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Research Article

Immunomodulatory Activity of *Momordica charantia* L. (Cucurbitaceae) Leaf Diethyl Ether and Methanol Extracts on *Salmonella typhi*-Infected Mice and LPS-Induced Phagocytic Activities of Macrophages and Neutrophils

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Infections due to *Salmonella* strains constitute one of the major health problems in humans, particularly in Africa. The use of traditional herbs has proven effective in reducing the incidence of infection in some high-risk groups. To assess the effects of *Momordica charantia* leaf extracts that influence blood infection, an *in vitro* study of the effect on macrophages and neutrophils and treatment of mouse model of *S. typhi* infection was done. Methanol and diethyl ether extracts were concerned by this study. *In vitro* study was to assess the effects of extracts on phagocytosis, and related intracellular killing mechanisms of macrophages were examined. Later, mobilization of leukocytes and production of antibodies against *S. typhi* were measured followed by quantitating cultures evaluation of the blood infection of orally inoculated mice with *S. typhi*. Ingestion or attachment of carbon particles, production of superoxide anion, nitric oxide, and that of lysosomal acid phosphatase by macrophages and neutrophils were significantly increased by methanol and diethyl extracts at concentrations ranging from 40 μg/ml to 640 μg/ml. Antibody titer and mobilization of leukocytes, particularly lymphocytes against *S. typhi*, were highly increased by both methanol and diethyl extracts at concentrations of 500 and 1000 mg/kg. At the same time, the extracts have reduced the rate of blood colonization in mice inoculated with 10^8 CFU of *S. typhi* for 28 days. Reduction in blood colonization or infection rates was similar for the levamisole mice group. The results of this study should prove that the leaves of *Momordica charantia* are useful for the treatment of infections by *Salmonella* strains and for the assessment of drugs for therapeutic intervention.

1. Background

The immune system has a fundamental role in protecting the body against pathogenic microbial agents [1]. Once activated, the immune system produces immediate response by the activation of immune component cells and the production of various cytokines, chemokines, and inflammatory mediators. In several conditions, the system is a target of numerous drugs and herbs known as immunomodulators act by achieving immunostimulation (as in the treatment of AIDS) or achieving immunosuppression (e.g., the treatment of autoimmune disease) [2].

Salmonella infections are extremely common in Cameroon. Frequently asymptomatic, salmonellosis imposes costs upon the public sector, on the industry, in particular the wholesale and retail food industry, and very importantly upon the infected person and their family. Given both the wide distribution of *Salmonella* in foodstuffs and the frequency of asymptomatic *Salmonella* carriage, it is difficult to envision how any restaurant might prevent the

occasional case of *Salmonella* transmission despite the emphasis on hygienic practices. *Salmonella* infection is, therefore, a risk of everyday life, especially for persons who dine out frequently. As in all diseases, containment of *Salmonella* infection depends on an intact T-lymphocyte system including macrophage function. Persons with impaired T-cell function because of lymphoproliferative disorders or immunosuppressive medication and persons with disorders that cause "macrophage blockade" such as hemoglobinopathies, malaria, and schistosomiasis are well known to be persons at risk of serious consequences of *Salmonella* infection.

Modulation of immune response to alleviate disease conditions has long been of interest and increasingly recognized as a key component of effective disease control. Plant extracts have been widely investigated in recent times in different parts of the world for their possible immunomodulatory properties [3, 4]. They are very helpful in the prevention of infectious diseases or acquired immunodeficiency [5].

Since most of the drugs currently available for the treatment of salmonellosis are toxic, costly, and no longer effective, attempts are being made in laboratories around the world to discover new, safer, more cost-effective, and more potent molecules from medicinal plants with an ethnomedical history. Many plant extracts with immunomodulatory activities can be of great help in the control of bacterial infection notably salmonellosis. Plants such as *Caesalpinia bonducella* Flem. (Caesalpiniaceae), *Rhododendron spiciferum* Franch. (Ericaceae), *Curcuma longa* Linn. (Zingiberaceae), *Azadirachta indica* A., Juss. (Meliaceae), *Boerhaavia diffusa* Linn. (Nyctaginaceae), and *Ocimum sanctum* Linn. (Lamiaceae) are known to possess immunomodulatory activity [6].

Momordica charantia L. (*M. charantia*) is known to have both immunosuppressive and immunostimulant activities [7]. Plant fruits were demonstrated to promote the phagocytic activity and activation of splenocytes [8–12]. Several bioactive compounds of *M. charantia* fruit have been recorded in the literature. They are classified as carbohydrates, proteins, lipids, and more [13–15]. *M. charantia* contains triterpenoids [16–19], saponins [20–22], polypeptides [23], flavonoids [24], alkaloids [23, 25], and sterols [18]. The leaves of *M. charantia* are used in Cameroonian traditional medicine to treat typhoid. But, the biological activities and mode of action of the plant extracts are poorly understood and may act directly or indirectly.

This work was therefore designed to study the immunomodulatory activity of methanol and diethyl ether extracts of *M. charantia* leaves on *Salmonella typhi*-infected mice and phagocytic cells with the aim of having a better understanding of the therapeutic of *M. charantia* against *Salmonella* strains.

2. Materials and Methods

2.1. Reagents and Chemicals. Various reagents and chemicals were used to prepare the extracts and for the assays. They include 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT), Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), para-nitrophenylphosphate (*P*-NPP), phosphate buffered saline (PBS), lipopolysaccharide (LPS), penicillin-streptomycin (Pen-Strep), neutral red (NR), sulfanilamide, naphthylethylenediamine dihydrochloride, dimethyl sulfoxide (DMSO), triton-100, and nitroblue tetrazolium (NBT) which were purchased from Sigma Chemical, Germany. $fTiO_2$, a pure anatase sample with a mean diameter of about 250 nm, was also obtained from Sigma Aldrich (Germany). Methanol and diethyl ether used as solvents were obtained from Merck.

2.2. Plant Material. The leaves of *M. charantia* were collected in May 2018 from Mbui Division, Northwest Region, Cameroon. It was identified by Dr. Tacham Walter, a botanist at the Department of Biological Sciences, University of Bamenda, Cameroon. The identification was authenticated by the national herbarium in comparison with the collected material of Letouzey R6428, where the voucher specimen is registered under the following number: No: 8095/REF/CAM.

