidity were streaked on Mac-Conkey agar media. The isolates producing characteristic colonies were identified by conventional biochemical tests.

2.4. Experimental Design

Eighty five (85) animals were divided into 6 groups of 1 - 6. Group 1 (normal) had five (5) animals, Group 2 (negative control) had twenty (20) animals, while groups 3 - 6 each had fifteen (15) animals. Group 1 animals were not treated throughout the experiment but were given free access to normal animal feed and water *ad libitum*. Group 2 contained *Salmonella typhi*-infected rats not treated after disease induction. Group 3 contained *Salmonella typhi*-infected rats treated with 100 mg/kg (low dose) of MLECO. Group 4 contained *Salmonella typhi*-infected rats treated with 200 mg/kg (medium dose) of methanol leaf extract of *Chromolena odorata*. Group 5 contained *Salmonella typhi*-infected rats treated with 400 mg/kg (high dose) of methanol leaf extract of *Chromolena odorata*. Group 6 contained *Salmonella typhi*-infected rats treated with 500 mg/70kg of a standard antibiotic drug (Ciprofloxacin). On day 0, at five day intervals (*i.e.* 6, 11, and 16), 5 animals from each group were humanely sacrificed using diethyl ether as anesthesia, and blood was collected and the spleen removed for assessment of the hematological parameters and histopathological examination, respectively.

2.5. Challenging Apparently Healthy Animals with *Salmonella typhi*

Eighty (80) animals (groups 2 - 6) were orogastrically challenged with an infective dose (2.0×10^8 cfu/ml) of *Salmonella typhi*. After infection had set in (through observation of signs like weakness, anorexia, non-productive cough, watery stool, standing of the hairs as in cold condition and isolation of the organism from the stool of the infected animals) (day 0), five animals were sacrificed and blood samples and spleen tissues collected for preliminary screening while the other 75 animals were treated with the methanol leaf extract of *Chromolena odorata* according to the different doses specified for each sub-group and the standard antibiotic (Ciprofloxacin), once daily, for seventeen days.

2.6. Preparation of the Extract Concentrations and Antibiotic

Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500

mg of ciprofloxacin in sterile distilled water. DMSO was also used as vehicle control in the study.

2.7. Blood Collection and Dissection

Blood was collected from each animal by cardiac puncture method after the animals were anaesthetized with diethyl ether in a desiccator. The blood was immediately transferred into appropriately labelled sample bottles containing anti-coagulant and the spleen was removed aseptically and was weighed and a portion was kept for histological analysis.

2.8. Hematological Analysis

Hematological analysis was carried out as described by [11], within 24 h of sample collection, to determine the levels of red blood cells (RBC) and white blood cells (WBC), differential leucocyte (DLC), platelet, haemoglobin concentration (Hb), and red cell indices, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean cell hemoglobin concentration (MCHC).

3. Results

The effect of *Salmonella typhi* inoculation in Wistar rats resulted in a decrease in WBC and neutrophil levels, and an increase in lymphocytes and monocytes that were not statistically significant when compared with the normal group. These changes were reversed on administration of the standard drug and the test extract, except for the lymphocytes. Administration of the standard drug and the test extract resulted in an increase in WBCs (Table 1), neutrophil (Table 2), lymphocytes (Table 3) and a slight decrease in monocytes (Table 4). On the contrary the animals in the negative control group showed a consistent decrease in WBCs and neutrophils, as well as a persistent increase in lymphocytes and

Table 1. Effect of methanol leaf extract of *Chromolaena odorata* on Total White Blood Cell count [(WBC) ($\times 10^3/\mu\text{l}$)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Control	7.10 \pm 0.4	6.87 \pm 0.18	6.93 \pm 0.24	6.83 \pm 0.15
Neg. Control	5.63 \pm 0.75	3.60 \pm 0.26**	3.20 \pm 0.35**	1.43 \pm 0.28**
Low Dose	5.63 \pm 0.76	5.73 \pm 0.81***	5.80 \pm 0.76***	6.00 \pm 0.72***
Medium Dose	5.63 \pm 0.75	5.87 \pm 0.72***	6.03 \pm 0.72***	6.40 \pm 0.30***
High Dose	5.63 \pm 0.75	6.13 \pm 0.69***	6.27 \pm 0.58***	6.87 \pm 0.09***
Ciprofloxacin	5.63 \pm 0.75	5.90 \pm 0.70***	6.10 \pm 0.72***	6.60 \pm 0.23***

