Journal of Ethnopharmacology 151 (2014) 984–989

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Evaluation of the antidiarrhoeal activity of the hydroethanolic leaf extract of *Pupalia lappacea* Linn. Juss. (Amaranthaceae)

A.J. Akindele *, O.A. Salako, U.V. Ohonbamu

Department of Pharmacology, Therapeutics & Toxicology (PTT), Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form
21 November 2013
Accepted 7 December 2013
Available online 14 December 2013

Keywords:
Pupalia lappacea
Antidiarrhoeal activity
Castor oil
Loperamide
Intestinal transit

ABSTRACT

Ethnopharmacological Relevance: *Pupalia lappacea* is a medicinal plant found in savannah and woodland localities and forest path sides from Senegal to Southern Nigeria. It has been used in the management of diarrhoea in Nigerian traditional medicine. This study was designed to evaluate the antidiarrhoeal activity of the hydroethanolic leaf extract of *Pupalia lappacea* (PL).

Materials and methods: The antidiarrhoeal activity of PL was evaluated using the normal and castor oil-induced intestinal transit, castor oil-induced diarrhoea, gastric emptying and intestinal fluid accumulation tests in rodents.

Results: PL (100–400 mg/kg, p.o.) produced a significant dose-dependent decrease in normal and castor oil-induced intestinal transit compared with the control group (distilled water 10 ml/kg, p.o.). This effect was significantly ($P < 0.05$) inhibited by pilocarpine (1 mg/kg, s.c.) but not by yohimbine (10 mg/kg, s.c.), prazosin (1 mg/kg, s.c.), or propranolol (1 mg/kg, i.p.). The extract produced a dose-dependent and significant increase in the onset of diarrhoea. PL (100–400 mg/kg) also reduced the diarrhoea score, number and weight of wet stools. The *in-vivo* antidiarrhoeal index ($ADI_{in\ vivo}$) of 56.95% produced by the extract at the dose of 400 mg/kg was lower compared to that produced by loperamide 5 mg/kg (77.75%). However, PL (400 mg/kg) significantly increased gastric emptying in rats but significantly reduced the volume of intestinal content in the intestinal fluid accumulation test. Phytochemical analysis of the extract revealed the presence of alkaloids, saponins, and fixed oils and fats. The acute toxicity studies revealed that the extract is relatively safe when given orally; no death was recorded at a dose of 10 g/kg.

Conclusion: Results showed that the hydroethanolic leaf extract of *Pupalia lappacea* possesses antidiarrhoeal activity possibly mediated by antimuscarinic receptor activity.

1. Introduction

Diarrhoea is a symptom marked by rapid and frequent passage of semi-solid or liquid faecal material through the gastrointestinal tract. Various types of diarrhoea exist (based on their clinical manifestations), but it is broadly classified into acute and chronic for diagnostic and therapeutic purposes. Acute diarrhoea is the prevalent form of the disease which has a major impact on the morbidity and mortality worldwide in all age groups, particularly in infants and children under the age of three (Hirchhorn, 1980; Muriithi, 1996; Farthings, 2002). In less developed parts of the world (such as Latin America, India and Africa), children may experience between 3 and 10 episodes of diarrhoea yearly (Farthings, 2002). In 2009, diarrhoea was estimated to have caused 1.1 million deaths in people aged 5 and 1.5 million deaths in children under the age of 5 (World Health Organization, 2009). In terms of etiology, diarrhoea can be classified as infectious and non-infectious (de Hostos et al., 2011). Infectious diarrhoea is caused by a virus, parasite or bacterium while non-infectious diarrhoea can be caused by toxins, chronic diseases or antibiotics (NHDHHS, 2009). Pharmacological models of non-infectious diarrhoea were used in this study.

Pupalia lappacea Linn. Juss. (Amaranthaceae) is a plant found in savannah and woodland localities and forest path sides from Senegal to Southern Nigeria, but less common in the western part of the region. Distribution is widespread elsewhere in tropical Africa and in Asia. *Pupalia lappacea* also commonly known as ram's bur and locally as "Kaimin kadangari" (Hausa, Northern Nigeria), "Agbiriba" (Igbo, South-east Nigeria) or "Emo agbo" (Yoruba, South-west Nigeria) has been used in traditional medicine for the treatment of cough, syphilis, skin diseases and diarrhoea (Odugbemi, 2008). The leaves mixed with palm-oil, are used in Ghana to treat boils (Agyare et al., 2009). The leaves are also used in topical applications to cuts, or used as enema or

* Corresponding author. Tel.: +234 8062359726.
E-mail addresses: ajakindele@cmul.edu.ng, jakindele@unilag.edu.ng (A.J. Akindele).

0378-8741/$ - see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.12.013

febrifuge (Agyare et al., 2009). In Ivory Coast, a decoction is taken in draught and applied in frictions for oedema of the legs and is used in various remedies for dysentery and diarrhoea. It is used as an anti-emetic in the south-western region of Nigeria and the Republic of Benin (Adjanohoun and Akjakidje, 1989).

