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Document heading

In vivo anti-salmonella activity of aqueous extract of *Euphorbia prostrata* Aiton (Euphorbiaceae) and its toxicological evaluation

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PEER REVIEW

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Comments

In the present study, it was observed that the aqueous extract of *E. prostrata* Aiton can be used in the treatment of typhoid fever with satisfactory efficacy and safety. However, hematological, biochemical and histopathological analyses indicated that, at relatively higher doses, the liver and kidney could be damaged. Information generated in this study could be the reference for the routine use of aqueous extract of the aforementioned plant in the treatment of typhoid fever after extrapolation in human being.

Details on Page 317

ABSTRACT

Objective: To investigate the *in vivo* anti-salmonella activity and the safety of aqueous extract of *Euphorbia prostratra* (*E. prostratra*), a plant commonly used in Cameroon by traditional healers.

Methods: A *Salmonella typhimurium*-infected rat model was used for the study. The physiological, biochemical and histopathological markers of possible side effects of this extract were studied using standard methods.

Results: The extract had a significant effect on the number of viable *Salmonella typhimurium* recovered from faeces, and could stop salmonellosis after 8 and 10 days of treatment for male and female rats, respectively, with non-toxic doses. However, the biochemical and histopathological analyses revealed that at relatively high doses (≥ 73.48 mg/kg for female and ≥ 122.71 mg/kg for male) the extract could induce liver damage, as illustrated by a rise of serum transaminases' levels and significant inflammation of the parenchyma and portal vein. Side effects were also observed on the kidneys, as shown by both serum and urinary creatinine, and urinary proteins.

Conclusions: The overall results indicate that the aqueous extract of *E. prostrata* has the potential to provide an effective treatment for salmonellosis, including typhoid fever. However, it is necessary to extrapolate these results in large animals, in further studies.

KEYWORDS
Euphorbia prostrata Ait., Typhoid fever, *Salmonella*, Safety

1. Introduction

Salmonella enterica, which is a group of Gram-negative bacterial pathogens capable of infecting humans and animals, cause significant morbidity and mortality worldwide[1]. Certain serotypes adapted to human, such as *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*), usually cause severe diseases in humans, such as enteric fevers (typhoid and paratyphoid fevers). In most endemic areas like Africa, Asia, and Latin America[2], approximately 90% of enteric fever is typhoid. This disease is an important global health problem with an estimated 16 million cases and 600 000 deaths each year[3]. Typhoid is transmitted by the faecal-oral route via contaminated food and water and is therefore common where sanitary conditions are inadequate and access to clean water is limited. The use of antibiotics is a major strategy for the fight against these bacteria, and antimicrobial agents are commonly used therapeutically and prophylactically in human and animal salmonellosis. However, conventional antityphoid drugs are becoming more and more unavailable to the common man in Africa due to increased cost[4]. Moreover, the typhoid causative organism, *S. typhi*, has rapidly gained resistance to the previously efficacious drugs

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Article history:
Received 11 Sep 2014
Received in revised form 12 Sep 2014
Accepted 20 Nov 2014
Available online 11 Mar 2015

like ciprofloxacin[5]. Hence, there is a need for new antityphoid agents.

Studies have shown that the pathogenicity and virulence of *Salmonella* is host specific[6]. *S. typhi* induces a systemic infection in humans but not in mice or rats, while *Salmonella typhimurium* (*S. typhimurium*) induces a systemic infection in mice and rats (similar to that induced by *S. typhi* in humans) and just a localized gastroenteritis in humans. Thus, salmonellosis induced by *S. typhimurium* in rats has many similarities to the typhoid fever in human, with the primary site of colonization being ileum in both species[7]. So *S. typhimurium*-infected rats or mice have been extensively used as models for the understanding of the pathophysiology of typhoid fever[6,8].

Recently, there has been considerable interest in the use of plant materials as an alternative method of controlling pathogenic microorganisms[9], and many compounds from plants have been shown to be effective against resistant pathogenic bacteria[10]. According to WHO[11], medicinal plants are the best sources to obtain a variety of new herbal drugs. About 80% of individuals from developing countries use traditional medicine, which has substances derived from medicinal plants[11]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy[12].

Euphorbia prostrata (*E. prostrata*) is an annual herb, which belongs to family Euphorbiaceae and is abundantly found in India and Africa. It has been traditionally used in several digestive system disorders[13,14]. In Burkina Faso, the leaves are used as a remedy against the bites of venomous insects (wasps, scorpions, *etc.*). In Togo, this plant is used to fight against infertility and menstrual pain[15] and in the western rural parts of Cameroon, the whole plant of *E. prostrata* is very often used for the treatment of dysentery and typhoid fever. The *in vitro* antimicrobial activity of *E. prostrata* extracts against *S. typhi*, *S. paratyphi* A, *S. paratyphi* B and *S. typhimurium* has been demonstrated in our previous work[16]. It was also shown that the aqueous extract of *E. prostrata* could be considered as practically non-toxic, since the LD_{50} values of the extract were 23.2 g/kg and 26.4 g/kg for female and male mice, respectively[16]; *i.e.* the LD_{50} values were greater than 5 g/kg, as stated by the Hodge and Sterner criteria[17].

In order to evaluate the therapeutic potentials of *E. prostrata* for the treatment of salmonellosis (*e.g.* typhoid fever, gastroenteritis), we investigated the *in vivo* anti-salmonella activity of the aqueous extract against *S. typhimurium*. The safety of this extract was also evaluated through subacute toxicological study.

2. Materials and methods

2.1. Plant material

The whole plants of *E. prostrata* Ait were collected from Dschang (West region of Cameroon) in April 2010, and identified by the Cameroon National Herbarium (Yaoundé), where a voucher specimen was deposited (Ref N 33585/HNC).