* = Significant difference between normal and test groups; ** = Significant difference between normal and negative control; *** = Significant difference between normal and negative control and test groups; 1) Normal (Animals not exposed to any form of treatment but were fed *ad libitum*); 2) Negative Control (Animals inoculated with *Salmonella typhi* without treatment); 3) Low Dose (100 mg/kg of extract); 4) Medium Dose (200 mg/kg of extract); 5) High Dose (400 mg/kg of extract); 6) Ciprofloxacin (500 mg/70kg).

Table 2. Effect of methanol leaf extract of *Chromolaena odorata* on neutrophil count ($\times 10^3/\mu\text{l}$) in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Control	0.21 \pm 0.006	0.21 \pm 0.05	0.21 \pm 0.006	0.20 \pm 0.06
Neg. Control	0.14 \pm 0.03	0.11 \pm 0.01	0.13 \pm 0.02	0.04 \pm 0.02**
Low Dose	0.14 \pm 0.03	0.16 \pm 0.03	0.17 \pm 0.04	0.18 \pm 0.05
Medium Dose	0.14 \pm 0.03	0.16 \pm 0.04	0.18 \pm 0.05	0.20 \pm 0.06***
High Dose	0.14 \pm 0.03	0.18 \pm 0.05	0.19 \pm 0.04	0.22 \pm 0.01***
Ciprofloxacin	0.14 \pm 0.03	0.17 \pm 0.04	0.19 \pm 0.05	0.20 \pm 0.6***

Table 3. Effect of methanol leaf extract of *Chromolaena odorata* on Lymphocyte count ($\times 10^3/\mu\text{l}$) in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Control	6.13 \pm 0.26	5.90 \pm 0.49	6.13 \pm 0.20	6.10 \pm 0.46
Neg. Control	7.16 \pm 1.02	7.27 \pm 0.13	7.53 \pm 0.75**	7.97 \pm 0.09**
Low Dose	7.16 \pm 1.02	7.16 \pm 1.24	7.25 \pm 1.01	7.32 \pm 0.94
Medium Dose	7.16 \pm 1.02	7.20 \pm 1.27	7.30 \pm 0.17	7.39 \pm 0.23
High Dose	7.16 \pm 1.02	7.24 \pm 1.29	7.35 \pm 0.33	7.42 \pm 0.29
Ciprofloxacin	7.16 \pm 1.02	7.19 \pm 1.26	7.38 \pm 0.36	7.37 \pm 0.32

Table 4. Effect of methanol leaf extract of *Chromolaena odorata* on monocyte count ($\times 10^3/\mu\text{l}$) in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Control	0.11 \pm 0.02	0.11 \pm 0.02	0.11 \pm 0.02	0.11 \pm 0.02
Neg. Control	0.15 \pm 0.03	0.17 \pm 0.04	0.18 \pm 0.03**	0.18 \pm 0.02**
Low Dose	0.15 \pm 0.012	0.14 \pm 0.02	0.13 \pm 0.03	0.12 \pm 0.01***
Medium Dose	0.15 \pm 0.012	0.13 \pm 0.01	0.12 \pm 0.02	0.12 \pm 0.01***
High Dose	0.15 \pm 0.012	0.12 \pm 0.02	0.11 \pm 0.01	0.11 \pm 0.01***
Standard	0.15 \pm 0.012	0.13 \pm 0.02	0.12 \pm 0.01	0.10 \pm 0.01***

monocytes. ANOVA comparison showed a significant difference ($p < 0.05$) between negative control and normal and between negative control and other treatment groups on days 6, 11 and 16 in WBC count; between negative control and normal and between negative control and other treatment groups on day 16 for neutrophils and monocytes. Inoculation of a single contagious dose of *salmonella typhi* caused a significant decrease ($p < 0.05$) in the mean levels of thrombocytes, red blood cells (RBC), hemoglobin concentration (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC), when compared to the normal rats. On the contrary, administration of the standard drug and extract reversed these changes leading to a gradual increase in all these parameters.

