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

Original article

Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 16 December 2013
Accepted 2 April 2014

Keywords:
Carica papaya
Diabetes
Streptozotocin
Steroids
Tannins
Quinones
Alkaloids

ABSTRACT

The extraction of plant constituents is essential to isolate biologically active compounds, aimed to understand their role on the treatment of diabetes. This study was designed to explore the preliminary phytochemical and physicochemical analysis of *Carica papaya* L., Caricaceae, leaf, and further evaluation of its hypoglycemic effect on diabetic rats. *C. papaya* leaves were extracted using chloroform, *n*-hexane or ethanol. For each extract a phytochemical screening was performed. The tests were conducted in triplicate and the qualitative and quantitative determination of the various metabolites was done using analytical standards proposed by Mexican Herbal Pharmacopoeia. The chloroform extract, containing steroids and quinones as major components, was chosen to study *C. papaya* biological effects. The chloroform extract was evaporated to dryness, and doses 0, 31, 62, 125 mg/kg were orally administered in 300 µl polyethylene glycol to diabetic rats; and 0 and 62 mg/kg to non-diabetic rats. After a 20-day treatment with the chloroform extract, the animals were sacrificed and blood was obtained for biochemical studies. The main effect observed was a decrease in serum glucose, triglycerides and transaminases in diabetic rats after the administration of *C. papaya* chloroform extract. These results confirm the potential beneficial action of *C. papaya* to treat the symptoms of diabetic patients.

Introduction

Diabetes mellitus is possibly the world's largest growing metabolic disorder. Global prevalence of diabetes has dramatically continued to increase. The difficulty of managing hyperglycemia in diabetes is the most important factor in reducing the risks associated with diabetes and its complications (Polonsky, 2012). Both fasting and postprandial glucose regulation are critical to achieve a long-term proper control in diabetic patients. The number of diabetic patients is rapidly increasing, and in consequence the control of their complications is a challenge. In this regard, medicinal plant

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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.2014.07.012

extracts have an ancient background in this issue and modern medicine can gain valuable benefits from them. Phytochemicals and their derived products have been an extraordinary source of compounds with therapeutic and drug development potential (De D et al., 2012). These molecules are novel and complex structures that can be used in their original form, or can serve as lead molecules to develop derivatives with higher specificity and fewer side effects (Koehn and Carter, 2005). The World Health Organization has been particularly attentive to the potential offered by herbal medicine, the main subfield of traditional medicine practiced in different countries (WHO, 2012). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. Mexico has an extensive and diverse medicinal flora whose properties are part of the ancestral knowledge. Currently, natural compounds used as hypoglycemic agents have a strong impact on diabetic patients.

Carica papaya L., is an herbaceous plant with prominent leaves (20-60 cm long), and is a member of the Caricaceae family, indigenous to the tropical region of Mexico, Central America and northern South America. *C. papaya* is distributed throughout the tropics and subtropics where it is extensively cultivated. The characterized metabolites from the plant are chitinase, glutaminyl cyclase and cysteine endopeptidases of class-II and III from Carica latex (Azarkan et al., 2006); linalool in fruit pulp, and alkaloids such as carpaine, pseudocarpaine, dehydrocarpaine I and II (Lim, 2012); and kaempferol and quercetin (Miean and Mohamed, 2001) in the leaves.

On the other hand, there are reports that describe the therapeutic effect of *C. papaya* leaf on dengue and malaria (Ahmad et al. 2011) and as anti-inflammatory (Owoyele et al., 2008). Other reports suggest that a fermented papaya preparation significantly reduces plasma glucose levels in healthy subjects and in patients with type 2 diabetes (Danese et al., 2006). The hypoglycemic activities of *Carica papaya* have been previously described for its fruit and leaves (Aruoma et al., 2010), nevertheless, the available information regarding the leaves is incomplete (Sasidharan et al., 2011). The present study was designed to perform phytochemical and physicochemical analyses of *Carica papaya* leaves, and to evaluate its hypoglycemic effect in diabetic rats.

Materials and methods

Plant material

Leaves from *Carica papaya* L., Caricaceae, were collected from June to September 2010 from Cintalapa, in the state of Chiapas, Mexico. The plant was authenticated at the Academic Division of Biological Sciences (DACB, acronym in Spanish) in the Juarez Autonomous University of Tabasco (UJAT, initials in Spanish) as *Carica papaya*. A voucher specimen was deposited in the herbarium (No. 32307) of this institution in Tabasco, Mexico.

Chemical products

Streptozotocin (STZ) was purchased from Sigma (St Louis, MO, USA). Insulin (Humulin* N) was obtained from Sanofi Aventis. All other chemicals of analytical grade were obtained from Merck. Kits for different enzyme assays were purchased from Biosystems S.A., Mexico.

Assays

Chemical analysis and quantitative assays of alkaloids, tannins, steroids, quinones and flavonoids content in C. papaya extract

Preliminary qualitative tests were carried out to determine the metabolites present in greater proportion in the leaf of *C. papaya*. Alkaloids, flavonoids, saponins, tannins, steroids and/or terpenes (triterpenoids), and quinones were identified. A total of 30 g of dried and ground *C. papaya* leaves were placed in a 250 ml round-bottom flask, where they were macerated and extracted with hexane, chloroform and ethanol. All procedures were developed at room temperature. The extracts were used for the subsequent qualitative analysis of metabolites. Once the main secondary metabolites were determined, a quantitative assay was developed using spectroscopic techniques as described by Mexican Herbal Pharmacopoeia (FHEUM, 2001). Each assay on the hexanic, ethanolic and chloroform extracts was performed in triplicate; 10 g of dried and ground leaves of *C. papaya* were exhaustively extracted with the corresponding solvent employing a Soxhlet system. After the extraction the solvent was removed under vacuum. Steroids were quantified by the modified Lieberman-Burchard reaction (Robin, 1945) at an absorbance of 550 nm. A cholesterol standard curve (2-8 mm) was made to report the concentration of steroids (Barreto, 2005). The quantification of tannins was performed by the reaction with ferric citrate, and then absorbance was read at 525 nm. A tannic acid standard curve (0.1-0.5 mg/ml) was prepared to report the concentration of tannins (ISO, 1988). Alkaloids were quantified by the reaction with bromocresol green read at 470 nm; a calibration curve with atropine (0.1-4 mg/ml) was constructed to report the alkaloid concentration (Fazel et al., 2008). For the quantification of quinones, the reaction with ferric chloride was employed and the absorbance was measured at 390 nm. A standard curve was prepared with a solution of 8-hydroxyquinone (0.010 to 0.06 mg/ml) to evaluate the concentration of quinones (Pochapski et al., 2011).

Preparation of C. papaya leaf chloroform extract

The leaves of *C. papaya* were washed with tap water and cut into small slices. The slices were pulverized after being air-dryed. Dry samples (100 g) were placed in the Soxhlet system, where the extraction was conducted for 8 h with 500 ml of chloroform. Afterwards, the solvent was evaporated under vacuum until the extract was completely dry, and was preserved at -20°C. The chloroform extract of *C. papaya* was used to a final concentration of 1 mg/ml.

Animals

Experiments were performed on adult male Wistar rats (body weight range: 250-300 g), 10 to 11 weeks of age. Animals were