2.2. Test bacterium and culture medium

S. typhimurium was used in this study and was provided by the Centre Pasteur, Yaoundé, Cameroon. Bacterial strain was maintained on agar slant at 4 ℃ and sub-cultured on a fresh appropriate agar plate 24 h prior to antimicrobial test.

Salmonella-Shigella agar was used for the activation of *Salmonella*, and during *in vivo* assays in rats for bacterial counts and identification.

2.3. Experimental animals

Wistar Albino rats (aged 7-8 weeks, weighing 170-210 g) of either sex were used in the study. They were bred at the animal house of Department of Biochemistry, University of Dschang in the ambient environmental conditions [(23 ± 2) ℃].

2.4. Chemicals for antimicrobial assay

Ciprofloxacin and cyclophosphamid were used as reference antibiotic and immunosuppressor, respectively.

2.5. Preparation of plant extract

The whole plant of *E. prostrata* was collected and air-dried at room temperature and then pulverised. The extraction (infusion) was done according to traditional healer indications. Thus 97.20 g of the powder were soaked for 15 min in 2 L of boiled distilled water. The preparation was filtered using Whatman No. 1 filter paper. The filtrate was then concentrated by allowing it to stand in an oven (Memmert) set at 45 ℃.

2.6. In vivo assay using rats

Male and female Wistar Albino rats were used for this study. Each sex was divided into 7 groups of 5 animals each (*i.e.* M_0, M_1, M_2, M_3, M_4, M_5, and M_6 for males; F_0, F_1, F_2, F_3, F_4, F_5, and F_6 for females). The rats were acclimatized [room temperature (23 ± 2) ℃, and a 12 h photoperiod] in cages (1 rat/cage) for one week before the commencement of the experiment. Salmonellosis was induced using the method proposed by Pan, *et al*[7], with modification. Briefly, rats were immunosuppressed by intraperitoneal injection with cyclophosphamid as described by Abhishek *et al*.[18] to facilitate the infection. At the third days of immunosuppression, the rats were fasted overnight and given, by gavage, 1 mL of saline solution (0.9% NaCl) containing 1.5 × 10^8 CFU of *Salmonella typhimurium*, except animals of groups M_0 and F_0 (which were neither infected nor treated, and used as neutral control; they received distilled water). Animals of groups M_1 and F_1 (which were infected, but not treated) received distilled water during the treatment period, hence were used as negative control groups; and those of M_6 and F_6 received ciprofloxacin, and thus were used as positive control groups. To verify that infection has occurred, the bacterial load of the faeces of the animals was determined one day before infection and during four days following the infection: a steady increase in the bacterial load during the four days indicated the establishment of the infection. Graded doses (*i.e.* 26.34, 44.00, 73.48, 122.71 mg/kg) of the aqueous extract of *E. prostrata* were administered to rats in groups M_2 and F_2, M_3 and F_3,

M_4 and F_4, and M_5 and F_5, respectively, by gastric intubation for 14 consecutive days. These doses were chosen based on the traditional healer indications (*i.e*. 44 mg/kg body weight) and the minimum inhibitory concentration (MIC) result against *S. typhimurium* obtain in the previous work[16] (*i.e*. 26.34, 73.48, and 122.71 mg/kg body weight). The animals were maintained at room temperature [(23 ± 2) ℃] with a 12 h light-dark cycle and standard animal feed and water were provided *ad libitum*. After administration of the bacterial suspension (inoculum), faecal samples were then collected every day and the numbers of the bacteria per gram of faeces were determined. In fact, faeces collected were dissolved in saline distilled water (0.9% NaCl) at the proportion of 1 g for 2 mL of suspension. Aliquots (100 μL) of faecal suspensions were serially diluted in saline distilled water (0.9% NaCl) and then plated on duplicate *Salmonella-Shigella* agar plates, which were subsequently incubated overnight at 37 ℃. Typical colonies were then identified and counted on the plates. At the end of the treatment period, the biochemical, haematological and histological markers of possible side effects were evaluated in rats using standard methods and kits.

2.7. Collection of blood and preparation of serum sample

This was done according to the method used by Gatsing *et al*[19]. At the end of the treatment period, rats were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected by cardiac puncture into two different tubes, one containing anticoagulant [ethylene diamine tetraacetic acid (EDTA)] and the other without anticoagulant. The blood in the tube with EDTA was used for the determination of haematological parameters, whereas that in the tube without EDTA was used for the preparation of serum samples. For serum preparation, the blood was allowed to clot by standing at room temperature for one hour and then refrigerated for another 1 h. The resultant liquid part was centrifuged at 1 058 r/min for 10 min, and then the clear serum (supernatant) was isolated and stored at -30 ℃ until required for analysis.

2.8. Relative organ weight and preparation of homogenates

After taking the blood, the abdominal cavity of each animal was opened and organs, namely the heart, liver, lungs, spleen and kidneys, were quickly removed, cleaned with ice-cold saline, weighed and stored at 80 ℃. The relative organ weight (organ to body weight ratio) of each animal was then calculated. The homogenates of various organs were prepared at 15% (15 g organ per 100 mL of 0.9% NaCl) as described by Gatsing *et al*[19]. This was done by grinding 500 mg of each organ in a mortar containing 3.34 mL saline distilled water (0.9% NaCl), placed on a block of ice. The homogenates were centrifuged at 1 058 r/min for 15 min and the supernatants were then used for protein assays.

2.9. Haematological assays

White blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Ht) and haemoglobin (Hb) concentration were all determined by use of an automated blood analyzer (QBC Autoread Plus, UK). The blood samples were first pipetted into QBC capillary tubes and spurn in a parafuge centrifuge (Becton Dickson, UK) for 5 min and read by means of an autoread analyzer.

