Journal of Ethnopharmacology 137 (2011) 553–561

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

# Microencapsulation enhances the anti-ulcerogenic properties of *Entada africana* leaf extract

I.C. Obidike[a], M.O. Emeje[b,c,*]

[a] Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu, P.M.B.21 Garki, Abuja, Nigeria
[b] Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development, Idu, P.M.B.21 Garki, Abuja, Nigeria
[c] Nanoscience Group, Physical and Materials Chemistry Division, National Chemical Laboratory, Pune, India

ARTICLE INFO

*Article history:*
Received 25 March 2011
Received in revised form 27 April 2011
Accepted 8 June 2011
Available online 17 June 2011

*Keywords:*
Alginate
Calcium chloride
Microcapsules
Antiulcer
*Entada africana*

ABSTRACT

*Ethnopharmacological relevance:* The antiulcer potentials of most plants still remain largely unexplored, despite their prospects evidenced by their use as ethnomedicine. *Entada africana* (Mimosaceae) has been widely used in Africa for the treatment of skin infections, wounds, tonic for stomach troubles and against diphtheria-like throat complaints. The aim of the present study was to evaluate the anti-ulcer properties of *Entada africana* (EA) ethanol leaf extract and to obtain a novel multiparticulate pharmaceutical formulation (ACE) with it.
*Materials and methods:* Ethanol or Indomethacin was administered to rats after oral administration of EA (200, 400 and 800 mg extract/kg b.w), ACE (400 and 800 mg/kg bw), cimetidine (100 mg/kg bw), misoprostol (40 μg/kg bw) or distilled water/saline (vehicle). Anti ulcer property was evaluated by examining and scoring stomach lesions.
*Results:* The extract exhibited significant ($P < 0.01$) cytoprotective effect against ethanol and indomethacin induced gastro ulceration. The microcapsules showed enhanced cytoprotective effect against ethanol and indomethacin induced gastro ulceration. Histopathologically, the effects of EA and ACE on mucus epithelia were mild with reduced neutrophil, eosinophil and lymphocytic infiltration in stomach tissues of rats ulcerated with ethanol.
*Conclusions:* Our current findings show that EA and its multiparticulate formulation may be a useful preparation in peptic ulcer disease.



## 1. Introduction

Although delivery systems such as micelles, liposomes, nanoemulsions, and biopolymeric nanoparticles have found numerous applications in the pharmaceutical sector (Svenson, 2006; Pertuit et al., 2007), their use as vehicles for the delivery of natural bioactive extracts is relatively new in the Pharmaceutical or Nutraceutical industry. The use of biopolymer nanoparticles offers a promising means to improve the bioavailability of poorly soluble substances such as functional lipids (Svenson, 2006). Recently, nanoparticles based on food proteins such as casein and lacto globulin have been studied for nutraceutical delivery (Caillard et al., 2009; Elzoghby et al., 2011). However, there are very scarce reports of the application of polysaccharide based microcapsules to the delivery of bioactive herbal extracts.

Microencapsulation is the process of enclosing a substance inside a membrane to form a microcapsule. It provides a simple and cost-effective way to enclose bioactive materials within a semi-permeable polymeric membrane. Both synthetic/semi-synthetic polymers and natural polymers have been extensively utilized and investigated as the preparation materials of microcapsules (Freemantle, 2005). Although the synthetic polymers display chemical stability, their unsatisfactory biocompatibility still limits their potential clinical applications (Passl, 1989; Fritz and Widmann, 1993; Coviello et al., 2007). Because the natural polymers always show low/non toxicity, low immunogenicity and thereafter good biocompatibility, they have been the preferred polymers used in microencapsulation systems. Among the natural polymers, alginate has become one of the most common materials used to form microcapsules (Gåserød et al., 1998). It was used in this study to encapsulate extract of *Entada africana* intended for management of peptic ulcer disease.

* Corresponding author at: Nanoscience Group, Physical and Materials Chemistry Division, National Chemical Laboratory, Pune, India. Tel.: +91 8698359336.
*E-mail address:* martinsemeje@yahoo.com (M.O. Emeje).

0378-8741/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2011.06.012

Peptic ulcer disease (PUD) is a chronic, multifactorial disease characterized by benign lesions of gastric or duodenal mucosa. Estimates show that almost 20% of global population is affected by PUD during their lifetime (Levenstein, 2000). Thus, efforts are being re-directed towards finding effective alternatives that can be targeted towards ulcer healing, with minimal side effects. In this regard, plants and their products are promising alternatives as a good number of plants are used folklorically as medicine for their wound healing properties, some of which have been shown to possess antiulcer properties (Aniagu et al., 2005; Gupta et al., 2005; Pattanayak and Sunita, 2008; Alam et al., 2009). The antiulcer potentials of most of these plants still remain largely unexplored, despite their prospects evidenced by their use as ethnomedicine. For example, the plant *Entada africana* (Mimosaceae) is used for haemostatic and antiseptic purposes on wounds, sores and skin infections. It is employed as an emetic as antidote in food poisoning, as an antipyretic, antirheumatic and hepatoprotective agent. It is also used as a wound healer and against malaria fever in Mali. An infusion of *Entada africana* leaves is used in Ghana and northern Nigeria for wound dressing, as a tonic for stomach troubles and against diphtheria-like throat complaints. No report was found in the literature that demonstrates the antiulcer properties of this plant.