ANOVA comparison showed a significant difference ($p < 0.05$) between negative control and normal in all these parameters throughout the study. Similarly, a significant difference was observed between the negative control and other treatment groups on days 11 and 16 for platelets and RBCs (Table 5 and Table 6 respectively) as well as between negative control and other treatment groups on days 6, 11 and 16 in Hgb, HCT, as shown in Table 7 and Table 8 respectively. The occurrence of MCV, Table 9 was similar to those of platelets and RBC, while the occurrence of MCH and MCHC (Table 10 and Table 11) was similar to Hgb and HCT.

Spleen histo-architectural examination of normal control rats (Plate 1) revealed normal morphology with clearly defined red and white pulp and marked,

Table 5. Effect of methanol leaf extract of *Chromolaena odorata* on Platelet count ($\times 10^3/\mu\text{l}$) in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	652.33 \pm 4.10	652.33 \pm 4.10	652.33 \pm 4.10	652.33 \pm 4.10
Neg. Control	306.67 \pm 7.22**	273.00 \pm 37.10**	239.00 \pm 30.01**	217.00 \pm 9.29**
Low Dose	306.67 \pm 7.22*	341.00 \pm 28.92*	*437.33 \pm 27.51***	545.67 \pm 13.38***
Medium Dose	306.67 \pm 7.22*	418.67 \pm 13.13*	*504.00 \pm 8.89***	627.00 \pm 15.50***
High Dose	306.67 \pm 7.22*	474.00 \pm 21.66*	595.67 \pm 9.02***	667.67 \pm 22.36***
Ciprofloxacin	306.67 \pm 7.22*	427.00 \pm 9.29*	*572.33 \pm 17.84***	631.00 \pm 11.85***

Table 6. Effect of methanol leaf extract of *Chromolaena odorata* on Red Blood Cell count [(RBC) ($\times 10^6/\mu\text{l}$)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	8.35 \pm 0.64	8.35 \pm 0.64	8.35 \pm 0.64	8.35 \pm 0.64
Neg. Control	3.70 \pm 0.29**	3.57 \pm 0.21**	3.17 \pm 0.26**	2.97 \pm 0.09**
Low Dose	3.70 \pm 0.29*	3.87 \pm 0.24*	*4.93 \pm 0.09***	*5.27 \pm 0.12***
Medium Dose	3.70 \pm 0.29*	4.57 \pm 0.23*	*5.67 \pm 0.26***	*6.37 \pm 0.29***
High Dose	3.70 \pm 0.29*	4.87 \pm 0.33*	*6.10 \pm 0.38***	8.60 \pm 0.36***
Ciprofloxacin	3.70 \pm 0.29*	4.20 \pm 0.23*	*5.00 \pm 0.17***	*6.37 \pm 0.29***

Table 7. Effect of methanol leaf extract of *Chromolaena odorata* on Hemoglobin concentration [(Hgb) (g/dl)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	16.20 \pm 1.15	16.20 \pm 1.15	16.20 \pm 1.15	16.20 \pm 1.15
Neg. Control	6.70 \pm 0.79**	6.63 \pm 1.06**	6.20 \pm 0.75**	5.47 \pm 0.58**
Low Dose	6.70 \pm 0.79*	*11.50 \pm 0.23***	12.70 \pm 0.12***	13.43 \pm 0.26***
Medium Dose	6.70 \pm 0.79*	*12.50 \pm 0.32***	14.73 \pm 0.44***	15.50 \pm 0.15***
High Dose	6.70 \pm 0.79*	*13.43 \pm 0.15***	15.27 \pm 0.35***	16.23 \pm 0.29***
Ciprofloxacin	6.70 \pm 0.79*	*11.67 \pm 0.15***	14.83 \pm 0.09***	15.63 \pm 0.18***

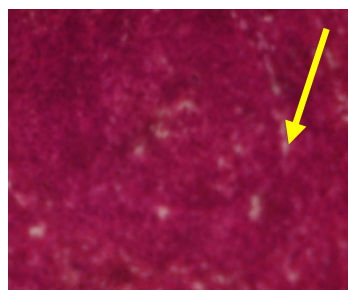


Plate 1. Histological sections of the spleen of normal rats with clearly defined red and white pulp. The picture does not show any germinal centres but the trabecular are marked (**Normal-Day 6**).

