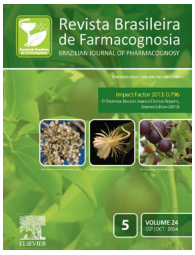
Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Original article

Safflower oil: an integrated assessment of phytochemistry, antiulcerogenic activity, and rodent and environmental toxicity

Walber Toma[a,*], *Luciana L. Guimarães*[a], *Alba R.M.S. Brito*[b], *Aldo R. Santos*[c], *Fernando S. Cortez*[c], *Fábio H. Pusceddu*[c], *Augusto Cesar*[d], *Landulfo S. Júnior*[e], *Marcos T.T. Pacheco*[a,e], *Camilo D.S. Pereira*[a,d]

[a]*Laboratório de Pesquisa em Produtos Naturais, Mestrado em Ecologia, Programa de Pós-graduação em Sustentabilidade de Ecossistemas Costeiros e Marinhos, Universidade Santa Cecília, Santos, SP, Brazil*
[b]*Laboratório de Produtos Naturais, Departamento de Fisiologia e Biofísica, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[c]*Laboratório de Ecotoxicologia, Universidade Santa Cecília, Santos, SP, Brazil*
[d]*Instituto do Mar, Campus Baixada Santista, Universidade Federal de São Paulo, Santos, SP, Brazil*
[e]*Instituto de Engenharia Biomédica, Universidade Camilo Castelo Branco, São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 5 June 2014
Accepted 18 September 2014

Keywords:
Antiulcerogenic activity
Ecotoxicology
Raman spectroscopy
Safflower oil
Toxicology

ABSTRACT

Gastric ulcers are a significant medical problem and the development of complications lead to significant mortality rates worldwide. In Brazil, *Carthamus tinctorius* L., Asteraceae, seeds essential oil, the safflower oil, is currently used as a thermogenic compound and as treatment for problems related to the cardiovascular system. In this study, by Raman spectroscopy, it was shown that oleic and linoleic acids are the compounds present in higher concentrations in the safflower oil. We demonstrated that safflower oil (750 mg/kg, *p.o.*) decrease the ulcerogenic lesions in mice after the administration of hydrochloric acid-ethanol. The gastric ulcers induced by non-steroidal anti-inflammatory drug (NSAID) in mice treated with cholinomimetics were treated with four different doses of safflower oil, of which, the dose of 187.5 mg/kg (*p.o.*) showed significant antiulcerogenic properties ($^{**}p < 0.01$). Moreover, the safflower oil at doses of 187.5 mg/kg (*i.d.*) increased the pH levels, gastric volume ($^{**}p < 0.01$) and gastric mucus production ($^{***}p < 0.001$), and decreased the total gastric acid secretion ($^{***}p < 0.001$). The acute toxicity tests showed that safflower oil (5.000 mg/kg, *p.o.*) had no effect on mortality or any other physiological parameter. Ecotoxicological tests performed using *Daphnia similis* showed an EC_{50} at 223.17 mg/l, and therefore safflower oil can be considered "non-toxic" based on the directive 93/67/EEC on risk assessment for new notified substances by European legislation. These results indicate that the antiulcer activity of Safflower oil may be due to cytoprotective effects, which serve as support for new scientific studies related to this pathology.

* *Corresponding author.*
E-mail: walbertoma@unisanta.br (W. Toma).
0102-695X/$ - see front matter © 2014 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.
http://dx.doi.org/10.1016/j.bjp.2014.09.004

Introduction

The development of peptic ulcers is one of the world's major gastro-intestinal disorders, including both gastric and duodenal ulcers, which affects 10% of the global population (Zapata-Colindres et al., 2006). The pathophysiology of peptic disease is attributed to an imbalance between aggressive factors like acid, pepsin, and an *Helicobacter* infection, and local mucosal defenses like bicarbonate, mucus and prostaglandin secretion (Jain et al., 2007). *Helicobacter pylori* infection, the use of non-steroidal anti-inflammatory drugs (NSAID), emotional stress, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer development (Malfertheiner et al., 2009).

There are several drug treatments used to treat gastric ulcer. These include gastric acid neutralizing therapeutic drugs (*e.g.* aluminum hydroxide and magnesium), H2 receptor antagonists (*e.g.* ranitidine), and proton pump inhibitors (*e.g.* omeprazole). However, despite the large range of treatments gastric ulcer recurrence has long been thought to be an unavoidable feature of peptic ulcer disease, and therefore, maintenance treatment has been necessary to prevent recurrence (Arakawa et al., 2012).