2.3. Experimental Animals. Adult male outbred albino mice (10–12 weeks old; 18–25 g) were used for the study. They were obtained from the National Veterinary Laboratory, Garoua, Cameroon, where they were raised under constant temperature (25–27°C) and light (12 hours light/dark). The animals were taken to the animal house of the Department of Biological Sciences, where they were given standard rodent feed and water ad libitum. Animal studies were in compliance with the ethical procedures of the Animal Use and Care Committee, Faculty of Sciences, University of Bamenda, which corresponds with the National Institutes of Health (NIH) guidelines [26].

2.4. Salmonella Strain. Salmonella typhi was used for this study. It was isolated from clinically sick patients. It was maintained in the department by serial cultures in the SDS medium. This organism was grown on MacConkey's agar, containing 2% agar, at 37°C for 18 hours and harvested into sterile saline. The bacteria were washed three times in saline and finally suspended in 0.5% formalinized saline. This suspension was incubated at 37°C to kill the organisms and then tested for sterility. This sterile suspension constituted the stock antigen and was stored at 2°C. The antigens for inoculation into mice or for the agglutination test were diluted from the stock antigen with sterile saline to a density of tube of McFarland nephelometer (10^9 organisms per ml).

2.5. Preparation of Plant Extracts. Fresh leaves of *M. charantia* were washed with distilled water and dried at 30°C. The dried leaves were ground and weighed. Subsequently, the dried powder was extracted with 98% diethyl ether (ratio: 1 : 5) for 3 days at room temperature. The solvent-containing extract was then filtered and the filtrate was evaporated using a rotary evaporator to provide the diethyl ether extract (D-extract). The residue was dried and extracted with 80% methanol with a ratio of 1 : 6. The filtrate was evaporated to provide the methanol extract (M-extract). To further ensure that all the water was removed, the extracts were freeze-dried

using a dry ovum. For *in vitro* and *in vivo* uses, extracts (5 mg) were dissolved in the 0.25 ml DMSO and diluted with PBS (1% DMSO), and this 1% DMSO PBS was as a control test.

2.6. Animals and Blood Collection. Blood samples were collected from mice for the experiment by cardiac puncture under anesthesia by a mixture of Ketamine/Xylazine administered at 2 different doses (50 mg/kg–5 mg/kg), 0.1 ml/100 g b.w. intraperitoneally.

2.7. Cell Preparation. Total white blood cells were obtained by collecting the plasma from heparinized mice blood and diluted in an equal volume of RPMI-1640 medium. Cells were harvested after 2 successive washings by centrifugation (1800 rpm, 10 min) in the medium. Peritoneal cells (neutrophils and macrophages) were obtained using elicitation methods after an intraperitoneal injection of fetal bovine serum [27]. Neutrophils (PNs) were isolated 21 hours after injection of FBS while macrophages were harvested 3 days after injection of FBS. Ten millilitres of cold RPMI was injected in the peritoneal and the exudate was collected in sterile assay tube by syringe. The exudates containing the cells were then centrifuged at 1200 rpm for 10 min at 4°C, and the cells were washed twice and resuspended in complete RPMI medium. Cells were counted using a haemocytometer, and viability was assessed by trypan blue exclusion. Cell number was therefore adjusted to the needed density.

2.8. Preparation of Carbon Particle Suspension. A stable suspension of carbon particles was obtained by suspending the ultrafine carbon powder in complete RMPI 1640. Then, the mass concentration of carbon particles in suspension was quantified by measuring the optical density at 800 nm with a spectrophotometer. A linear relationship is shown between the mass concentration of carbon particles and optical density (Figure 1).

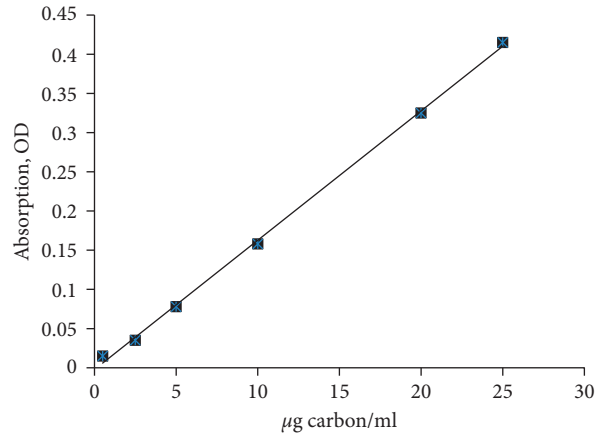

Figure 1: Correlation between the concentration of suspended carbon particles and optical density at 800 nm.

3. *In Vitro* Immunomodulation Studies

3.1. Cell Stimulation. Neutrophils or macrophages were cultured with the extracts in 96-well plate and then incubated at 37°C in a 5% CO_2 humidified atmosphere in RMPI 1640 medium with the extracts for final concentrations (20 to 640 μg/ml) and LPS (4 μg/ml). Cell cultures added to wells containing LPS only and medium only were taken as positive and negative controls, respectively. All solutions used were ensured to be lipopolysaccharide-free, and all assays were performed in triplicate and under sterile conditions. The cells were stimulated as described for all the various assays.

3.2. Phagocytosis Assay. In order to examine whether extracts affect the phagocytic activity of macrophages and neutrophils, *carbon particles* were used as test particles in the studies of phagocytosis. A modification of the method as described by Margot et al. [28] was used for the assessment of phagocytic function. Briefly, 1 ml of carbon particles in complete medium (25 μg/ml) containing the extracts was added to test tubes with and without 1.5×10^6 peritoneal cells. The samples were placed in a shaking water bath at 37°C for 12 h in order to obtain cells (macrophages and neutrophils) with attached and ingested carbon particles. The tubes were then centrifuged at 1000 tr/min for 15 min. The supernatants from the tubes with and without cells were measured in the spectrophotometer and the difference in carbon particle concentration was taken as a measure of attached and ingested particles.