Table 8. Effect of methanol leaf extract of *Chromolaena odorata* on Hematocrit [(HCT) (%)] levels in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	43.00 ± 1.15	43.00 ± 1.15	43.00 ± 1.15	43.00 ± 1.15
Neg. Control	18.00 ± 2.08**	17.00 ± 1.53**	15.67 ± 1.20**	14.00 ± 0.58**
Low Dose	18.00 ± 2.08*	*26.00 ± 1.15***	*29.67 ± 0.88***	*33.33 ± 1.45***
Medium Dose	18.00 ± 2.08*	*30.33 ± 3.28***	*32.67 ± 2.73***	*38.67 ± 0.88***
High Dose	18.00 ± 2.08*	*33.00 ± 1.73***	39.00 ± 2.65***	42.67 ± 1.76***
Ciprofloxacin	18.00 ± 2.08*	*31.00 ± 3.79***	*33.00 ± 2.65***	40.00 ± 1.73***

Table 9. Effect of methanol leaf extract of *Chromolaena odorata* on Mean Corpuscular volume [(MCV) fL (μm^3)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	51.00 ± 2.08	51.67 ± 1.45	51.67 ± 1.45	51.67 ± 1.45
Neg. Control	21.00 ± 2.31**	19.67 ± 0.88**	18.00 ± 0.58**	16.33 ± 0.88**
Low Dose	21.00 ± 2.31*	*24.00 ± 3.46	*31.67 ± 2.73***	*39.33 ± 2.19***
Medium Dose	21.00 ± 2.31*	*25.33 ± 2.73	*34.00 ± 1.15***	*43.00 ± 1.15***
High Dose	21.00 ± 2.31*	*32.00 ± 1.15***	*41.33 ± 0.88***	51.00 ± 2.65***
Ciprofloxacin	21.00 ± 2.31*	*27.00 ± 1.15***	*36.33 ± 1.45***	*43.67 ± 1.76***

Table 10. Effect of methanol leaf extract of *Chromolaena odorata* on Mean Corpuscular Hemoglobin [MCH (pg)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	18.77 ± 1.03	18.83 ± 1.08	18.83 ± 1.02	18.80 ± 1.00
Neg. Control	7.17 ± 1.31**	6.67 ± 0.78**	6.30 ± 0.58**	6.37 ± 0.64**
Low Dose	7.17 ± 1.31*	*8.97 ± 0.67	11.33 ± 0.50***	14.07 ± 1.12***
Medium Dose	7.17 ± 1.31*	*11.30 ± 0.64***	14.73 ± 1.18***	16.03 ± 1.07***
High Dose	7.17 ± 1.31*	*14.40 ± 1.10***	16.70 ± 1.31***	17.77 ± 1.90***
Ciprofloxacin	7.17 ± 1.31*	*11.97 ± 1.23***	15.47 ± 1.49***	16.37 ± 1.21***

trabecular while splenic tissues of rats infected with *S. typhi* without treatment after 5 days (**Plate 2**) showed numerous splenic venous stenosis in the red pulp. In

Table 11. Effect of methanol leaf extract of *Chromolaena odorata* on Mean Corpuscular Hemoglobin Concentration [MCHC (g/dl)] in *Salmonella typhi* infected Wistar rats.

Group	Day 0	Day 6	Day 11	Day 16
Normal	34.77 ± 1.13	35.13 ± 1.85	34.87 ± 2.07	35.17 ± 1.48
Neg. Control	11.83 ± 1.22**	11.33 ± 0.78**	10.43 ± 1.13**	9.23 ± 0.77**
Low Dose	11.83 ± 1.22*	*13.73 ± 1.20	*15.83 ± 2.57	*21.90 ± 4.14***
Medium Dose	11.83 ± 1.22*	*17.37 ± 1.48***	*24.53 ± 2.73***	29.60 ± 3.18***
High Dose	11.83 ± 1.22*	*23.63 ± 2.00***	27.83 ± 2.34***	35.07 ± 0.09***
Ciprofloxacin	11.83 ± 1.22*	*17.90 ± 1.20***	27.60 ± 2.95***	32.00 ± 1.37***

**Plate 2.** Photomicrograph of splenic tissues of rat infected with *S. typhi* without treatment after 6 days. Numerous splenic venous stenosis are seen in the red pulp (**Negative control-Day 6**).

