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(Research Article)

In vivo anti-salmonella activities of methanol extract of *Harungana madagascariensis* (Hypericaceae) and its toxicological evaluation

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Abstract

The decline in efficiency and the frequent toxicity effects observed with antibiotics urge researchers to investigate medicinal plant-based therapies. This study aimed to evaluate the anti-salmonella activity of the methanol extract of *Harungana madagascariensis*, a plant commonly used in Cameroon by traditional healers. Thirty *Wistar* albinos male were divided into six groups (normal control, negative control, positive control and three test groups). Test groups received the plant extract at 75, 150 and 300 mg/kg body weight (b.w). The disease evolution and treatment effectiveness were monitored by feces culture from the second-day post-infection until the end of the treatment. In addition, the effects of treatment on growth performances and feed conversion efficiency of rats were evaluated. The extract significantly affected the number of viable *Salmonella typhimurium* recovered from feces and could stop salmonellosis after 12 days of treatment. The hematological parameters were assessed after infection and treatment. The biological parameter and some serum and tissue oxidative stress markers were also evaluated. Moreover, the plant extract increased the weight of infected rats and normalized levels of TCMH (3.06; 2.83 and 3.16 Pg), CCMH (30.00; 26.66 and 20.00 g/dL) and lymphocytes (3.20; 3.20 and 3.10 µL). The results indicate that the extract of *Harungana madagascariensis* could be potentially used in salmonellosis treatment.

Keywords: *Harungana madagascariensis*; Methanol extract; Anti-salmonella activity; Toxicological evaluation

1. Introduction

The microbial safety of food is a major concern to consumers, regulatory agencies and food industries worldwide [1]. About 600 million people globally suffer from ill health due to consuming contaminated food, and approximately 420.000 deaths are recorded annually [2]. Foodborne diseases of bacterial etiology are commonly caused by enterohaemorrhagic.

Salmonella contamination and resultant infection have a high economic burden on food production due to a broad host range and significant morbidity and mortality in the human population [3-4]. In 2015 alone, invasive non-typhoidal *Salmonella* (iNTS) disease was estimated to cause 680.000 deaths per year worldwide, more than half of which were in

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Africa [4]. Therefore, *Salmonella* control will continue to be a crucial task at all stages of the food chain, from production to processing, distribution and consumption, to decrease food contamination incidence and ensure food safety [5].

Salmonella spp. belongs to the Enterobacteriaceae family. It is a Gram-bacilli and facultative anaerobic bacterium. The genus is divided into three main species, *Salmonella enterica*, *Salmonella bongori* and *Salmonella subterranean* [6]. It is one of the four leading causes of diarrheal diseases worldwide (OMS). There were about 94 million cases of gastroenteritis, with 155.000 deaths globally every year [7]. Out of these cases, it is considered that 80.3 million were of food-borne origin [8]. In poultry, the epidemiologically important serotypes associated with most human salmonellosis are *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella newport* and *Salmonella Heidelberg* [9-12]. Typhoid fever is encountered worldwide but is primarily prevalent in developing countries. It is a systemic prolonged febrile illness caused by specific *Salmonella* serotypes. *S*erovars *Typhi* and *Paratyphi* of this serotype, *Salmonella enteric* subspecies *enterica,* are the etiologic agents that cause typhoid and paratyphoid fevers, respectively. It commonly presents with a sudden onset of fever, headache, abdominal pain, and diarrhea. It can quickly progress to potentially fatal complications, including gastrointestinal haemorrhage and intestinal perforation [13].

H. madagascariensis (Hypericaceae) is a flowering plant species widely spread in inter-tropical Africa and Madagascar [14]. It is used in traditional medicine to treat some diseases; the leaves and stem bark are used as herbal medicines in treating anemia, malaria and skin diseases [14]. In Cameroon, the leaves of *H. madagascariensis* are commonly used as traditional medicines for the treatment of typhoid fever. Previous studies confirm the use of aqueous root extract in treating drug-induced hepatotoxicity [15]. Other properties, including antiamoebic, antidiarrhoeal and spasmolytic [16] antioxidant [17], as well as anti-trichomonal [18] activities have also been documented. This study aimed to investigate the *in vivo* anti-salmonella activity of the methanol extract of *Harungana madagascariensis* (MEHM) against *Salmonella typhimurium* for potential salmonellosis treatment.