3.3. Assay of Oxidative Metabolism. The oxidative metabolism was measured by using the ability of the produced superoxide to reduce yellow nitroblue tetrazolium (NBT) to blue formazan. The assay was done to scrutinize the production of superoxide anion which is proportional to the reduction of the NBT. The assay was performed as previously described [29] in macrophages and neutrophils (1.5×10^5 cells/mL) from three rats. After 48 h of incubation with or without extracts at 37°C in a 5% CO_2 humidified incubator, 50 μl of freshly prepared 1.5 mg/ml NBT dye solution was added. Then, the adherent phagocytes were rinsed vigorously after incubation for 60 min with RPMI medium and washed four times with methanol. After air drying, 2 M KOH and DMSO were added, and the absorbance was measured at 570 nm using a microplate reader.

3.4. Nitric Oxide Determination. Nitric oxide (NO) concentration was determined after 48-hour incubation with samples in 96-well plates. NO levels in each well were identified using the Griess reagent according to a previous study. [29] After preincubation of macrophages or neutrophils (1.5×10^5 cells/mL) with LPS (4 μg/mL) for 48 h, the quantity of nitrite in the culture medium was measured as an indicator of NO production. Amounts of nitrite, a stable metabolite of NO, were measured using Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) in 100 μl of the supernatant. The supernatant was mixed with an equal volume of

Griess reagent and the absorbance at 540 nm was measured in a microplate reader after incubation for 30 min.

3.5. Acid Phosphatase Determination. Macrophages or neutrophils (1.5×10^5 cells/mL) were incubated with LPS (4 μg/mL). After the desired length of time (48 hours) of incubation, the culture media were removed. Plates were washed twice with PBS. The adherent cells were then lysed with 100 μl of cold lysis buffer (0.2% Triton X-100 in 0.05 M acetate buffer pH 5.4) and sonication (the cell plates were placed on ice) for 30 minutes.

The acid phosphatase activity was measured using nitrophenylphosphate as a substrate [29, 30], which was buffered to pH 5.6 by sodium acetate. The release of nitrophenol was determined spectrophotometrically by measuring absorbance at 405 m after stopping the reaction by alkalinization with NaOH. [29] In detail, cell extracts (100 μL) were mixed with 100 μl of an assay mixture containing 20 μl glacial acetic acid, 6 mg/mL *P*-NPP, and 0.1 mol/L acetate buffer (pH, 5.4). At 65 minutes, the reaction was stopped by the addition of 100 μL 1 N NaOH. The color was measured at 410 nm in a microplate reader. The activity of the extract (% stimulation) was calculated using the absorbance of treated and untreated wells.

3.6. Expression of Percentage of Stimulation. The assay was carried out in triplicate. The activity of the extract was expressed as a percentage of stimulation in each of the test wells. The % of stimulation was calculated according to the following formula:

$$\%\ \text{of stimulation} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100\%. \quad (1)$$

The OD control is the optical density of negative control and OD sample, the optical density of the sample.

4. *In Vivo* Immunomodulation Studies

4.1. In Vivo Leucocytes Mobilization. Leucocytes mobilization method as described by Ribeiro et al. [31] was used with few modifications to study the effect of the extracts on *in vivo* leucocytes migration induced by the inflammatory stimulus. Thirty adult male mice infected intraperitoneally with *Salmonella typhi* were divided into five groups of 6 each. On days 3 and 7 after infection, three groups were given 250, 500, and 1000 mg/kg weight of extract, respectively, by gavage. One of the remaining groups has received 7.5 mg/kg body weight of levamisole and the last group was left as a control receiving the 1% DMSO PBS. One hour later, each mouse received an intraperitoneal injection of 0.5 mL of 3% agar suspension in normal saline. Four hours later, the mice were sacrificed under anesthesia and the peritoneum was washed with 5 mL of phosphate buffer saline containing 0.5 mL of 10% EDTA. The peritoneal fluid was recovered, total leucocytes counts (TLCs) were determined with haemocytometer, and the differential cell count was determined by microscopic counting of Giemsa stained perfusate smear on a glass slide.

4.2. Antibody Titrations. Salmonella typhi agglutinins were measured by an agglutination test using an antigen equivalent to 10^9 organisms per ml and doubling dilutions of antisera starting as 1/10. The tests were incubated at 37°C for 12 hours and then read. A second reading was made after a further incubation for 15 hours at room temperature. The titer of the serum was taken as the highest dilution in which definite agglutination was detected.

4.3. Infection Model. Inoculum containing a dose of *S. typhi* (10^8 CFU in 1 ml of saline) was given to mice orally by gavage. Twenty-seven days after inoculation, the animals were euthanized with diethyl ether and evaluated for blood infestation by *S. typhi*. Heparinized blood samples were obtained by tail vein puncture, and duplicate 100 μl aliquots were plated on SDS. The carriage rate was defined as the number of animals with ≥1 CFU divided by the total number of mice per group.

4.4. Data Analysis. Experiments were done in triplicate or quadruplicate. Experimental results are presented as mean ± standard deviation. Data analysis was performed by one-way analysis of variance test followed by Tukey's multiple comparison tests. The analysis was done using the program Graphpad Prism version 5.0. A p value <0.05 was considered statistically.

5. Results

5.1. Carbon Particles Uptake. Table 1 shows a comparison of the percentage of carbon particles uptake by macrophages and neutrophils treated with M-extract and D-extract carbon particles. In all samples, exposure to extracts increased ingested particles per peritoneal macrophages and neutrophils, the accumulated attachment, and the ingested fraction. The values for all extract concentrations (40, 160, and 640 μg/ml) were highly different compared to untreated macrophages and neutrophils ($p < 0.05$) showing that PNs and PMs have reduced the particles put in presence. For PMs and PNs, the reduced carbon particles were 11.84 and 14.87 μg/10^6 cells, respectively, at concentrations 640 μg/ml.

5.2. Superoxide Anion Production. M. charantia through D-extract and M-extract increased NBT dye reduction relative to untreated cells of LPS-induced mouse neutrophils in a dose-dependent fashion at concentration ≥320 μg/ml (Figure 2). In detail, in presence of D-extract, the percentage of stimulation of NBT dye reduction in neutrophils at a concentration of 640 and 320 μg/ml for 48 h was 68.48 ± 3.74%, 58.85 ± 3.23%, and 46.52 ± 3.18% of LPS control, respectively. With M-extract at a concentration of 640 and 320 μg/ml, the NBT dye reduction was 88.28 ± 7.43%, 74.47 ± 6.72%, and 50.00 ± 9.08% of LPS control, respectively. A dose-dependent increase of NBT

Table 1: Effect of methanol and diethyl ether extracts of *M. charantia* on the uptake of carbon particles by macrophages and neutrophils.