animals treated with 100 mg/kg of the extract for 5 days (**Plate 3**), the splenic tissue showed central white pulp with germinal center and in the field was the presence of a central artery. In animals treated with 100 mg/kg of the extract for 5 days (**Plate 4**), the splenic tissues of rats showed destroyed tissues, and in those animals treated for with 200 mg/kg of the extract for 5 days (**Plate 5**), the splenic tissues showed infected tissue with white pulp having germinal centers. For those treated with 400 mg/kg of *C. odorata* for 5 days (**Plate 6**), the spleen showed non-specific tissue disruption. **Plate 7** also showed normal histomorphology of the spleen in animals of normal group after 10 days exposure to normal laboratory conditions, while **plate 8** is the photo micrograph of the spleen in animals exposed to *Salmonella typhi* for 10 days; it showed numerous splenic venous stenosis and tissue disruption in the red pulp. In animals treated with 500 mg/70kg of ciprofloxacin for 10 days (**Plate 9**), the spleen revealed white pulp with the presence of germinal center, while for those animals treated with 100 mg/kg of extract for 10 days (**Plate 10**), the spleen histology still showed infected tissues. For those animals treated with 200 mg/kg of extract (**Plate 11**), the spleen tissues revealed a non-specific tissue disruption, while for those animals treated with 400 mg/kg of extract for 10 days (**Plate 12**), the spleen tissues shows normal morphology. **Plate 13** also showed normal histomorphology of the spleen in animals of normal group after 15 days exposure to normal laboratory conditions, while **Plate 14** is the photo micrograph of the spleen in animals exposed to *Salmonella typhi* for 15 days, also showing

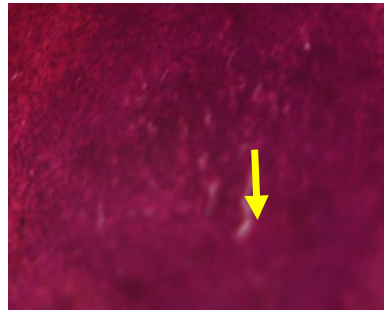


Plate 3. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 500 mg of ciprofloxacin for 5 days. The splenic tissue presented a central white pulp with germinal center and in the field was the presence of a central artery.

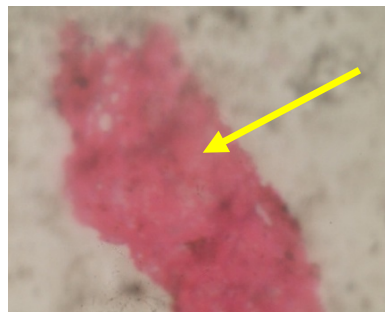


Plate 4. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 100 mg/kg of *C. odorata* for 5 days, showing destroyed tissues.

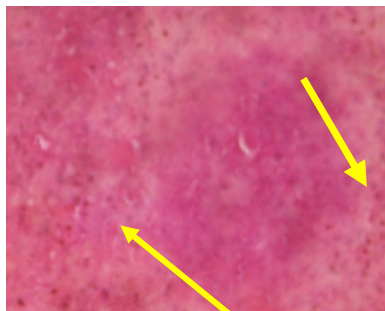


Plate 5. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 200 mg/kg of *C. odorata* for 5 days, showing disruption and infected tissue and white pulp with germinal centres.

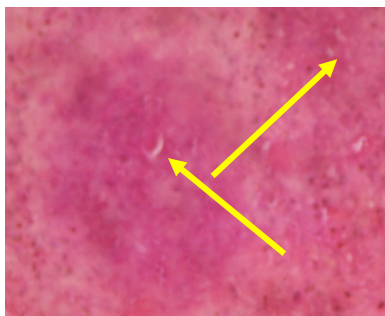


Plate 6. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 400 mg/kg of *C. odorata* for 5 days, showing non-specific tissue disruption.

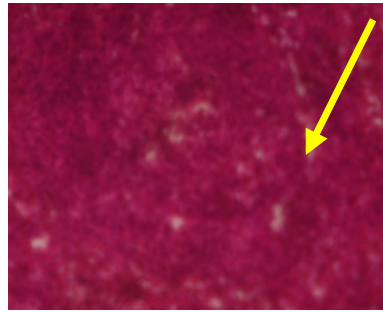


Plate 7. Histological sections of the spleen of normal rats with clearly defined red and white pulp. The picture does not show any germinal centres but the trabecular are marked (**Normal Day 11**).