2. Material and methods

2.1. Materials

2.1.1. Plant collection and identification

H. madagascariensis stem barks were harvested in Douala in the Littoral Region (Cameroon), more precisely at PK17. *H. madagascariensis* stem barks were harvested in August 2019 in the afternoon (6 p.m.). The plant identification was confirmed at the National Herbarium of Cameroon by comparison with the reference sample No 4224 HNC.

2.1.2. Microorganism and culture media

The clinical *Salmonella typhimurium* isolates were collected from the stool of patients who came for consultation at Laquintinie hospital in Douala with gastrointestinal problems and were used in the study. The culture media used included Mueller Hinton Agar (MHA) and Salmonella-Shigella Agar (SSA). MHA was used for activating the bacterial isolate and SSA for cultivation, identification and counting of *Salmonella typhimurium* colonies in fecal matter from the animals during *in vivo* therapeutic studies.

2.1.3. Animal models

In vivo experiments were performed using *Wistar* albino male adult rats (10-12 weeks old, 200 ± 30 g) bred in the animal house of the Department of Biochemistry (University of Douala, Cameroon). They were fed a standard diet. Food and water were given *adlibitum* throughout the experimental period. Animals were maintained at ambient temperature (28 ± 2°C). They were handled according to standard protocols for using laboratory animals as recommended by the OECD guideline for handling experimental animals [19]. The experimental procedure adopted in this study was following the United States National Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [20].

2.2. Methods

2.2.1. Preparation of plant extract

The stem barks of *Harungana madagascariensis* were cut into small pieces and dried in the shade (24 ± 2°C) for approximately 30 days. Samples were ground at ambient temperature using a mechanical grinder (Waring commercial laboratory blender) to obtain a fine powder. The powder obtained was extracted, as reported by [21]. For this purpose, powder (300 g) was mixed with 1L methanol and maintained at room temperature for 72 h with twice daily

homogenization. The obtained solution was then filtered using Whatman N° 1 filter paper. The filtrate was concentrated using a rotary evaporator (Büchi rotavapor R-205) at 55°C. The extract was dried in an oven (Binder-78532) for 48 h at 40°C to remove residual solvent and then stored at 4°Cfor subsequent experiments.

2.2.2. In vivo antimicrobial assay

Before their utilization, *Wistar* albino male were immunosuppressed by oral administration of 30 mg/kg of cyclophosphamide for three consecutive days, as described by [22]. Thirty rats were arranged into six groups of five, each according to their average weight. Except for animals of group 1, which were not infected, the rest were infected by oral administration of 1 mL of a suspension containing 1.5×10^8 CFU of *Salmonella typhimurium* prepared at 0.5 Mc Farland turbidity scale. The animals were treated as follows:

- Group I (neutral control): rats were uninfected and untreated (UNT) and received 5% DMSO during the treatment.
- Group II (negative control): rats were infected and untreated (INT). They received only 5% DMSO during the treatment.
- Group III (positive control, CIP): rats were treated from the 7th day after infection with ciprofloxacin at 2.5 mg/kg b.w.
- Group IV: rats were treated from the 7th day after infection with MEHM at 75 mg/kg b.w.
- Group V: rats were treated from the 7th day after infection with MEHM at 150 mg/kg b.w.
- Group VI: rats were treated from the 7th day after infection with MEHM at 300 mg/kg b.w.

2.2.3. Food intake, body weight and relative organ weight

Food intake was evaluated as the difference between the animal's body weight on day X and the animal's initial body weight before infection. The weight gain was determined as the difference between the animal's body weight on day X and the animal's initial body weight before infection. The relative organ weight (ROW) of each animal was calculated as follows:

ROW (%) = [Absolute organ weight (g) 100]/ [Body weight of chicken on the day of sacrifice (g)]

2.2.4. Biochemical parameters and stress markers

Biochemical parameters such as proteinemia, ASAT, ALT, uremia and serum creatinine levels were analyzed from serum using commercial kits (Hospitex Diagnostics std, Roma, Italia). The activity of catalase (CAT) and superoxide dismutase (SOD), thiol groups and thiobarbituric acid reactive substances (TBARS) were also determined [23-26].