Samples	Methanol extract		Diethyl ether extract	
	% reduction of OD	$\mu g/10^6$ cells	% reduction of OD	$\mu g/10^6$ cells
Attached and ingested particles per macrophage controls				
(−) control	31.87 ± 1.54	5.31	31.87 ± 1.54	5.31
(+) control	$52.99 \pm 1.154^{***}$	8.83	$52.99 \pm 1.154^{***}$	8.83
Extracts (μg/ml)				
40	$^{a}53.98 \pm 1.32^{***}$	8.99	$^{a}42.01 \pm 1.94^{***}$	7.00
160	$^{b}70.81 \pm 1.12^{***}$	11.80	$^{b}57.25 \pm 1.66^{***}$	9.54
640	$^{c}85.53 \pm 0.83^{***}$	14.25	$^{c}71.07 \pm 0.41^{***}$	11.54
Attached and ingested particles per neutrophil controls				
(−) control	43.14 ± 3.70	7.19	43.14 ± 3.70	7.19
(+) control	$82.67 \pm 1.79^{***}$	13.77	$82.67 \pm 1.79^{***}$	13.77
Extracts (μg/ml)				
40	$^{a}85.63 \pm 0.14^{***}$	14.27	$^{a}83.08 \pm 0.97^{***}$	13.84
160	$^{ab}87.51 \pm 0.92^{***}$	14.58	$^{ab}86.12 \pm 0.09^{***}$	14.35
640	$^{b}90.46 \pm 0.98^{***}$	15.07	$^{b}89.23 \pm 0.56^{***}$	14.87

Three samples in three mice were studied. The amounts of carbon taken up by the cells were estimated by measurements of optical density (OD) of the carbon particle suspension (25 μg/ml) without the 1.5×10^6 cells/ml being added and after the cells were removed by centrifugation. The asterisks indicate the significant difference relative to (−) control determined by Tukey's test ($p \leq 0.05$). In the same column, the letters indicate the significant difference determined by Tukey's test ($p \leq 0.05$).

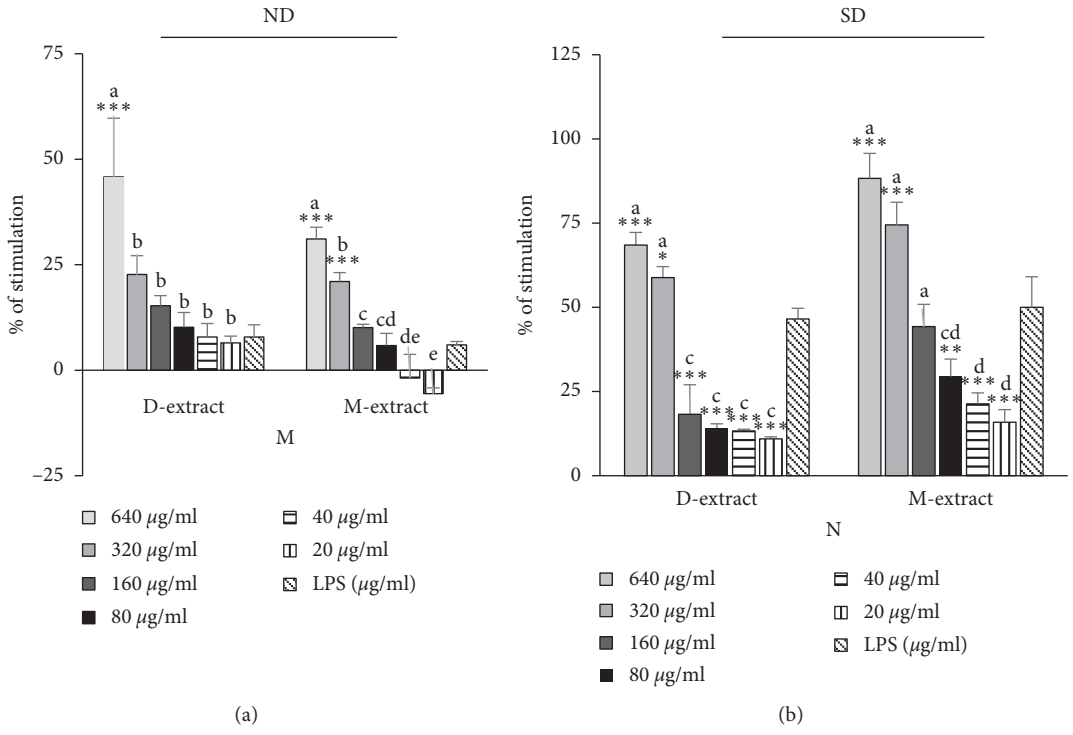

Figure 2: Stimulatory properties of the diethyl ether extracts (D-extract) and methanol extract (M-extract) on superoxide anion production by lipopolysaccharide- (LPS-) induced peritoneal mouse macrophages (M) and neutrophils (N). The histogram expressed the mean ± SD ($n = 4$). The asterisks indicate the significant difference relative to LPS control (4 μg/ml) determined by Tukey's test ($p \leq 0.05$). The letters indicate the significant difference determined by Tukey's test ($p \leq 0.05$). SD and ND indicate the significant difference and the absence of difference in the action of the two extracts determined by Tukey's test, respectively ($p \leq 0.05$).

dye reduction was also observed in macrophages. A significant increase was observed with D-extract at 640 μg/ml where the percentage of stimulation was $45.83 \pm 13.88\%$ and $7.87 \pm 2.89\%$ for LPS control, while the incubation with M-extract has resulted in augmentation of NBT reduction at 640, 320, and 150 μg/ml where the effects were $31.11 \pm 2.77\%$, $21.01 \pm 2.12\%$, $10.09 \pm 0.80\%$, and $6.01 \pm 0.80\%$ for LPS control, respectively.