Plate 8. Photomicrograph of splenic tissues of rat infected with *S. typhi* without treatment after 12 days. Numerous splenic venous stenosis and tissue disruption are seen in the red pulp (**Negative control-Day 11**).



Plate 9. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 500 mg of ciprofloxacin for 10 days. White pulp shows the presence of germinal center.



Plate 10. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 100 mg/kg of *C. odorata* for 10 days, showing infected tissues and disruption.

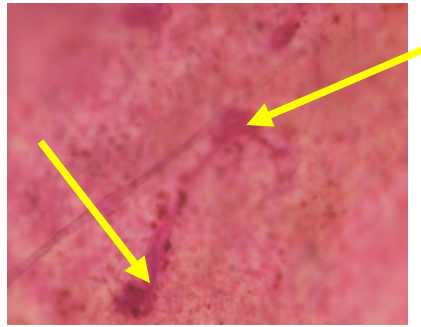


Plate 11. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 200 mg/kg of *C. odorata* for 10 days, showing non-specific tissue disruption.



Plate 12. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 400 mg/kg of *C. odorata* for 10 days showing normal cells.

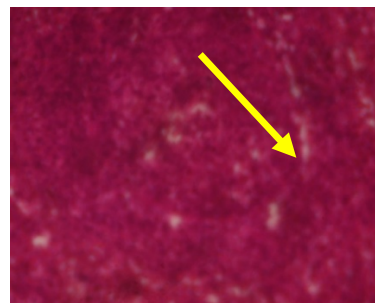


Plate 13. Histological sections of the spleen of normal rats with clearly defined red and white pulp. The picture does not show any germinal centres but the trabecular are marked (**Normal Day 16**).



Plate 14. Photomicrograph of splenic tissues of rat infected with *S. typhi* without treatment after 12 days. Numerous splenic venous stenosis and disruption are seen in the red pulp (**Negative control-Day 16**).

numerous splenic venous stenosis and tissue disruption in the red pulp. For animals treated with 500 mg/70kg of ciprofloxacin for 15 days (**Plate 15**), the Splenic tissue appeared normal with clearly defined white and red pulp, while animals treated with 100 mg/kg of extract for 15 days (**Plate 16**), still showed non-specific tissue disruption, while those treated with 200 mg/kg of extract (**Plate 17**) and 400 mg/kg of extract (**Plate 18**) showed tissues with normal morphology.

4. Discussion

During typhoid fever infection, the common hematological changes include; anemia, monocytosis and lymphocytosis [2], leucopenia, eosinophilia, thrombocytopenia, and sub-clinical disseminated intravascular coagulation [3],

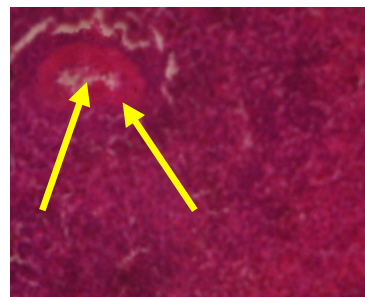


Plate 15. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 500 mg of ciprofloxacin for 15 days. Splenic tissue appeared normal with clearly defined white and red pulp. The germinal centers were clearly defined.

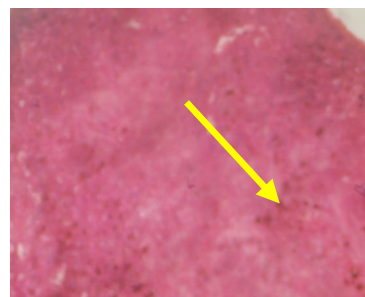


Plate 16. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 100 mg/kg of *C. odorata* for 15 days, with non-specific tissue disruption.



Plate 17. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 200 mg/kg of *C. odorata* for 15 days, showing normal tissues.