2.2.5. Hematological parameters

Red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), White blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (TCMH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM), MID (monocytes + eosinophiles) and granulocytes (GRAM) were determined using an automated hematological reader (QBC Autoread plus, United Kingdom) after administration of *S. typhimurium* and treatment.

2.3. Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA assessed differences between groups with Waller-Duncan Multiple Comparison Test SPSS. P values < 0.05 were considered statistically significant.

3. Results

3.1. *In vivo* antibacterial activity of MEHM

The evolution of *Salmonella typhi* in the feces of uninfected animals and treated and untreated infected animals showed in figure 1. Except for infected and untreated animals, the viable number of *S. typhimurium* recovered from feces increased during the first day following the infection. The administration of *H. madagascariensis* extract markedly decreased the number of viable *S. typhimurium* recovered from feces. Animals treated with different extract doses did not shed any viable *S. typhimurium*in feces between the 8th and 10th day of the treatment. The same trend was observed for those treated with standard antibiotics between the 4th and 6th day of the treatment. The number of viable *S. typhimurium* also decreased in the feces of infected and untreated control animals, but this only occurred 3 to 4 days after that of the treated animals.

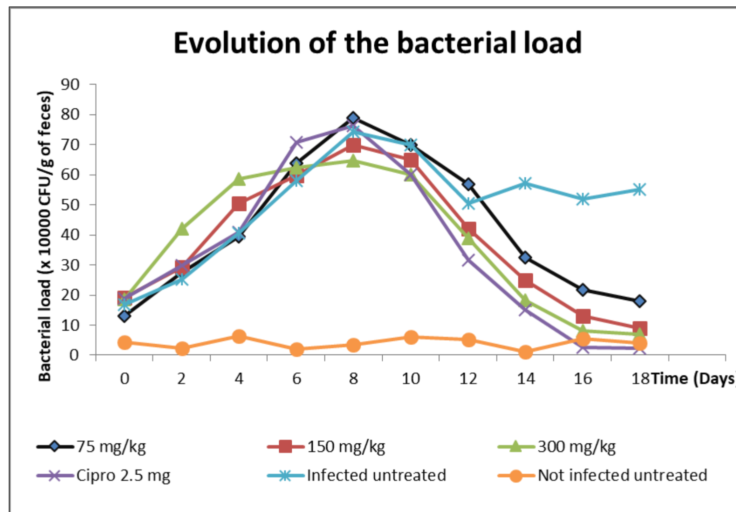

Figure 1 Number of colonies of *Salmonella typhimurium* in feces of infected rats. Data are expressed as means ± SD, n=5

3.2. Body weight, food consumption and relative organ weights

The effect of MEHM on weight gain in rats for days post-infection is presented in figure 2. The infection caused a significant decrease in weight gain compared to the uninfected group. Daily extract and ciprofloxacin administration tends to restore the weight gain evolution from the 5th day. The resumption of weight growth was observed on the 7th day for the untreated group.

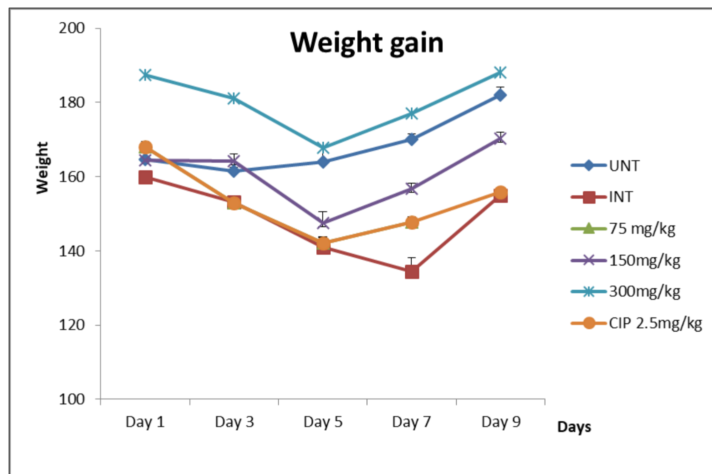

Figure 2 The effect of MEHM on weight gain in rats for days post-infection. UNT: uninfected and untreated; INT: Infected and untreated; CIP: ciprofloxacin; Data are expressed as means ± SD, n = 5