5.3. Nitric Oxide Concentration. The results of this study as presented in Figure 3 demonstrated that *M. charantia*

through its D-extract and M-extract at various concentrations caused a significant increase of NO production by both neutrophils and macrophages when compared with the LPS control (Figure 4). For the M-extract which has stimulated the NO production in macrophages only, the details of the production at concentrations of 640, 320, 150, 80, 40, and 20 μg/mL for 48 h were 232.98 ± 28.34%, 128.76 ± 5.52%, 94.27 ± 7.38%, 83.50 ± 6.35%, 47.97 ± 12.11%, and 34.83 ± 18.56%, respectively, compared to 89.32 ± 1.84% of the control group treated with media only. On neutrophils, both D-extract and M-extract have promoted the production of NO ($p < 0.05$). In detail, the production of NO in LPS-induced neutrophils incubated with D-extract at concentrations of 640, 320, 150, 80, 40, and 20 μg/mL for 48 h was 108.80 ± 1.40%, 84.70 ± 12.21%, 60.52 ± 20.21%, 23.63 ± 3.11%, 24.14 ± 8.70%, 4.67 ± 1.80%, and 37.58 ± 3.89% of the group treated with LPS only, respectively. The production of NO in LPS-induced neutrophils incubated with M-extract at the same concentrations was 297.91 ± 62.21%, 171.45 ± 39.42%, 129.50 ± 27.07%, 79.37 ± 12.47%, 47.54 ± 14.51%, 36.03 ± 19.06%, and 37.58 ± 3.89% of the group treated with LPS only, respectively.

5.4. Acid Phosphatase Activity. Acid phosphatase activity was measured in LPS-induced neutrophils and macrophages after two days of incubation with the extracts. Our results showed that AcP activity, with the presence of D-extract and M-extract, was significantly increased in a dose-dependent manner (Figure 4). The 640 μg/mL extracts only showed significantly higher augmentation of AcP activity in neutrophils ($p < 0.05$). At 640 μg/ml of D-extract and M-extract, the percentage of stimulation was 63.28 ± 6.53%, 59.90 ± 6.03%, and 36.23 ± 8.19% for LPS control, respectively. Both the diethyl ether and methanol extracts showed significant augmentation dose-dependent of AcP activity in macrophages ($p < 0.05$). The percentages of stimulation in LPS-induced macrophages incubated with D-extract at concentrations of 640, 320, and 150 μg/mL were 328.99 ± 3.58%, 216.89 ± 2.15%, and 194.64 ± 5.84%, respectively. In cells incubated with M-extract at concentrations of 640, 320, and 150 μg/mL, the percentages of stimulation were 794.33 ± 79.26%, 568.19 ± 60.58%, 309.49 ± 32.46%, and 134.15 ± 17.54% of the LPS-treated group value, respectively.

5.5. Leucocytes Mobilization. The effect of the extracts on *in vivo* leucocytes mobilization led to augmentation in total leucocytes count. With M-extract, the concentrations 500 mg/kg and 1000 mg/kg significantly increased the total leucocytes count from the control group, with the effect being higher than the levamisole group. In differential leucocytes mobilization, all the tested doses significantly increased the lymphocyte count, while monocyte count has been increased in 1000 mg/kg group. In contrast, the basophil and eosinophil of the extract-treated group showed a decrease in number when compared with the control group (Table 2). Concentrations 500 mg/kg and 1000 mg/kg of D-extract also significantly increased the total leucocytes count in a concentration-dependent manner compared to the control group. In differential leucocytes mobilization, all the tested doses significantly increased the lymphocyte count, while the basophil and eosinophil showed a decrease in number when compared with the control group (Table 3).

5.6. Anti-Salmonella typhi Antibody Titers. Antibodies against *Salmonella typhi* were determined by the HA technique. The results showed that HA titers increased after treatment at day 14 and day 28 in animals. In the M-extract-treated group, the primary antibody titers ranged from 160 to 640 (mean of 228 and 17 in the control group) at 500 mg/kg, whereas those of animals receiving 1000 mg/kg were from 320 to 1280 (mean of 1280). For this extract, there was also an increase of antibodies at day 28 with a mean of 1664 and 4096 at the doses of 500 and 1000 mg/kg, whereas that of the control group was 44. An increase in antibody titers was also seen in the levamisole-treated group as well as on day 14 with a mean of 960 and 2304 on day 28 (Figure 5).

Besides the results of this study in animals treated with D-extract, antibody titer against *S. typhi* was significantly increased in group receiving 1000 mg/kg with a mean of 168 at day 14, while at day 28, an increase of antibody titer was seen in groups receiving the extract at 500 and 1000 mg/kg with means of 416 and 1152, respectively. HA titer means at days 14 and 28 were 18 and 448 in the levamisole-treated group, whereas the means were 8 and 11 in the control group (Figure 6).

5.7. Effect on the Blood Infestation Rate. At the time of blood collection after three experiments carried out independently, the percentage of mice having blood infected with ≥1 CFU of *S. typhi* in 500 and 1000 mg/kg treated groups was significantly lower than that of control groups. The proportion of infected animals was found to be 83.33% and 44.44%, respectively, for 500 and 1000 mg/g/kg against 94.44% for the control group. Levamisole also significantly decreased the proportion of mice with infected blood (38.88%) compared to the control group (Figure 7).

6. Discussion

Many studies have addressed the immunomodulatory activities of *M. charantia* but very little is known about phagocytic mechanisms and factors that influence blood infestation by bacterial strain. Passage of *Salmonella* strain in the blood is a multifactorial process that requires a variety of adaptive mechanisms, including adherence to host tissues, and host defenses. Identifying the immunotherapeutic factors of *M. charantia* that influence blood infestation by this bacterium is the best way for the use of this plant. We have studied the phagocytic activity of the M-extract and D-extract of the leaves of *M. charantia in vitro* in macrophages and neutrophils. Furthermore, we treated oral-infected mice with extracts to know the role of *M. charantia* leaves in the treatment of salmonellosis pretending the traditional therapists in the Northwest Region of Cameroon.