## 2. Materials and methods

### 2.1. Plant material

Fresh leaves of *Entada africana* were collected from Suleja, Niger State in June 2009. The leaves were authenticated by Mallam Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (NIPRD/H/6412) was prepared and deposited in the NIPRD herbarium. The leaves were air-dried for one week and milled to coarse powder.

### 2.2. Extraction of plant material

The powdered plant material (200 g) was extracted by maceration in 2 l ethanol (70%, v/v) with intermittent agitation for 48 h using a mechanical shaker (GFL 37, Germany). Afterwards, the mixture was filtered using filter paper (Whatman size 11) and the filtrate concentrated by rotary evaporation. The concentrate was dried to a constant weight on a hot water bath at maintained at 50 °C. The dark green extract (EA) was transferred to an air tight bottle and refrigerated till use.

### 2.3. Animals

Adult wistar rats of either sex weighing 120–160 g and Swiss albino mice weighing 15–25 g were obtained from the animal facility centre of the National Institute for Pharmaceutical Research and Development, Abuja. They were kept at standard condition of temperature (26 ± 1 °C), 12 h light/dark cycle, fed with standard rodent feed and allowed free access to potable drinking water. All experiments were carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals (1985) and NIPRD's standard operating procedures.

### 2.4. Drugs and reagents

Indomethacin, cimetidine, activated charcoal, absolute ethanol (Sigma–Aldrich, Seelze, Germany), misoprostol (Pharmacia, Kent, UK) and atropine sulphate (Laborate Pharmaceutical, Panpat, India). Sodium alginate (viscosity of 2% (w/v) solution 250 cps at 25 °C) was procured from Sigma Chemicals, St Louis, MO, USA. All other chemicals were of analytical grade and used as received.

### 2.5. Acute toxicity

Acute toxicity and median lethal dose of the extract was estimated using the method of Tahraoui et al. (2010). Acute toxicity test was carried out in two phases. In the first phase, nine rats were divided into three groups of three rats each and treated orally with 10, 100 and 1000 extract/kg body weight (b.w), respectively, and observed for 24 h post treatment for clinical signs of toxicity including but not limited to disturbance of gait, physical inactivation, abdominal stretching, or mortality. Three groups of three rats each were used in the second phase and treated orally with 1250, 2500 and 5000 mg extract/kg b.w, respectively. The rats were observed after treatment for 24 h for clinical signs of toxicity and mortality.

### 2.6. Phytochemistry

Phytochemical screening of the crude extract and fractions was carried out in accordance with standard test procedures (Chhabra et al., 1984; Tanira et al., 1994; Sandabe et al., 2006) to detect the presence of secondary metabolites such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, sterols, resins and balsams.

### 2.7. Ethanol-induced gastric ulceration

The test was performed according to the method described by Corrêa Dias et al. (2000) was used with slight modification. Thirty-five rats were randomized into seven groups of five rats each such that mean body weights of the groups were kept close. The rats were deprived of food for 18 h but allowed access to drinking water. At the end of 18 h, treatment was carried out as follows; groups 1 and 2 (negative and positive controls) were administered 5 ml distilled water/kg body weight (b.w) and cimetidine (100 mg/kg b.w), respectively, while groups 3, 4 and 5 received 200, 400 and 800 mg EA/kg b.w, respectively. Group 6 (normal control) received normal saline (2.5 ml/kg b.w) orally. Thirty minutes after treatment, all the rats were given 1 ml absolute ethanol orally except the normal control, which received 1 ml distilled water. After 1 h, the rats were anaesthetized by chloroform inhalation and their stomachs excised. Each stomach was cut open along the greater curvature, rinsed in clean water and examined with the aid of a hand magnifying lens. The lesions observed were scored as follows:

0 = No ulcer; 1 = Haemorrhagic and slightly dispersed ulcers less than 2 mm length; 2 = 1 ulcer, haemorrhagic and up to 5 mm length; 3 = More than 1 ulcer, each up to 5 mm length; 4 = 1 ulcer above 5 mm length; 5 = More than 1 ulcer above 5 mm length. The mean ulcer score for each group was taken as the ulcer index for the group. Preventive ratio was calculated using the relation:

$$\frac{\text{Ulcer score}_{\text{control}} - \text{Ulcer score}_{\text{treated}}}{\text{Ulcer score}_{\text{control}}} \times 100$$

The stomach tissues were transferred to a cold solution of 10% formal saline before histopathological examination. Tissue slices were embedded in paraffin and sections stained with haematoxylin and eosin. Light microscopic examination of multiple tissue sections from each organ in all groups was performed in all groups.