Table 1 Effect of MEHM on food intake in rats for days post-infection

Groups		UNT	INT	MEHM (75 mg/kg)	MEHM (150 mg/kg)	MEHM (300 mg/kg)	CIP (2.5 mg/kg)
Food intake (g)	5 DPI	126.0 ± 0.0^{e}	57.0 ± 0.0^{a}	59.0 ± 3.4^{a}	77.0 ± 1.7^{c}	70.0 ± 1.73^{b}	97.0 ± 1.73^{d}
	10 DPI	121.0 ± 1.7^{d}	43.0 ± 1.7^{a}	96.0 ± 0.0^{b}	98.0 ± 1.7^{c}	98.0 ± 2.31^{c}	121.0 ± 5.7^{d}

UNT: uninfected and untreated; INT: infected and untreated; MEHM: methanol extract of *Harungana madagascariensis*; CIP: ciprofloxacin; DPI: days post-infection. Data are expressed as means ± SD, n = 5. ^{a,b,c} Values in the same column with different superscript letters are statistically different at p < 0.05 (Waller Duncan test).

The food intake decreased significantly (p<0.05) in infected and untreated rats compared to the uninfected and untreated rats. Administration of MEHM and ciprofloxacin significantly ($p < 0.05$) increased food intake (Table 1). No significant differences between animal groups were observed in relative organ weight (Table 2).

Table 2 Relative organ weights recorded at the end of the study from experimental animals after oral administration of MEHM

Groups	Liver (mg/g)	Kidney (mg/g)	Heart (mg/g)	Lung (mg/g)	Spleen (mg/g)
UNT	4.2 ± 0.5	0.8 ± 0.0	0.4 ± 0.1	1.4 ± 0.4	0.3 ± 0.1
INT	3.3 ± 0.5	0.7 ± 0.1	0.4 ± 0.0	1.1 ± 0.2	0.3 ± 0.0
MEHM (75 mg/kg)	3.3 ± 0.0	0.8 ± 0.0	0.4 ± 0.0	0.9 ± 0.1	0.2 ± 0.0
MEHM (150 mg/kg)	3.7 ± 0.3	0.8 ± 0.0	0.4 ± 0.0	1.0 ± 0.2	0.4 ± 0.0
MEHM (300 mg/kg)	4.1 ± 0.7	0.9 ± 0.0	0.4 ± 0.0	1.2 ± 0.3	0.4 ± 0.1
CIP (2.5 mg/kg)	4.1 ± 0.4	0.8 ± 0.0	0.6 ± 0.0	1.1 ± 0.3	0.2 ± 0.0

UNT: uninfected and untreated; INT: infected and untreated; MEHM: methanol extract of *Harungana madagascariensis*; CIP: ciprofloxacin. Data are expressed as means ± SD, n = 5. No significant difference was observed between the groups.

3.3. Hematological, biochemical and oxidative stress parameters

The infection did not affect the level of red and white blood cells. On the other hand, it induces a significant increase in TCMH, MCHC, platelets and lymphocytes (Table 3). Treatment with the extract at 75, 150 and 300 mg/kg did not affect hematocrit, VGM, TCMH and MCHC levels compared to those obtained after infection. It also caused a significant increase in red blood cells and a significant decrease in white blood cells. Overall, no significant difference in creatinine, urea and proteins was observed in animals treated with different extract doses compared to the UNT group (Table 4). AST activity was significantly decreased in the INT group and extract-treated groups. However, the extract caused an increase in ASAT at 300 mg/kg b.w. On the other hand, treatment with extract induced a significant increase in ALAT at 75 mg/kg b.w. Superoxide dismutase activity significantly increased ($p < 0.05$) in the liver and spleen in the INT group compared to the UNT group (Table 5). Likewise, extract administration at 75 and 150 mg/kg b.w brought back the superoxide dismutase activity to normal in the liver and spleen. Moreover, the extract treatment at 75 and 300 mg/kg b.w increased superoxide dismutase activity in the spleen.