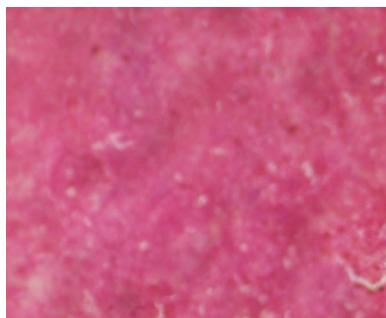


Plate 18. Photomicrograph of splenic tissues of rat infected with *S. typhi* and treated with 400 mg/kg of *C. odorata* for 15 days, showing normal tissues.

as well as a significant decrease in mean levels of RBC, Hb, PCV, MCV, MCH and MCHC [2] and [12]. There is also a significant leucopenia [reduction of white blood cells (WBC)], reduction in red blood cells (RBC), blood platelet counts as well as haemoglobin concentration and PCV in patients with typhoid fever [13] and [14].

In our study, the haematological profile following inoculation of animals with *Salmonella typhi* revealed a decrease in total white blood cell count (WBC) and neutrophil levels, and an increase in the lymphocytes and monocytes levels, which agrees with the findings of [2] and [3]. The leucopenia and neutropenia may be a result of invasion of the hemopoietic organs such as the spleen and bone marrow with *Salmonella* which is said to slow down leucopoiesis [15], while the lymphocytosis and monocytosis may be due to an increased release of cells from lymphoid/myeloid tissues [16]. Other hematological changes observed consequent to *Salmonella typhi* infection include a decrease in platelets, red blood cell count (RBC), Hemoglobin concentration (HB), percentage hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) which agrees with the reports of [17] and [12]. It was explained by [12] that destruction of RBCs and decrease in Hb were responsible for the decrease in MCV, MCH and MCHC. Also, it has been reported by [18], that hemophagocytosis and bone marrow suppression are essential mechanisms in generating hematological changes such as decreased PCV, Hb, hematocrit, MCV, MCH and MCHC and RBC. Similarly, [2], reported that a decreased level of RBC, Hb and PCV were caused by inhibition of hematopoiesis which led to anemia. Administration of *Chromolaena odorata* reversed these hematotoxicological changes observed with *Salmonella typhi* infection. Subsequent to the treatment with *Chromolaena odorata* and ciprofloxacin, there was a gradual increase in WBCs, neutrophils, lymphocytes and monocytes, platelets, RBCs, Hb, hematocrit, MCV, MCH and MCHC indicating a restoration to normal from the pathological changes caused by *Salmonella typhi*. These findings agree with the report of [19] who, attributed the changes to an effect of *Chromolaena odorata* extract reversing bone marrow depression with attendant improvement in erythrocyte membrane stability

through the antioxidant potential of the plant extract, thus reducing haemolysis.

Infection of Wistar rats with *Salmonella typhi* caused numerous splenic venous stenosis in the red pulp which agrees with the reports of [2]. This may be as a result of the organism's ability to replicate in the spleen of infected animals as reported by [20], in a study in mouse models for the human pathogen *Salmonella typhi*, which according to [21], could lead to bacteremia and subsequent abscess. The effects may also be as a result of cytotoxins generated and released by *Salmonella typhi* [3]. Animals treated with 100 mg/kg of MLECO revealed non-specific tissue disruption, indicating that this dose was not effective in reversing the pathological changes caused by *Salmonella typhi* infection. However, for the animals treated with 200 mg/kg of MLECO a reversal of the pathological changes in the splenic tissues was noted only on day 15. The high dose of 400 mg/kg of the extract normalized the diseased tissues from day 10, quicker than it took ciprofloxacin, which produced effects similar to the medium dose of 200 mg/kg (reversal of pathological changes on day 15). These observed antibacterial effects of *Chromolaena odorata* on the spleen agrees with the reports of [5] and [6], who reported in vitro anti-bacterial activity of the extract against *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Escherichia coli* and *Salmonella typhi* respectively, which may be bactericidal in nature since it was able to cause reversal of the pathological changes. This may imply that the extract eliminated the bacteria from the spleen of the animals and stopped the release of cytotoxins produced by *S. typhi*.

5. Conclusion

MLECO reversed the adverse hematological and pathological changes in the spleen induced by *S. typhi* infection at 200 and 400 mg doses, with the 400 mg dose being more effective at reversing splenic changes than ciprofloxacin, suggesting that MLECO may have antibacterial activity against *S. typhi*.

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