HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

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

Donald Sédric Tala[1], Donatien Gatsing[1*], Siméon Pierre Chegaing Fodouop[1,2], Charles Fokunang[3], Fabrice Kengni[1], Merline Namekong Djimeli[1]

[1]*Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon*

[2]*Department of Biomedical Sciences, University of Ngaoundéré, P.O. Box 454 Ngaoundéré, Cameroon*

[3]*Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon*

PEER REVIEW

Peer reviewer

Dr. Guelmbaye Ndoutamia Anaclet, University of Doba, Chad.
Tel: 235 66 32 46 67
E-mails: ndoutamiaanaclet@yahoo.fr; ndoutamia@gmail.com

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

*Corresponding author: Prof. Donatien Gatsing, Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.
Tel: +237 677 51 6740
E-mail: gatsingd@yahoo.com

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,