Table 3 Hematological parameters in experimental animals after oral MEHM administration

Groups		UNT	INT	MEHM (75mg/kg)	MEHM (150 mg/kg)	MEHM (300 mg/kg)	CIP (2.5 mg/kg)
RBC (mm^3)	1	2.4 ± 0.5^{ab}	3.0 ± 0.1^{a}	3.0 ± 0.1^{a}	3.6 ± 0.1^{ab}	4.6±0.5^{a}	4.3 ± 0.2^{a}
	2	5.2 ± 0.0^{a}	6.2 ± 0.0^{b}	5.8 ± 0.5^{b}	5.2 ± 0.1^{ab}	5.8±0.5^{b}	4.3 ± 0.6^{c}
WBC (mm^3)	1	4.0 ± 1.0^{ab}	4.0 ± 1.0^{ab}	5.6 ± 1.1^{a}	3.3 ± 0.5^{b}	3.3±0.5^{b}	3.2 ± 1.0^{b}
	2	5.2 ± 0.0^{d}	3.0 ± 1.0^{c}	2.6 ± 0.5^{cd}	1.6 ± 0.5^{d}	1.6±0.5^{b}	8.0 ± 0.0^{a}
Ht (%)	1	4.4 ± 5.0^{a}	5.2 ± 1.0^{bc}	5.3 ± 1.1^{d}	4.2 ± 1.7^{b}	5.2 ± 1.1^{cd}	4.7 ± 0.5^{cd}
	2	5.1 ± 0.5^{a}	5.2 ± 1.5^{a}	5.1 ± 1.0^{a}	4.2 ± 1.1^{a}	5.3 ± 0.5^{a}	4.4 ± 0.5^{a}
Hb (g/dL)	1	11.0 ± 1.0^{d}	14.6 ± 0.5^{bc}	16.3 ± 0.5^{a}	14.0 ± 0.0^{c}	15.3 ± 0.5^{ab}	15.8 ± 0.1^{a}
	2	16.3 ± 0.5^{a}	16.3 ± 0.5^{a}	14.3 ± 0.5^{a}	16.0 ± 0.0^{a}	15.0 ± 1.0^{a}	14.9 ± 2.3^{a}
VGM (µm3)	1	8.6 ± 1.1^{bc}	8.2 ± 0.5^{a}	9.4 ± 3.7^{a}	9.2 ± 1.1^{c}	9.1 ± 1.1^{a}	8.8 ± 2.0^{b}
	2	8.9 ± 0.5^{ab}	8.7 ± 2.0^{ab}	8.7 ± 2.6^{ab}	8.0 ± 1.5^{ab}	8.0 ± 2.5^{b}	8.6 ± 1.5^{bc}

[Parameter]	[Time]	[UNT]	[INT]	[MEHM 75 mg/kg]	[MEHM 150 mg/kg]	[MEHM 300 mg/kg]	[CIP 2.5 mg/kg]
MCH (Pg)	1	2.4 ± 4.5^{c}	2.9 ± 0.5^{d}	2.8 ± 0.5^{a}	3.1 ± 1.0^{a}	2.9 ± 0.5^{ab}	3.0 ± 0.0^{bc}
	2	3.1 ± 0.5^{a}	29.3 ± 0.5^{a}	3.0 ± 0.5^{a}	2.8 ± 0.5^{b}	3.1 ± 0.5^{b}	2.8 ± 0.5^{a}
TCMH (Pg)	1	2.4 ± 4.5^{c}	2.9 ± 0.5^{d}	2.8 ± 0.5^{a}	3.1 ± 1.0^{a}	2.9 ± 0.5^{ab}	3.0 ± 0.0^{bc}
	2	3.1 ± 0.5^{a}	29.3 ± 0.5^{a}	3.0 ± 0.5^{a}	2.8 ± 0.5^{b}	3.1 ± 0.5^{b}	2.8 ± 0.5^{a}
MCHC (g/dL)	1	2.6 ± 4.5^{b}	3.1 ± 0.5^{a}	3.2 ± 0.0^{a}	3.1 ± 0.5^{a}	3.4 ± 1.0^{a}	3.3 ± 0.2^{a}
	2	3.4 ± 0.0^{a}	31.6 ± 0.5^{b}	3.2 ± 1.0^{a}	3.2 ± 0.0^{b}	3.1 ± 1.0^{a}	3.3 ± 0.1^{b}
PLT (mm^{3})	1	147.3 ± 4.0^{b}	244.6 ± 1.0^{a}	198.3 ± 5.4^{a}	183.6 ± 5.7^{a}	243.6 ± 1.2^{a}	196.6 ±6.4^{a}
	2	157.6 ± 1.1^{a}	433.3 ± 1.5^{b}	157.6 ± 2.5^{b}	416.6 ± 3.2^{b}	420.0 ± 1.0^{b}	192.0 ±6.7^{a}
LYM (µL)	1	30.0 ± 0.0^{c}	33.3 ± 1.5^{bc}	66.6 ± 5.7^{ab}	43.3 ±5.7^{abc}	63.3 ± 5.7^{abc}	68.8 ± 3.5^{b}
	2	50.0 ± 0.0^{a}	13.3 ± 5.7^{b}	30.0 ± 1.0^{b}	26.6 ± 5.7^{b}	20.0 ± 0.0^{b}	52.8 ± 1.8^{b}
MID (µL)	1	66.6 ± 2.0^{ab}	83.3 ± 5.7^{a}	53.3 ± 5.7^{b}	16.6 ± 5.7^{c}	50.0 ± 0.0^{b}	23.0 ± 7.0^{c}
	2	60.0 ± 0.0^{a}	33.3 ± 5.7^{ab}	50.0 ± 0.0^{a}	16.6 ± 1.1^{ab}	16.6 ± 5.7^{ab}	44.5 ±3.4^{ab}
GRAM (%)	1	16.9 ± 4.0^{b}	45.3 ± 0.5^{a}	52.0 ± 0.0^{a}	15.3 ± 0.5^{b}	25.3 ± 1.1^{b}	48.6 ± 0.5^{a}
	2	62.2 ± 0.0^{a}	58.8 ± 0.5^{a}	35.5 ± 0.5^{bc}	15.5 ± 0.5^{c}	18.8 ± 0.5^{c}	25.5 ± 1.5^{b}