It has been found that the exposure of neutrophils and macrophages to M-extract and D-extract stimulates both their capacity to ingest foreign particles and their

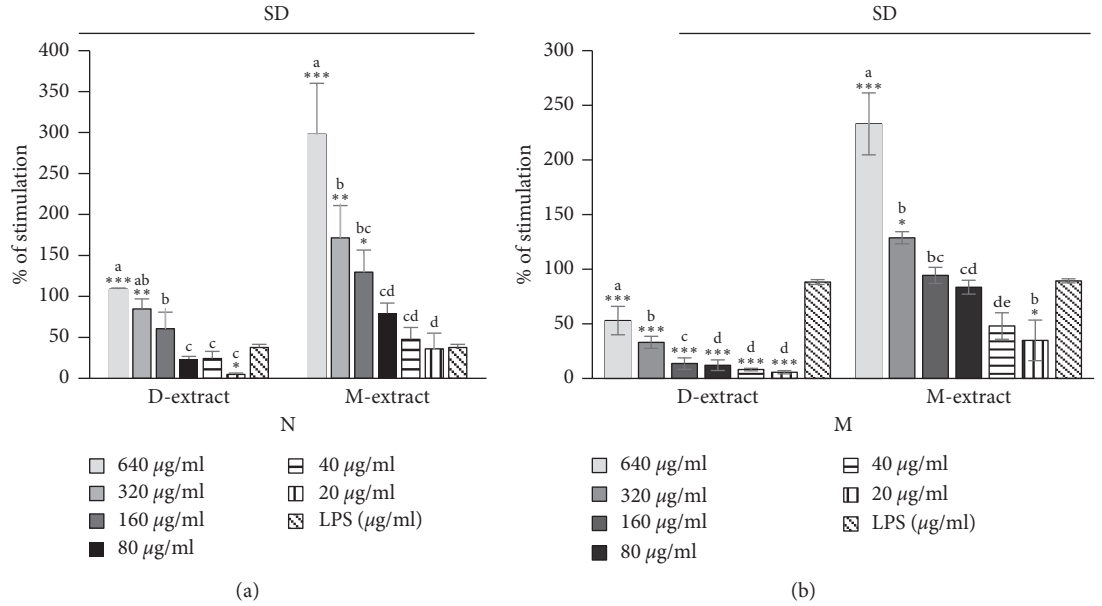

Figure 3: Stimulatory properties of the diethyl ether extracts (D-extract) and methanol extract (M-extract) on nitric oxide production by lipopolysaccharide- (LPS-) induced peritoneal mouse macrophages (M) and neutrophils (N). The histogram expressed the mean ± SD ($n = 4$). The asterisks indicate the significant difference relative to LPS control (4 μg/ml) determined by Tukey's test ($p \leq 0.05$). The letters indicate the significant difference determined by Tukey's test ($p \leq 0.05$). SD indicates the significant difference in the action of the two extracts determined by Tukey's test ($p \leq 0.05$).

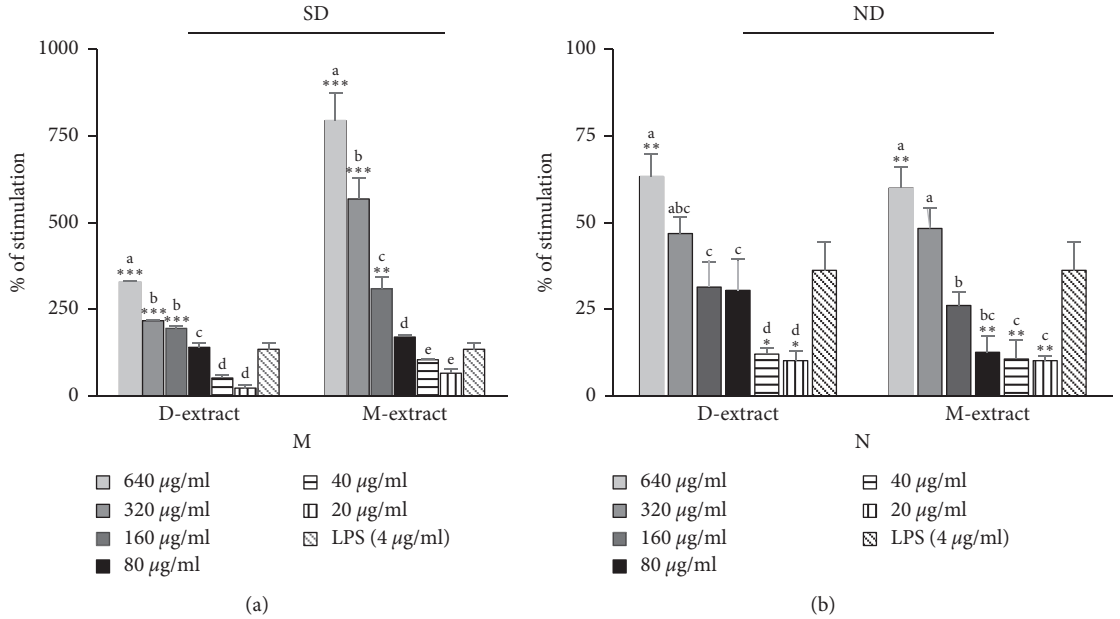

Figure 4: Stimulatory properties of the diethyl ether extracts (D-extract) and methanol extract (M-extract) on acid phosphatase activity in lipopolysaccharide- (LPS-) induced peritoneal mouse macrophages (M) and neutrophils (N). The histogram expressed the mean ± SD ($n = 4$). The asterisks indicate the significant difference relative to LPS control (4 μg/ml) determined by Tukey's test ($p \leq 0.05$). The letters indicate the significant difference determined by Tukey's test ($p \leq 0.05$). SD and ND indicate the significant difference and the absence of difference in the action of the two extracts determined by Tukey's test, respectively ($p \leq 0.05$).

intracellular killing activities. This activity demonstrates an immunostimulation of phagocytosis [5, 32]. After the exposure to extracts, neutrophils and macrophages were found to be more functional, as shown by the release of oxygen radicals. The results follow the earlier studies exhibiting *in vivo* and *in vitro* stimulatory effect of phagocytic activity by fruits of this plant [8–10]. Production of the reactive oxygen species (ROS) is known to be increased during infection through activation of NADPH oxidase; therefore, *M. charantia* leaves may stimulate the synthesis or activation of NADPH oxidase.

Table 2: Effect of *M. charantia* leaf methanol extract on total and differential leucocytes mobilization (cells/ml) in mice.