2.10. Biochemical assays

The sera prepared above were used to estimate total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, serum triglycerides (TG), low-density lipoprotein (LDL) cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Total cholesterol, HDL cholesterol and triacylglycerol were determined by enzymatic methods as described by Roeschlau, *et al*[20], using the kit Hospitex Diagnostics s.r.l. (Italy). The LDL cholesterol was calculated using the formula of Friedewald *et al*.[21]:

LDL = TC - HDL - (TG/5)

The atherosclerosis index (LDL/HDL) was calculated[22]. AST and ALT were assayed with the method described by IFCC[23], using the kit Hospitex Diagnostics s.r.l. (Italy). Serum creatinine and urinary creatinine were quantified by kinetic method of Newman and Prince[24], using the kit Hospitex Diagnostics s.r.l. (Italy). Total serum protein and organ proteins (liver, lungs, heart and spleen) were assayed by the Biuret method[25], while urinary proteins and kidney proteins were quantified by the method of Bradford[26].

2.11. Measurement of the percentage of weight gain, daily feed and water intakes

The amounts of feed and water consumed were measured daily from the quantity of feed and water supplied and the amount remaining after 24 h. The weight increase or weight loss of animals was evaluated daily and the percentage of weight gain was determined.

2.12. Histopathological analysis

Tissue cross sections were prepared and analyzed using conventional techniques described by Di Fiore[27]. After sacrificing the animals, small pieces of liver were fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue were embedded in paraffin wax and sectioned into five micrometres thick with the rotary microtome, then stained with hematoxylin and eosin. Then the sections were examined with light microscope and photographed using a microscopic camera.

2.13. Statistical analysis

Data obtained were expressed as mean ± SEM and were statistically analysed using One-way ANOVA. The Waller Duncan test was used to compare means of different groups. A P-value of < 0.05 was considered statistically significant.

2.14. Ethics

This work was carried out with respect for the welfare of animals, as recommended by WHO[28].

3. Results

3.1. In vivo antibacterial activity of aqueous extract of E. prostrata in rat

In general, the number of viable *S. typhimurium* recovered from faeces increased during the first day following the infection. The administration of *E. prostrata* extract was found to induce marked decreases on the number of viable *S. typhimurium* recovered from faeces as indicated by Figure 1. Animals (female and male) treated with different doses of the extract did not shed any viable *S. typhimurium* in faeces between the eighth and tenth days of the treatment period. The same observation was made for those treated with standard antibiotics between the fourth and sixth days of the treatment period. The number of viable *S. typhimurium* also decreased in the faeces of infected and untreated control animals, but this only occurred three to four days after that of the treated animals.

3.2. Effect of the extract on body weight trends, feed and water consumption

There were generally no significant changes ($P > 0.05$) in food and water consumption patterns (Table 1). However, an increase of percentage of weight gain in general for animals of both sexes was observed during the 14 days treatment period as shown by Figure 2.

Table 1

Food and water consumption of rats during *in vivo* studies.

Sex	Doses (mg/kg)	Food consumption (g)		Water consumption (mL)	
		1st week	2nd week	1st week	2nd week
Female	0	97.67 ± 28.32^{a}	109.14 ± 8.30^{a}	97.21 ± 7.07^{a}	90.60 ± 7.08^{a}
	0i	76.43 ± 35.52^{b}	92.86 ± 24.75^{b}	88.40 ± 3.36^{a}	74.00 ± 1.87^{c}
	26.34	91.43 ± 27.35^{ab}	101.14 ± 23.28^{ab}	94.00 ± 7.25^{a}	87.00 ± 3.70^{abc}
	44.00	91.86 ± 21.74^{ab}	104.29 ± 13.99^{a}	86.80 ± 7.26^{a}	76.40 ± 3.23^{bc}
	73.48	83.71 ± 19.43^{b}	109.57 ± 15.17^{a}	101.40 ± 3.37^{a}	95.60 ± 5.37^{ab}
	122.71	96.33 ± 33.41^{a}	109.14 ± 20.61^{a}	96.33 ± 7.26^{a}	94.64 ± 5.31^{ab}
Male	0	98.14 ± 24.77^{a}	116.57 ± 21.65^{a}	121.80 ± 4.96^{a}	106.57 ± 8.04^{a}
	0i	78.86 ± 35.21^{b}	94.71 ± 26.97^{c}	97.40 ± 4.79^{a}	94.20 ± 10.96^{a}
	26.34	92.86 ± 26.96^{ab}	102.14 ± 25.47^{bc}	107.80 ± 5.00^{a}	109.20 ± 6.18^{a}
	44.00	91.86 ± 21.74^{ab}	105.43 ± 15.59^{b}	104.00 ± 2.27^{a}	96.80 ± 5.46^{a}
	73.48	95.14 ± 26.90^{ab}	110.00 ± 15.74^{ab}	103.04 ± 2.25^{a}	106.00 ± 7.30^{a}
	122.71	94.29 ± 31.32^{ab}	110.57 ± 20.90^{ab}	113.80 ± 12.47^{a}	98.57 ± 11.52^{a}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: non infected and untreated control group; 0i: infected and untreated group.