1: Post infection; 2: Post treatment; UNT: uninfected and untreated; INT: infected and untreated; MEHM: methanol extract of *Harungana madagascariensis*; CIP: ciprofloxacin; RBC: red blood cell count; WBC: white blood cell count; Hb: hemoglobin; Ht: hematocrit; VGM: mean globular volume; MCH: mean cell hemoglobin; TCMH: mean corpuscular hemoglobin content; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet count; LYM: lymphocytes; MID: monocytes + eosinophiles GRAN: granulocytes. Data are expressed as means ± SD, n = 5. ^{a,b,c,d}Values in the same column with different superscript letters are statistically different at $p < 0.05$ (Waller Duncan test).

Table 4 Biochemical parameters in experimental animals after oral MEHM administration

Groups	**UNT**	**INT**	**MEHM (75mg/kg)**	**MEHM (150mg/kg)**	**MEHM (300 mg/kg)**	**CIP (2.5 mg/kg)**
AST (IU/L)	8.3 ± 0.6^{c}	1.3 ± 0.3^{a}	1.3 ± 0.3^{a}	1.9 ± 0. 6^{a}	2.9 ± 0.5^{b}	1.9 ± 0.3^{a}
ALT (IU/L)	6.7 ± 2.4^{a}	7.1 ± 2.2^{a}	17.2 ± 7.2^{b}	9.5 ± 6.4^{ab}	10.0 ± 4.2^{ab}	11.8 ± 5.9^{ab}
Creatinine (mg/dL)	2.0 ± 2.8^{a}	2.6 ± 1.7^{a}	6.5 ± 4.1^{a}	11.2 ± 1.8^{a}	1.1 ± 1.3^{a}	0.5 ± 0.5^{a}
Urea (mg/dL)	7.2 ± 12.5^{a}	21.7 ± 0.0^{a}	14.4 ± 12.5^{a}	7.2 ± 12.5^{a}	7.2 ± 12.5^{a}	14.4 ± 1.2^{a}
PT (mg/g)	21.9 ± 3.0^{a}	31.5 ± 0.7^{ab}	16.7 ± 3.1^{a}	44.2 ± 2.9^{b}	39.7 ± 11.4^{b}	42.6 ± 13.2^{b}
PL (mg/g)	14.4 ± 2.7^{ab}	17.9 ± 0.5^{b}	12.6 ± 1.3^{a}	16.8 ± 1.2^{b}	14.8 ± 0.3^{ab}	15.1 ± 2.8^{ab}
PK (mg/g)	14.9 ± 3.4^{ab}	15.2 ± 0.8^{b}	16.9 ± 1.6^{b}	15.4 ± 0.2^{b}	15.4 ± 0.2^{b}	11.5 ± 1.5^{a}
PH (mg/g)	25.6 ± 1.1^{a}	13.6 ± 1.0^{a}	29.2 ± 2.7^{a}	23.6 ± 7.8^{a}	17.1 ± 1.2^{a}	31.6 ± 1.6^{a}
PL (mg/g)	13.5 ± 9.2^{a}	7.9 ± 4.8^{a}	18.7 ± 1.2^{a}	7.8 ± 4.0^{a}	7.1 ± 4.8^{a}	7.2 ± 2.0^{a}
PS (mg/g)	2.9 ± 1.9^{a}	1.9 ± 0.8^{a}	3.1 ± 0.7^{a}	2.7 ± 2.1^{a}	6.6 ± 5.1^{a}	3.8 ± 3.2^{a}