	Extract			Levamisole	Control
	250 mg/kg	500 mg/kg	1000 mg/kg	7.5 mg/kg	—
TLC	1416.6 ± 166.55^{a}	$1675.6 \pm 144.60^{**b}$	$1981 \pm 461.18^{***b}$	1184.4 ± 149.24	962.28 ± 144.41
NEUT	318.35 ± 147.30 (22.09)	391.04 ± 167.52 (22.95)	462.40 ± 132.83 (23.44)	$283{,}69 \pm 75.77$ (23.82)	445.53 ± 197.16 (45.68)
LYMPH	$1069.60 \pm 146.38^{**a}$ (75.88)	$1266.06 \pm 111.73^{***ab}$ (75.97)	$1467.92 \pm 364.80^{***b}$ (73.85)	$892.12 \pm 108.61^{*}$ (75.47)	477.33 ± 153.21 (50.186)
BASO	4.38 ± 4.51 (0.29)	$0.03 \pm 0.07^{**}$ (0.00)	$0.00 \pm 0.00^{**}$ (0.00)	$0.46 \pm 0.60^{**}$ (0.04)	10.11 ± 6.24 (1.07)
MONO	15.10 ± 5.12^{a} (1.07)	$1.22 \pm 1.14^{*b}$ (0.07)	$30.95 \pm 7.59^{***c}$ (1.61)	$1.64 \pm 0.94^{*}$ (0.13)	14.06 ± 7.90 (1.50)
EOSIN	0.03 ± 0.06 (0.03)	4.92 ± 2.44 (0.29)	3.93 ± 2.34 (0.21)	5.38 ± 7.09 (0.42)	9.27 ± 6.00 (0.93)

Mean ± SD with different superscript letters is significantly different ($p < 0.05$).

Table 3: Effect of *M. charantia* leaf diethyl ether extract on total and differential leucocytes mobilization (cells/ml) in mice.

	Extract			Levamisole	Control
	250 (mg/kg)	500 (mg/kg)	1000 (mg/kg)		
TLC	1356 ± 191.94^{a}	$1515.8 \pm 311.80^{*a}$	$1804.15 \pm 322.71^{***a}$	1151.32 ± 172.10	962.28 ± 134.41
Neutrophil	311.90 ± 165.23^{a} (22.09)	400.09 ± 119.91^{a} (26.37)	472.64 ± 116.89^{a} (26.02)	303.11 ± 62.80 (26.22)	411.43 ± 160.73 (42.92)
Lymphocyte	$1016.38 \pm 95.17^{**a}$ (75.88)	$1102.57 \pm 243.62^{***a}$ (72.78)	$1289.80 \pm 215.62^{***a}$ (71.65)	$842.42 \pm 121.06^{*}$ (73.28)	504.96 ± 170.48 (52.33)
Basophil	$4.27 \pm 4.49^{***a}$ (0.29)	$0.03 \pm 0.07^{***a}$ (0.00)	0.00^{***a} (0.00)	$0.45 \pm 0.52^{***}$ (0.04)	15.51 ± 4.04 (1.60)
Monocyte	16.39 ± 9.32^{a} (1.2)	$0.63 \pm 0.79^{***b}$ (0.04)	19.60 ± 3.82^{a} (1.08)	$1.75 \pm 0.48^{***}$ (0.15)	20.23 ± 4.72 (2.10)
Eosinophil	$0.40 \pm 0.91^{***a}$ (0.03)	4.81 ± 1.68^{b} (0.32)	5.29 ± 0.95^{b} (0.30)	$0.20 \pm 0.46^{***}$ (0.02)	7.05 ± 2.53 (0.71)

Mean ± SD with different superscript letters is significantly different ($p < 0.05$).

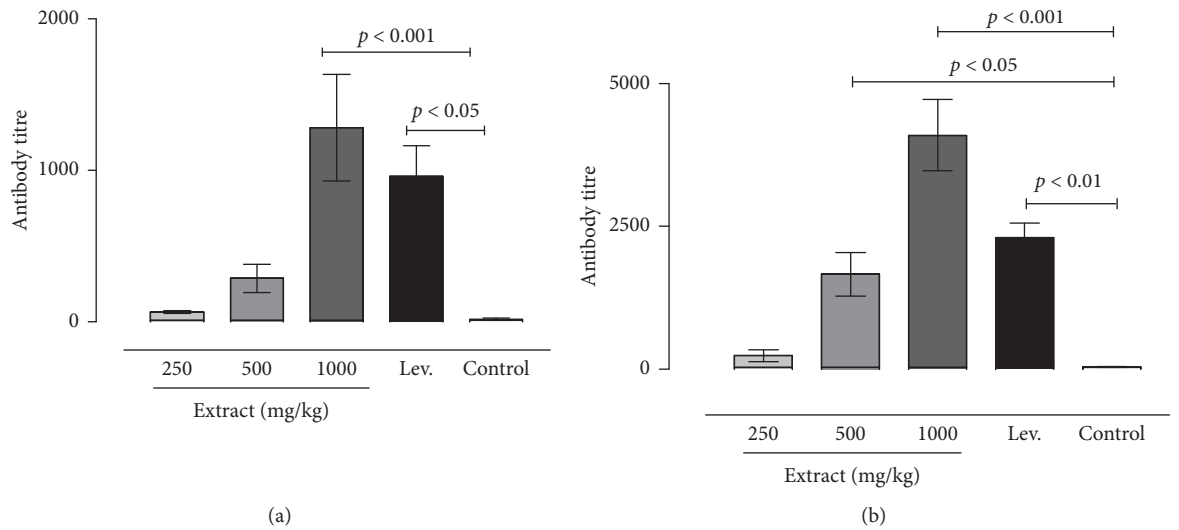

Figure 5: Effect of methanol extract on the antibody titer against *Salmonella typhi* in mice. The probability is the result of the Tukey test indicating the difference of the doses against control. (a) Primary response. (b) Secondary response.

Furthermore, it has been demonstrated that the extracts stimulate the production of nitric oxide. The role of this nitrogen reactive species is well known during immune reactions [33], and its production by neutrophils and macrophages is a result of iNOS synthesis. The effect of *M. charantia* leaf extracts in salmonellosis may be also attributed to that reactive species production.

Under circumstances as bacteria activation, neutrophils or macrophages synthesis acid phosphatase, what it has been seen in neutrophils and macrophages exposed to extracts.Thus, *M. charantia* leaves might contribute to the elimination of bacteria by stimulating lysosomal enzyme synthesis.