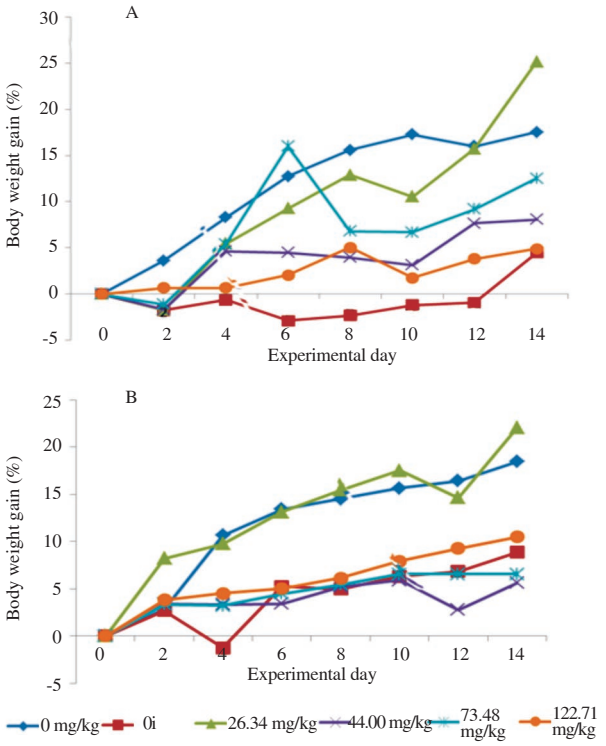

Figure 2. Body weight gain trend for female Wistar rats (A) and male Wistar rats (B) fed with aqueous extract of *E. prostrata* for 14 days. 0i: infected and untreated rats; 0 mg/kg: non infected and untreated rats (neutral and control). Each data point represents the mean ± SEM. ($n = 5$).

3.3. Effect of the extract on the relative organ weights after two weeks of treatment

The relative organ weights of liver, heart and spleen did not show any significant ($P > 0.05$) differences between the treated groups of both sexes and the neutral control groups (non infected and untreated groups). Meanwhile, in female rats there was a significant increase of the relative organ weight of kidneys at the dose of 122.71 mg/kg. A significant ($P < 0.05$) increase of the relative organ weight of lungs was observe at the doses of 26.34 mg/kg in female and 73.48 mg/kg in male (Table 2).

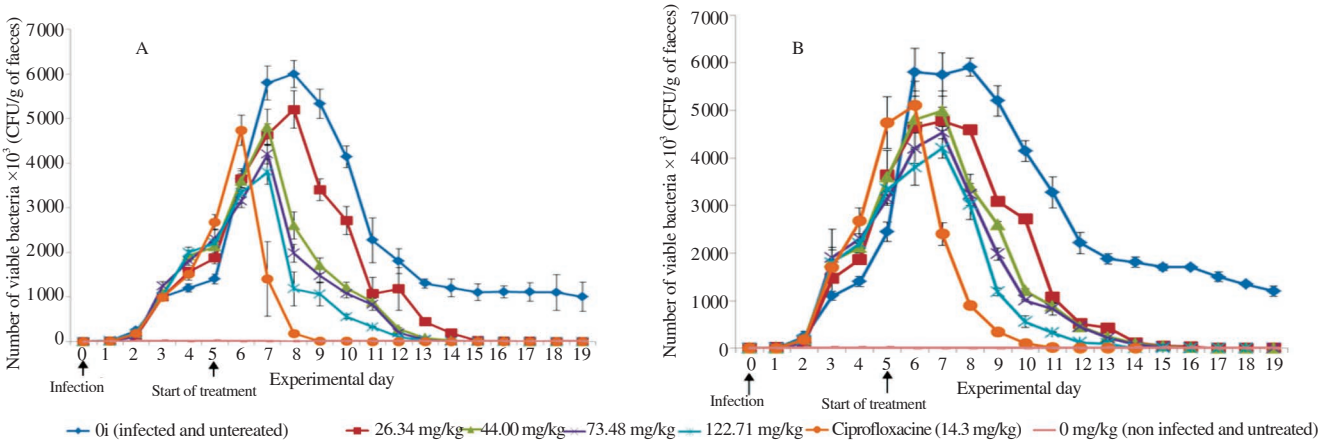

Figure 1. Effects of treatment with aqueous extract of *E. prostrata* on fecal shedding of *S. typhimurium* (CFU/g) by female (A) and male (B) rats.

Table 2

Effects of aqueous extract of *E. prostrata* on proteins profiles of liver, kidney, heart, spleen and lungs after 14 days of treatment.

Sex	Doses (mg/kg)	Proteins levels (mg/g of organs)				
		Liver	Spleen	Kidney	Heart	Lungs
Female	0	39.21 ± 0.49^{ab}	5.25 ± 1.07^{ab}	11.06 ± 0.57^{c}	3.93 ± 0.06^{a}	7.10 ± 0.52^{b}
	0i	36.84 ± 2.10^{b}	4.09 ± 0.42^{b}	11.49 ± 1.11^{bc}	3.65 ± 0.37^{a}	7.79 ± 2.62^{b}
	26.34	43.28 ± 3.99^{a}	6.51 ± 2.55^{a}	12.33 ± 0.63^{b}	4.21 ± 0.19^{a}	11.67 ± 1.75^{a}
	44.00	37.72 ± 2.99^{ab}	3.34 ± 1.59^{b}	10.82 ± 1.25^{c}	4.10 ± 0.26^{a}	8.55 ± 0.58^{b}
	73.48	43.21 ± 7.96^{a}	4.84 ± 0.31^{ab}	11.61 ± 0.27^{bc}	3.97 ± 0.36^{a}	8.55 ± 1.62^{b}
	122.71	43.47 ± 2.27^{a}	5.48 ± 1.36^{ab}	13.64 ± 0.82^{a}	4.81 ± 0.87^{a}	8.79 ± 0.56^{b}
Male	0	42.38 ± 6.45^{a}	5.23 ± 0.92^{a}	10.99 ± 1.15^{ab}	3.82 ± 0.56^{a}	7.17 ± 1.64^{b}
	0i	34.91 ± 6.05^{b}	5.06 ± 0.93^{a}	13.50 ± 3.28^{a}	4.13 ± 0.64^{a}	10.92 ± 1.77^{a}
	26.34	36.69 ± 3.78^{ab}	4.73 ± 0.94^{a}	10.43 ± 0.58^{b}	4.23 ± 0.13^{a}	6.80 ± 0.68^{b}
	44.00	38.45 ± 1.63^{ab}	4.12 ± 0.72^{a}	10.66 ± 1.39^{ab}	3.7 ± 0.14^{a}	8.19 ± 0.67^{b}
	73.48	40.84 ± 2.30^{ab}	5.02 ± 0.43^{a}	12.05 ± 1.10^{ab}	4.02 ± 0.18^{a}	9.98 ± 1.16^{a}
	122.71	39.75 ± 1.22^{ab}	4.74 ± 0.52^{a}	13.23 ± 2.47^{ab}	3.76 ± 0.11^{a}	7.17 ± 0.80^{b}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: uninfected and untreated control group; 0i: infected and untreated group.