UNT: uninfected and untreated; INT: infected and untreated; MEHM: methanol extract of *Harungana madagascariensis*; CIP: ciprofloxacin; AST: aspartate transaminase; ALT: alanine transaminase; PT: total proteins; PL: liver proteins; PK: kidney proteins; PH: heart proteins; PL: lung proteins; PS: spleen proteins. Data are expressed as means ± SD, n = 5. ^{a,b,c} Values in the same column with different superscript letters are statistically different at $p<0.05$ (Waller Duncan test).

Table 5 Oxidative stress parameters in experimental animals after oral MEHM administration

Parameters		UNT	INT	MEHM (75mg/kg)	MEHM (150mg/kg)	MEHM (300mg/kg)	CIP (2.5 mg/kg)
SOD (unit/ mg protéin)	Serum	122.7 ± 25.5^{d}	63.3 ± 1.4^{b}	99.6 ± 5.2^{c}	41.0 ± 7.9^{a}	37.7 ± 0.0^{a}	60.6 ± 2.4^{b}
	Liver	186.3 ± 16.4^{d}	353.7 ± 16.2^{e}	111.5 ± 4.5^{b}	118.9 ± 8.8^{b}	119.5± 2.4^{bc}	26.6 ± 2.3^{a}
	Kidney	60.9 ± 1.5^{b}	29.1 ± 2.5^{a}	148.7 ± 9.2^{d}	113.7±16.0^{c}	361.5±14.2^{e}	118.0±15.6^{c}
	Heart	33.1 ± 1.6^{a}	75.9 ± 2.4^{bc}	113.8±12.9^{d}	48.7 ± 1.3^{b}	63.5 ± 15.6^{b}	31.8 ± 5.0^{a}
	Lung	249.1 ± 15.0^{d}	264.6 ± 20.9^{d}	114.6 ± 5.4^{a}	163.6 ± 5.2^{b}	391.9±23.3^{e}	197.7±12.2^{c}
	Spleen	377.4 ± 87.5^{b}	1442.0±57.3^{d}	440.7 ±9.8^{bc}	403.7±49.5^{b}	1446.3±6.3^{d}	223.7 ± 8.3^{a}
MDA (unit/ mg protéin)	Serum	3.2 ± 0.7^{a}	6.1 ± 0.5^{b}	5.9 ± 0.9^{b}	3.2 ± 0.7^{a}	6.5 ± 1.3^{b}	3.7 ± 0.3^{a}
	Liver	6.5 ± 1.2^{a}	5.7 ± 1.0^{a}	21.5 ± 7.0^{c}	4.3 ± 0.4^{a}	13.5 ± 3.0^{b}	5.0 ± 0.5^{a}
	Kidney	23.7 ± 2.9^{d}	7.8 ± 0.2^{a}	13.6 ± 1.5^{c}	9.4 ± 2.3^{ab}	9.1 ± 0.6^{b}	12.7 ± 0.6^{c}
	Heart	11.8 ± 0.8^{b}	14.8 ± 5.5^{c}	6.5 ± 0.3^{a}	6.7 ± 0.3^{a}	7.1 ± 0.7^{ab}	7.8 ± 2.4^{ab}
	Lung	14.2 ± 4.4^{b}	7.4 ± 0.4^{a}	35.8 ± 4.4^{c}	7.1 ± 0.7^{a}	18.6 ± 0.1^{b}	19.0 ± 1.5^{b}
	Spleen	19.3 ± 3.4^{bc}	14.6 ± 3.8^{ab}	21.1 ± 2.9^{c}	12.5 ± 3.1^{a}	15.1 ± 1.3^{ab}	21.3 ± 2.4^{c}

UNT: uninfected and untreated; INT: infected and untreated; MEHM: methanol extract of *Harungana madagascariensis*; CIP: ciprofloxacin; MDA: malondialdehyde; GSH: thiol group; SOD: superoxide dismutase. Data are expressed as means ± SD, n = 5. ^{a,b,c,d} Values in the same column with different superscript letters are statistically different at p<0.05 (Waller Duncan test).