The result of this present study indicated that the extract increased the total count of leucocytes of the perfusate when

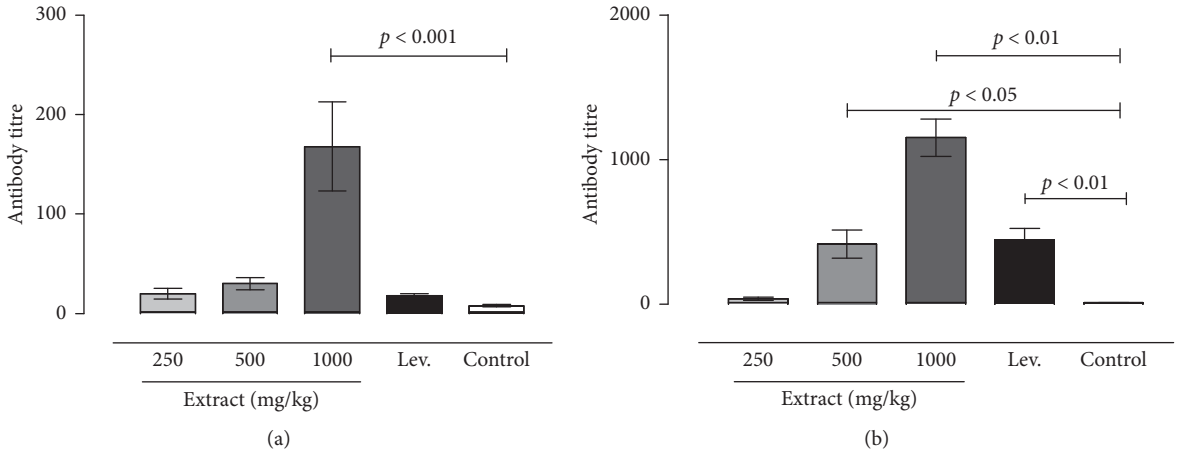

Figure 6: Effect of diethyl ether extract on the antibody titer against *Salmonella typhi* in mice. The probability is the result of the Tukey test indicating the difference of the doses against control. (a) Primary response. (b) Secondary response.

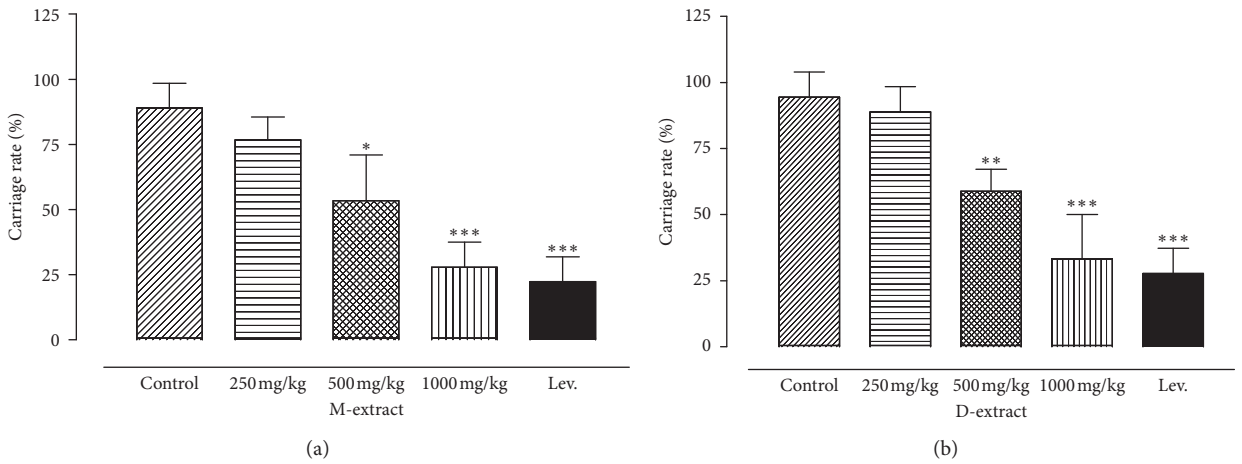

Figure 7: Blood infestation of mice with *Salmonella typhi* following the extract doses. The p values derived from the statistical comparison of carriage rate from the groups of untreated or treated extract-treated mice are shown below the graph. A $p < 0.05$ was considered significant.

compared with the control group. Migration of leucocytes is important for the transport of immunological information between different compartments of the immune system [34], stimulating the response to *S. typhi*. In addition, it has been found that all the tested doses improved the lymphocyte count. These cells are more important in the production of immunomodulatory cytokines and the production of antibodies. Particularly, Th2 lymphocytes are direct leukocyte producing IL-4, suggesting a prominent role for these cells of the adaptive immune system in the biology of B cells, notably the production of immunoglobulins what can be attributed to *M. charantia* leaf extracts.

In the current study, the primary antibody titer was found to be high in the extract-treated group for 1000 mg/kg. This effect of enhancement of the antibody production by the extracts may be associated with the effect on lymphoid cells as demonstrated by the high mobilization of these cells. When the mice were sensitized with the bacteria, the bacteria antigen was then taken up by macrophages and was processed. When a T lymphocyte sees the processed antigens on the B cell, the T cell then stimulates the B cells to undergo repeated cell divisions, enlargement, and differentiation to form a clone of antibody secreted by plasma cells. Hence, the antibody then binds to the antigen, making them easier to ingest by the white blood cells. In the present study, it has been demonstrated that the secondary antibody has been increased in 500 mg/kg and 1000 mg/kg treated groups. This indicates the enhanced responsiveness of macrophages and T and B lymphocytes involved in the antibody synthesis by the extracts.

When *S. typhi* was given to mice, it has been found that the strain passes into blood and multiplies. Using the extracts to avoid this blood infestation in mice, it was found that the proportion of animals having bacterium in their blood decreases for certain concentration as it is for levamisole, a well-known immunostimulant drug. This experiment might be used as a proof of the possible immunotherapeutic impact of *M. charantia* leaf extracts against *S. typhi* by simulating the phagocytic mechanisms or antibodies directed against such bacteria entering into the

bloodstream, both of which have proven to be successful in eliminating or preventing blood from infection by microbes.

7. Conclusion

The result of the current study suggests that immunomodulation may be a key factor in the therapeutic activity of the extracts of *Momordica charantia* leaf in the treatment of salmonellosis.

Data Availability

Data and materials are available when needed.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors' Contributions

All authors have read the paper and approved the publication of the manuscript.

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