3.4. Effect of aqueous extract of E. prostrata on the haematological parameters of rats

The hematological analysis (Table 3) showed no significant ($P > 0.05$) changes in the RBC and WBC of rats (female and male), while there was a significant ($P < 0.05$) increase of Hb in females compared to the neutral control group. Also, there was a significant ($P < 0.05$) increase of Ht in males at the dose of 122.71 mg/kg, and no significant changes in female.

Table 3

Haematological parameters following 14-day treatment of rats with aqueous extract of *E. prostrata*.

Sex	Doses (mg/kg)	RBC ($\times10^6$/mm^3)	WBC ($\times10^6$/mm^3)	Hb (g/dL)	Ht (%)
Female	0	6.2600 ± 0.9100^{a}	5.80 ± 0.84^{b}	12.00 ± 1.87^{b}	36.20 ± 1.92^{a}
	0i	2.6225 ± 1.1100^{c}	10.53 ± 1.30^{a}	16.05 ± 0.90^{a}	33.50 ± 2.36^{a}
	26.34	5.4600 ± 0.5400^{ab}	6.85 ± 0.60^{b}	15.15 ± 1.70^{a}	36.30 ± 7.95^{a}
	44.00	6.1000 ± 0.4100^{ab}	6.85 ± 2.41^{b}	15.05 ± 1.21^{a}	33.35 ± 2.36^{a}
	73.48	4.6800 ± 0.5060^{b}	5.08 ± 3.31^{b}	13.93 ± 1.88^{ab}	33.35 ± 1.85^{a}
	122.71	4.6300 ± 1.3800^{a}	6.10 ± 0.84^{b}	14.02 ± 1.86^{a}	35.50 ± 4.20^{a}
Male	0	6.2500 ± 0.9600^{a}	5.75 ± 1.26^{bc}	13.75 ± 1.50^{a}	35.00 ± 2.16^{bc}
	0i	4.8900 ± 0.9300^{a}	9.42 ± 2.71^{a}	15.28 ± 2.61^{a}	29.28 ± 4.25^{c}
	26.34	5.0000 ± 0.7070^{a}	5.70 ± 1.20^{bc}	15.00 ± 1.58^{a}	38.20 ± 5.59^{ab}
	44.00	5.9900 ± 0.6000^{a}	4.04 ± 0.95^{c}	13.40 ± 1.43^{a}	34.40 ± 5.27^{bc}
	73.48	5.8200 ± 1.3100^{a}	8.28 ± 4.03^{ab}	15.14 ± 1.59^{a}	39.08 ± 9.07^{ab}
	122.71	5.7500 ± 0.4400^{a}	5.00 ± 1.58^{c}	15.28 ± 2.61^{a}	42.86 ± 2.35^{a}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: uninfected and untreated control group; 0i: infected and untreated group.

3.5. Effect of aqueous extract of E. prostrata on serum and urinary creatinine, serum and urinary proteins of rats

In general, no significant difference in serum and urinary creatinine level were observed in animals of both sexes (treated with different doses), compared to the neutral controls (non infected and untreated group). However, the extract caused a significant decrease of serum proteins at doses greater than or equal to 44 mg/kg in females, and a significant increase of urinary proteins in the infected and untreated male group, compared to the neutral control group (Table 4). There were no significant differences in urinary proteins between the infected and untreated group and the treated male groups at all doses.

Table 4

Effects of aqueous extract of *E. prostrata* on serum creatinine, urinary creatinine, serum proteins and urinary proteins of rats after 14 days of treatment.

Sex	Dose (mg/kg)	Serum creatinine (mg/dL)	Urinary creatinine (mg/dL)	Serum proteins (mg/mL)	Urinary proteins (mg/mL)
Female	0	0.35 ± 0.16^{a}	0.85 ± 0.14^{b}	104.40 ± 6.69^{b}	0.34 ± 0.07^{bc}
	0i	0.35 ± 0.22^{a}	1.45 ± 0.33^{a}	115.09 ± 14.64^{ab}	0.35 ± 0.55^{bc}
	26.34	0.40 ± 0.14^{a}	1.00 ± 0.25^{b}	123.18 ± 21.40^{a}	0.38 ± 0.16^{bc}
	44.00	0.35 ± 0.22^{a}	1.05 ± 0.33^{ab}	63.64 ± 9.30^{cd}	0.54 ± 0.06^{a}
	73.48	0.35 ± 0.14^{a}	0.90 ± 0.22^{b}	76.45 ± 17.86^{c}	0.30 ± 0.04^{c}
	122.71	0.40 ± 0.14^{a}	1.00 ± 0.35^{b}	53.63 ± 7.01^{d}	0.45 ± 0.06^{ab}
Male	0	0.30 ± 0.11^{a}	0.95 ± 0.21^{b}	80.92 ± 3.00^{abc}	0.37 ± 0.08^{b}
	0i	0.50 ± 0.25^{a}	1.60 ± 0.45^{a}	103.55 ± 12.01^{a}	0.51 ± 0.05^{a}
	26.34	0.35 ± 0.14^{a}	1.25 ± 0.31^{ab}	89.09 ± 38.71^{ab}	0.41 ± 0.02^{ab}
	44.00	0.45 ± 0.33^{a}	1.60 ± 0.80^{a}	46.46 ± 7.43^{d}	0.48 ± 0.05^{ab}
	73.48	0.30 ± 0.11^{a}	1.05 ± 0.11^{ab}	61.54 ± 34.79^{bcd}	0.52 ± 0.05^{a}
	122.71	0.30 ± 0.11^{a}	0.95 ± 0.21^{b}	53.55 ± 6.37^{cd}	0.52 ± 0.15^{a}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: uninfected and untreated control group; 0i: infected and untreated group.