4. Discussion

Based on the information provided by both the traditional healer and the *in vitro* antibacterial test results, *in vivo* study was undertaken to verify the therapeutic efficacy of the extract. Results showed that MEHM administration inhibited the growth of *S. typhimurium* and thus reduced the number of viable *S. typhimurium* recovered from feces. This reduction was dose-dependent in animals infected and treated, and their bacterial load was cancelled out within 8 to 10 days of treatment. These observations suggested that antimicrobial activity against *S. typhimurium* observed *in vitro* was maintained *in vivo*. The marked decrease of the bacterial load in infected animals after the start of the treatment could be due to the combined actions of the extract and the immune system since a decrease was also observed in the negative controls (infected and untreated). However, this only occurred in the negative controls three to four days after that of the treated animals. The regeneration of the immune system could explain this reduction of bacterial load in the negative controls since the latter was weakened to let the infection occur. The above findings partially justified the use of *H. madagascariensis* in traditional medicine [27]. Several metabolites from plant species, including alkaloids, tannins and sterols, have previously been associated with antimicrobial activity [28-29]. Detecting these classes of secondary metabolites in the extract could explain the observed activities.

We observed a significant increase in lymphocytes in rats after the administration of *S. typhimurium* due to the activation of the defense system and inflammatory or necrosis process [30].The extract administration significantly increased platelets but did not affect RBC and Ht, indicating that MEHM did not induce anemia or polycythemia. Substances that affect red blood cell count and associated parameters affect the bone marrow, kidneys and hemoglobin metabolism [31]. Therefore, extract treatment could not adversely affect the bone marrow, kidneys and hemoglobin. White blood cells are involved in the host defense system against bacteria, fungi, viruses and other exogenous substances. An increase in white blood cells is generally linked to a defense mechanism by the immune system [32]. A significantly increased lymphocyte in extract-treated rats was observed compared to the controls, probably due to the activation of the defense system and inflammatory or necrosis process [30]. This suggests that MHEM has a stimulating immune system effect.

AST activity was significantly decreased in the infected and untreated control group and increased in serum ASAT at 300 b.w. ALAT activity significant increase of serum at 75 b.w. Transaminases are synthesized and are released in blood in case of cell damage or trauma. The AST and ALT are enzymes commonly used as markers of hepatic necrosis [33-34]. ALT is localized primarily in hepatocytes, whereas AST is found in most tissues, including the heart, kidney and liver [35].

The extract treatment significantly increased superoxide dismutase activity at 75 mg/kg and 300 mg/kg in serum and spleen, respectively, indicating that MHEM has antioxidant properties. These hepato-reno-cardioprotective properties could be attributed to secondary metabolites such as polyphenols and flavonoids in this extract [30]. Polyphenols, especially flavonoids, inhibit and trap lipid peroxidation and free radicals [36].

5. Conclusion

This work provides baseline information for the possible use of the methanol extract of *H. madagascariensis* in the treatment of salmonellosis, especially typhoid fever. In addition to antibacterial activity, the data reported from acute toxicity showed that the extract might be non-toxic. These observations can justify the traditional use of the plant in treating typhoid fever.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

The experimental procedure adopted in this study was following the United States National Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research

Authors' Contributions

Azaber Masoohe Ma Moune was the main field investigator. Raymond Simplice Mouokeu and Rosalie Anne Ngono Ngane designed and supervised the work. Boris Simo Noutsa, Cedric Laurel Pouaha Cidjeu and Sandrine Suzanne Beack Bayengue participated in the fieldwork. Alix Kevine Tchoupe, Herve Njike Ngamga and Aristide Laurel Mokale Kognou participated in the manuscript writing. All the authors read and approved the final manuscript.

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