Pupalia lappacea preparations have been reported to have antioxidant activity (Aladedunye and Okorie, 2008), anti-cancer activity (Ravi et al., 2012), antinociceptive and antipyretic activities (Neeharika et al., 2013). However, no scientific report of the antidiarrhoeal activity of *Pupalia lappacea* is available. The aim of this study is to evaluate the antidiarrhoeal effects of the hydro-ethanolic leaf extract of *Pupalia lappacea* (PL) in search for safe, effective and cheap remedies for diarrhoea management.

2. Materials and methods

2.1. Reagents and drugs

Castor oil (Finest Cold Drawn Commercial Castor oil, UK), loperamide hydrochloride (Imodium®, Janssen Pharmaceutical N.V., Belgium), methylcellulose (Koch-light Laboratories Ltd., England), pilocarpine hydrochloride, propranolol hydrochloride, prazosin hydrochloride and yohimbine (Sigma Chemical Company, St. Louis, USA) were used in this study.

2.2. Plant material

The fresh leaves of *Pupalia lappacea* were collected from the farm of a traditional herbal practitioner in Ogun State, Nigeria, and the plant was identified and authenticated at the herbarium of the Department of Botany, University of Lagos, Lagos, Nigeria, by Mr. T.K. Odewo. A voucher specimen with reference number LUH 5092 was deposited in the herbarium of the department.

2.3. Experimental animals

Adult albino Wistar mice (15–30 g) and rats (120–220 g) (8 weeks old) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria, were used for the experiments. Animals were maintained under standard environmental conditions (12 h light and 12 h dark cycle; 23–25 °C) and had free access to standard pelleted feed (RAAF Animal Feeds Ltd., Akute, Ogun State, Nigeria) and water ad-libitum in accordance with the Guidelines for Care and Use of Laboratory Animals in Biomedical Research (National Academy of Sciences, 2011).

2.4. Preparation of extract

The fresh leaves of the plant were collected and air-dried for a period of three weeks. The dried leaves were thereafter ground into powder with an electric blender. The powdered leaves were then macerated in hydroethanol (1:1; 50 g/L) for 48 h. Exhaustive extraction was done.

Filtration was carried out after 48 h and the combined filtrate was evaporated to dryness under reduced pressure at 40 °C using a Heidolph rotavapor. The dried hydroethanolic extract had a sticky consistency which was readily soluble in water. It had a dark brown colour and pH of 5.2. The percentage yield of the extract was 3.25%. The dried extract obtained was kept in the refrigerator at 4 °C and reconstituted in distilled water prior to each experimental session.

2.5. Phytochemical analysis

A portion of the dried extract was used for phytochemical screening in order to determine the presence of pharmacologically active constituents using the methods described by Trease and Evans (1989) and Edeoga et al. (2005).

2.5.1. Test for alkaloids

0.5 g of PL was added to 5 ml of 1% HCl with stirring on a water bath. Three portions of 1 ml each of the filtrate were then treated with few drops of Mayer's, Dragendorff's, and Wagner's reagent. Observation of turbidity of precipitation was taken as indication for the presence of alkaloids.

2.5.2. Test for saponins

2 g of PL was boiled in 20 ml of distilled water on a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and the mixture was vigorously agitated to obtain a stable froth. The froth was mixed with three drops of olive oil and shaken vigorously. Observation was made for the formation of emulsion. In the haemolysis test, two test tubes were labelled A and B. 0.2 ml solution of PL, prepared in 1% normal saline, and 0.2 ml of 10% (v/v) blood in normal saline was put into test tube A while B contained 0.2 ml of 10% blood in normal saline with 0.2 ml of normal saline. The observation of red supernatant in test tube A, absent in test tube B, confirmed the presence of saponins.

2.5.3. Test for phlobatannins

Observation of the deposition of a red precipitate upon the boiling of PL with 1% aqueous HCl was taken as indication for the presence of phlobatannins.

2.5.4. Test for reducing sugars

0.5 g of PL was diluted with 1 ml of distilled water and 1 ml of Fehling's solution (A and B) was added. This was heated on a water bath. A brown colouration indicates the presence of reducing sugars.

2.5.5. Test for fixed oils and fats

The filter paper and saponification tests were used in this respect. In the filter paper test, a small quantity of PL was pressed between two filter papers. The appearance of oil stain was taken as indication for the presence of fixed oils. In the saponification test, few drops of 0.5 M potassium hydroxide were added to a small quantity of PL along with a drop of phenolphthalein. On a water bath, the mixture was heated for 1–2 h. The presence of fixed oils and fats was indicated by the formation of soap.

2.6. Acute toxicity studies

Albino mice of either sex were fasted for 12 h prior to testing. The animals were randomly allotted to groups of five animals each. A dose of 10,000 mg/kg of extract was administered, in divided doses, by oral intubation to a group. The other groups of mice were given the extract intraperitoneally at the dose of 400 and 800 mg/kg respectively.

The general symptoms of toxicity such as restlessness, panting and mortality in each group within 24 h were recorded. The LD_{50} was estimated using Miller and Tainter's log-probit analysis method (Adeyemi et al., 2010). Animals were further observed for one week for any delayed toxic effects.