3.6. Effect of aqueous extract of E. prostrata on serum TC, HDL cholesterol, TG and index of atherosclerosis

The results of effect of aqueous extract of *E. prostrata* on the lipid profile after 14 days of treatment are summarized in Table 5. It appears from this table that the TG and the index of atherosclerosis showed non-significant changes in all treated animals (male and female, regardless of dose) compared to neutral control groups. The HDL cholesterol and TC showed significant ($P < 0.05$) decreases in treated females. In treated males, the TC and HDL cholesterol did not show any significant ($P > 0.05$) change compared to the neutral control. The LDL cholesterol showed significant ($P < 0.05$) decreases in females treated at doses of 26.34 and 73.48 and in male at dose of 26.34. The TC showed a significant ($P < 0.05$) decrease in infected and untreated male group.

Table 5

Effects of aqueous extract of *E. prostrata* on some lipid profiles after 14 days of treatment.

Sex	Dose (mg/kg)	TC (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	TG (mg/dL)	Index of atherosclerosis
Female	0	124.40 ± 8.35^{a}	45.60 ± 5.90^{a}	62.200 ± 1.818^{a}	81.00 ± 3.16^{a}	1.750 ± 0.300^{a}
	0i	82.00 ± 5.43^{c}	32.60 ± 4.03^{c}	35.400 ± 0.722^{c}	70.00 ± 3.39^{b}	1.540 ± 0.340^{a}
	26.34	88.40 ± 12.05^{c}	33.00 ± 7.25^{c}	39.760 ± 4.284^{bc}	78.20 ± 2.58^{a}	1.730 ± 0.310^{a}
	44.00	108.80 ± 6.05^{b}	37.40 ± 5.45^{bc}	55.600 ± 0.154^{a}	79.00 ± 2.23^{a}	1.970 ± 0.580^{a}
	73.48	101.60 ± 8.96^{b}	40.40 ± 3.13^{b}	45.280 ± 5.602^{b}	79.60 ± 1.14^{a}	1.530 ± 0.350^{a}
	122.71	101.80 ± 8.90^{b}	38.20 ± 1.78^{bc}	47.200 ± 6.328^{b}	80.20 ± 3.96^{a}	1.680 ± 0.360^{a}
Male	0	101.40 ± 4.39^{ab}	35.80 ± 4.21^{a}	50.360 ± 0.532^{a}	76.20 ± 356^{a}	1.870 ± 0.390^{a}
	0i	72.60 ± 2.88^{c}	33.20 ± 2.28^{a}	26.080 ± 0.186^{c}	66.60 ± 2.07^{b}	1.190 ± 0.140^{c}
	26.34	93.40 ± 9.63^{b}	37.60 ± 4.04^{a}	40.320 ± 4.918^{b}	77.40 ± 3.36^{a}	1.490 ± 0.230^{bc}
	44.00	105.20 ± 6.98^{a}	36.60 ± 5.68^{a}	53.120 ± 0.406^{a}	77.40 ± 8.53^{a}	1.910 ± 0.313^{a}
	73.48	102.60 ± 9.29^{ab}	36.20 ± 2.86^{a}	50.200 ± 6.110^{a}	81.00 ± 1.58^{a}	1.840 ± 0.180^{a}
	122.71	98.60 ± 11.15^{ab}	36.20 ± 3.96^{a}	46.040 ± 6.370^{ab}	81.80 ± 4.09^{a}	1.730 ± 0.140^{a}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: uninfected and untreated control group; 0i: infected and untreated group.