During the past decade, complementary and alternative medicines have become a topic of global importance. Current estimates suggest that in many developing countries, a large portion of the population relies heavily on traditional practitioners and medicinal plants to meet primary healthcare needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained their popularity for historical and cultural reasons (Saraf and Saraf, 2012).

Carthamus tinctorius L. is a member of the family Asteraceae, it possibly originated in Southern Asia and is known to have been cultivated in China, India, Iran and Egypt almost from prehistoric times. During the middle ages it was grown in Italy, France, and Spain, and afterwards it was introduced into North and South America from the Mediterranean region (Bae et al., 2002).

Carthamus tinctorius seeds are rich in edible oil (safflower oil), with similar content to olive, sunflower, and peanut oils (40% dry matter weight). This oil is composed of typically linoleic acid (63-72%), oleic acid (16-25%) and linolenic acid (1-6%) (Kim et al., 2000).

The oral administration of safflower oil at doses of 750 mg/kg in rodents has been proven to have thermogenic properties and may thus contribute to the treatment of obesity (Takeuchi et al., 1995). Other reports show that this oil has the capacity to relieve constipation and ease rheumatic pains, and that it has laxative and antifungal activities (Pintão and da Silva, 2008). However, despite the widespread use of safflower flowers for their pharmaceutical, cosmetic, and medicinal properties; there have been no studies regarding the use of this oil for the treatment of gastric ulcers, or tests to determine its rodent toxicity (side effects) and environmental risks of this oil.

Medicinal plant species that are being used to treat particular diseases on a large scale can have serious side effects (George, 2011). The likelihood of side effects increases when the production and sale of such products are largely uncontrolled and/or unregulated and the consumer is not adequately informed about their proper uses. Regulatory controls are therefore considered necessary to safeguard drug interactions with herbal drugs (WHO, 1997; 2002).

The monitoring of pharmaceutical compounds in environmental matrices has been addressed in several studies since the late 1990s. Drugs excreted by patients can contaminate rivers, even after treatment in wastewater-processing facilities. In addition, there is mounting evidence that effluents from pharmaceutical factories could also be carrying drugs into the rivers (Gilbert, 2012). The effects are already evident: they include the feminization of fish by residues of contraceptive pills, and the death of millions of vultures on the Indian subcontinent following the ingestion of the anti-inflammatory drug diclofenac. Antibiotic overuse has led to the emergence of resistant pathogenic bacteria in the environment, and not just in medical settings (Deplege, 2011).

Given the need for new therapeutic approaches to treat gastric ulcer with a low probability of toxicological effects; added to concerns regarding the ecological risks of chemical compounds with therapeutic properties, this study aimed to evaluate the phytochemistry, antiulcer activity, and rodent and environmental toxicity of safflower oil. Our results will serve as the basis for new pharmacological assays involving the Safflower oil treatment for the gastric ulcer diseases.

Material and methods

Drugs and reagents

Carboxymethylcellulose (CMC), lansoprazole and safflower oil from *Carthamus tinctorius* L. Asteraceae, seed were purchased from Sigma-Aldrich, USA. Safflower oil (test substance) was used at doses of 93.75; 187.5; 375 and 750 mg/kg; whereas lansoprazole (positive control) was administered at a dose of 30 mg/kg. Both substances were diluted in 0.5% CMC and the resulting solutions were administered at a concentration of 10 ml/kg by the oral route (*p.o.*).

Analysis of safflower oil by dispersive Raman spectroscopy

Safflower oil has been evaluated using dispersive Raman spectroscopy. For that, a portable near-infrared, dispersive Raman system (Dimension P-1 Raman system, Lambda Solutions, Inc., MA, USA), with an 830 nm excitation, adjustable laser of 230 mW, and spectral resolution of about 2 cm^{-1} in the range of 400 to 1800 cm^{-1} was used. The spectrometer was connected to a Raman probe (Vector probe, Lambda Solutions, Inc. MA, USA) of about 3 m long, with band pass and rejection pass filters. The 1320×100 pixel, back-thinned, deep-depleted CCD detector was cooled down (Peltier) to -75°C to decrease thermal noise. For spectral collection, the safflower oil sample was placed in an aluminum sample holder with wells of 5 mm in diameter and 100 µl capacity; then the probe was placed at a 10 mm distance perpendicular to the sample surface (probe's focal length). The sample spectra of oleic and linoleic acids (Sigma-Aldrich) were also obtained. The signal scattered by each sample was then collected by the probe and coupled to the signal port of the Raman spectrometer for dispersion and detection. The Raman signal was collected in 5 and 10 s scans