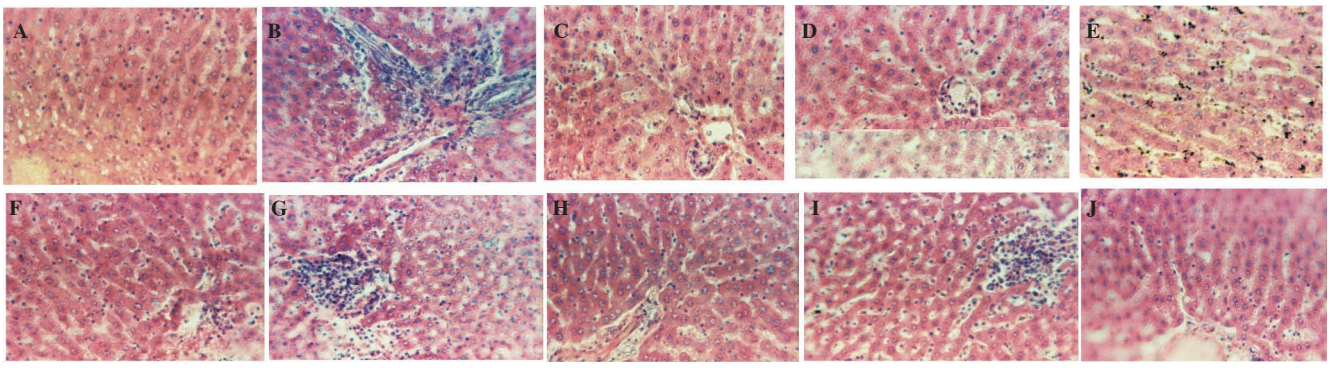

Figure 3. Histopathological changes in liver of infected rats after 14 days of treatment (400×). A: Intact lobular architecture and normal hepatocytes of infected and untreated rats; B: Dilation of sinusoid, inflammation of the parenchyma and the portal space, and cell degenerescence were observed within the liver of infected and untreated rats; Hepatocytes with well-preserved nuclear to cytoplasmic ratio and very slight inflammation in groups F_3 (C), M_3 (D), F_4 (E), M_4 (F), M_5 (H); Focal inflammatory cells infiltration (*i.e.* more injury pattern) in groups F_5 (G) and F_6 (I); J: Slightly inflammatory cells infiltration and vascular congestion in group M_6.

3.7. Effect of aqueous extract of E. prostrata on serum transaminases

Table 6 summarizes the results of effect of aqueous extract of *E. prostrata* on serum transaminases (ALT and AST) after 14 days of treatment. AST activity was significantly elevated in infected and untreated control group and at doses greater than or equal to 44 mg/kg, as compared to uninfected and untreated control group, for females and males. Also, ALT activity was significantly increased at doses of 26.34, 44.00 and 73.48 mg/kg for females and at doses greater than or equal to 44.00 mg/kg for males.

Table 6

Transaminases (ALT and AST) levels in serum samples from animals treated with aqueous extract of *E. prostrata*.

Sex	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)
Female	0	28.07 ± 2.98^{b}	60.51 ± 4.68^{d}
	0i	37.80 ± 3.42^{a}	83.60 ± 3.05^{bc}
	26.34	45.40 ± 5.77^{a}	76.80 ± 2.86^{cd}
	44.00	45.40 ± 10.16^{a}	96.40 ± 18.38^{ab}
	73.48	44.60 ± 6.54^{a}	102.40 ± 12.79^{a}
	122.71	37.75 ± 4.60^{b}	95.00 ± 23.10^{ab}
Male	0	35.68 ± 3.56^{c}	82.40 ± 5.03^{b}
	0i	40.20 ± 4.21^{bc}	91.00 ± 4.64^{b}
	26.34	38.00 ± 7.75^{bc}	88.80 ± 21.58^{b}
	44.00	46.27 ± 5.32^{ab}	114.40 ± 20.22^{a}
	73.48	55.00 ± 11.34^{a}	121.20 ± 21.29^{a}
	122.71	51.00 ± 8.71^{a}	124.00 ± 10.32^{a}

Values are mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different ($P < 0.05$). 0: uninfected and untreated control group; 0i: infected and untreated group.

3.8. Effect of aqueous extract of E. prostrata on the histology of the liver sections

Histology of the liver sections (Figure 3) of uninfected and untreated control animals (neutral control), and of those infected and treated at doses ⩽ 44 mg/kg showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and visible central veins. The liver sections of infected and untreated control rats showed dilation of sinusoid, inflammation of the parenchyma and the portal space. At doses ⩽ 44 mg/kg liver cross sections showed a normal appearance, similar to that of neutral control group. The histological architecture of liver sections of the animals treated with doses ⩾ 73.48 mg/kg (females) and ⩾ 122.71 mg/kg (males) of aqueous extract of *E. prostrata* showed injury pattern like significant inflammation of the parenchyma and the portal space, and vascular congestion.

4. Discussion

4.1. In vivo assay

Based on information provided by both the traditional healer and the *in vitro* antibacterial test results, *in vivo* study was undertaken in a view to verifying the therapeutic efficacy of the extract. Results of present research showed that the administration of aqueous extract of *E. prostrata* inhibited the growth of *S. typhimurium*, and thus reduced the numbers of viable *S. typhimurium* recovered from faeces. This reduction was dose dependent in animals infected and treated in both sexes, and their bacterial load was cancelled out within 8 to 10 days of treatment. All of 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. This reduction of bacterial load in the negative controls could be explained by the regeneration of the immune system, since the latter was weakened to let the infection occur.

The use of *E. prostrata* in traditional medicine[29,30] could partly be justified by the above findings, and we believe that this plant extract could be a potential novel antimicrobial for the treatment of salmonellosis, including typhoid fever. Several metabolites from plant species, including alkaloids, tannins and sterols, have

previously been associated with antimicrobial activity[31,32]. The detection of these classes of secondary metabolites in the extract[16] could explain the observed activities.

The protein must be eliminated in very small quantities in urine. Therefore, an increase in their removal is a sign of glomerular damage[33]. The extract appears to significantly increase the concentration of urinary protein in females treated at dose of 44.00 mg/kg, and in males at doses greater or equal to 73.48 mg/kg. This increased rate of urinary protein may indicate a renal toxicity that the extract could induce in these animals. Meanwhile, the significant increase observed in female between non infected and untreated, and infected and untreated may be attributed to the infection.

4.2. Hematological study

The hematological status after 14 days of oral administration of aqueous extract of *E. prostrata* was also assessed. In general the results showed that the values for the RBC, WBC, Hb and Ht were not significantly different as compared to the neutral control groups and fell within the normal range. The anaemia observed in infected and untreated female control as indicated by the RBC count could possibly be attributed to the various pathophysiological effects produced during *Salmonella* infection in rodent. Proliferation of *Salmonella* in rodents, controlled by a gene Nramp[34], could lead to chronic cell lysis due to the presence of free radicals produced during inflammatory reactions[35]. It has been established that the degree of anaemia always correlates well with increase in parasitemia[36].

4.3. Lipid profile

Numerous studies have pointed out the increased risk of coronary disorders with elevated levels of TC, TG, and LDL-cholesterol[37]. In a study on apparently healthy women, it has been observed that levels of TC, LDL-cholesterol, and the ratio of TC to HDL-cholesterol were significantly associated with increased risk of ischemic stroke[38]. High level of serum TC and LDL-cholesterol have been shown to pose a significant risk in ischemic stroke, whereas high levels of HDL-cholesterol indicated beneficial effects on the atherosclerotic process[39]. A high LDL/HDL ratio has also been reported to have a high predictive value of a first coronary event[22]. In the present study, it was observed that administration of aqueous extract of *E. prostrata* to rats did not induce significant changes in the serum levels of TC, TG, and HDL-cholesterol in male, while the levels of TC and HDL-cholesterol decrease significantly in female. Beside, no significant increase of LDL cholesterol and atherosclerosis index were observed in animals of both sex. This result suggests that *E. prostrata* contains component which may have no risk of cardiovascular and coronary diseases.

4.4. Transaminase and microscopic examination

Transaminases are enzymes that catalyze the transfer of an amino group from an amino acid to an α-keto acid. The increase in transaminase levels in the blood is indeed indicative of a number of ailments[40]. The AST and ALT are enzymes commonly used as markers of hepatic necrosis[41-43]. ALT is localized primarily in hepatocytes, whereas AST is found in most tissues including the heart, kidneys and liver[44]. In case of hepatotoxicity, ALT and AST increase simultaneously in serum, but the ALT increase persists longer than the AST[45]. In this study, AST was significantly elevated in infected and untreated control group and at doses greater than or equal to 44.00 mg/kg, as compared to uninfected and untreated control group, for females and males. Also, ALT activity was significantly increased at doses of 26.34, 44.00 and 73.48 mg/kg for females and at doses greater than or equal to 44 mg/kg for males, suggesting hepatic damage. The elevation of these parameters in infected and untreated control is due to the infection. In fact during typhoid fever, there is an increase of serum transaminase activity[46,47], which reflects liver damage, due to the accumulation of bacteria at this level[48].

The attribution of the increase in serum ALT and AST enzymes to liver damage was further strengthened by microscopic examination of liver sections. Histology of the liver sections of uninfected and untreated control animals (neutral control), and of those infected and treated at doses of 44 mg/kg showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and visible central veins. The liver sections of infected and untreated control rats showed dilation of sinusoid, inflammation of the parenchyma and the portal space that may be attributed to infection. The same observation was made by Choi *et al*.[49] on the liver of mice infected with *S. typhimurium* and untreated. At doses of 44.00 mg/kg liver cross sections showed a normal appearance, similar to that of neutral control group. Therefore, the extract at these doses would not only treat the infection, but might have corrected liver cell damage caused by the infection. The histological architecture of liver sections of the animals treated with doses of 73.48 mg/kg (females) and 122.71 mg/kg (male) of aqueous extract of *E. prostrata* showed injury pattern like significant inflammation of the parenchyma and the portal space, and vascular congestion. This may be explained by the fact that at relatively high doses, the extract had the ability to induce liver damage. However, the apoptosis or necrosis of hepatocytes remains one of the major signs of liver damage due to toxic compounds[50], and this was not observed in this study.

The overall results of the present work provide baseline information for the possible use of the aqueous extract of *E. prostrata* in the treatment of salmonellosis, especially typhoid fever. In addition to antibacterial activity, the data reported from acute toxicity showed that the extract may be non toxic. These observations can justify the traditional use of the plant in the treatment of typhoid fever. Moreover, the *in vivo* antibacterial activity revealed that the dose of extract obtained from (or used by the) traditional healer may be considered as relatively safe, as shown by the results of subacute toxicity evaluation. However, the extract may induce slight liver damage at high doses. Subchronic and chronic toxicity studies are necessary to further support the safe use of this plant. It is also necessary to extrapolate these results in large animals.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgements

We would like to express our gratitude to the Pasteur Centre, Yaoundé, Cameroon, for providing the test bacterium.

Comments

Background

Typhoid fever is an important global health problem in Africa where sanitary conditions are inadequate and access to clean water is limited. Since the use of conventional antibiotics is costly, there has been considerable interest in the use of plants as an alternative method to control the disease. This study showed that extract of *E. prostrata* Aiton could stop salmonellosis after 8-10 days of treatment of infected rats, with non toxic doses. However, the hematological, biochemical and histopathological analyses indicated that at higher doses, the extract could induce liver and kidney damage. This study showed that *E. prostrata* can be used for the treatment of typhoid fever with satisfactory efficacy and safety.

Research frontiers

The present study shows that extract of *E. prostrata* Aiton could stop typhoid fever at normal doses after 8 to 10 days and evaluated the hematological, biochemical and histopathological side effect of the use of relatively higher doses.

Related reports

Various parts (*i.e*., flowers, leaves, barks and roots) of *Cassia petersiana* were selected and tested against *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. This was with a view to ascertaining and assessing their traditionally claimed antityphoid properties.

Innovations and breakthroughs

It has been reported that *E. prostrata* Aiton can be used for the treatment of grade I and II of hemorrhoids with satisfactory efficacy and safety (Gupta, 2011). In this work authors have demonstrated that the aqueous extract of this plant can be used in the treatment of typhoid fever.

Applications

This study gave the scientific basis of the use of *E. prostrata* Aiton.

Peer review

In the present study, it was observed that the aqueous extract of *E. prostrata* Aiton can be used in the treatment of typhoid fever with satisfactory efficacy and safety. However, hematological, biochemical and histopathological analyses indicated that, at relatively higher doses, the liver and kidney could be damaged. Information generated in this study could be the reference for the routine use of aqueous extract of the aforementioned plant in the treatment of typhoid fever after extrapolation in human being.

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