Journal of Ethnopharmacology 181 (2016) 252–262

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Isolated flavonoids from *Ficus racemosa* stem bark possess antidiabetic, hypolipidemic and protective effects in albino Wistar rats

Amit K. Keshari [a,1], Ghanendra Kumar [a,1], Priya S. Kushwaha [a], Monika Bhardwaj [a], Pranesh Kumar [a], Atul Rawat [b,c], Dinesh Kumar [b], Anand Prakash [c], Balaram Ghosh [d], Sudipta Saha [a,*]

[a] Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow 226025, India
[b] Centre of Biomedical Research (CBMR), Sanjay Gandhi P ost-Graduate Institute of Medical Sciences Campus, Raebareli Road, Lucknow 226014, Uttar Pradesh, India
[c] Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow 226025, India
[d] Department of Pharmacy, BITS-Pilani at Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, India

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form
27 January 2016
Accepted 6 February 2016
Available online 8 February 2016

Keywords:
Ficus racemosa
Flavonoids
Antidiabetic
Hypolipidemic
PPARγ
GLUT1

Chemical compounds studied in this article:
Kaempherol (PubChem CID: 5280863)
Quercetin (PubChem CID: 5280343)
Naringenin (PubChem CID: 439246)
Baicalein (PubChem CID: 5281605)

ABSTRACT

Ethnopharmacological relevance: *Ficus racemosa* (FR) has been used for thousands of years in Ayurvedic system of medicine in India and is closely associated with prevention, treatment and cure of various human ailments like obesity and diabetes. It is popularly known as gular. A vast and wide range of chemical compounds like polyphenols, friedelane-type triterpenes, norfriedelane type triterpene, eudesmane-type sesquiterpene including various glycosides had been isolated from this plant. However, no detail studies related to isolation of flavonoids has been reported previously with their antidiabetic, hypolipidemic and toxicological consequences.
Aim of the study: The present study was undertaken to evaluate antidiabetic, hypolipidemic and toxicological assessments of flavonoids isolated from *Ficus racemosa* (FR) stem bark.
Materials and methods: We isolated four flavonoids from stem bark of FR and structures were confirmed by Infrared spectroscopy (IR), Nuclear Magnetic Resonance (NMR) (both 1D and 2D), mass spectroscopy (MS). Later, these flavonoids were administered to streptozotocin (STZ) rats once in a day for a period of seven days at 100 mg/kg dose. We measured blood glucose level and body weight changes at different days (1st, 3rd, 5th and 7th days). Serum lipid profiles were also estimated to investigate the hypolipidemic potential of flavonoids in the similar experiment. Various oxidative stress parameters in pancreas and liver and hepatic biomarker enzymes in plasma were also determined to investigate the toxicity potential of isolated flavonoids. Finally, we performed docking studies to find out the mechanism of action.
Results: Our results collectively suggested that four flavonoids reduced blood glucose level and restored body weight, signifying antidiabetic action. There were reduction of other lipid profile parameters and increase of high density lipoprotein (HDL) during administration of flavonoids, also signifying hypolipidemic action. Various oxidative stress biomarkers and hepatic enzymes levels were also normalized with respect to diabetic control at the same time. Docking studies revealed that isolated flavonoids showed their antidiabetic potential via binding to PPARγ and GLUT1 receptors.
Conclusion: The isolated four flavonoids demonstrated good antidiabetic, hypolipidemic and antioxidant properties in STZ diabetic rats which supported the use of FR stem bark as useful supplementary drug for future antidiabetic therapy.

1. Introduction

Diabetes mellitus is the major health problem worldwide in both developed and developing countries, which is occurred mainly due to either insufficient insulin secretion or inadequate carbohydrate, protein and fat metabolism (American Diabetes

* Corresponding author.
E-mail addresses: Keshariamitkeshari.pharma@gmail.com (A.K. Keshari), ghanendra20@gmail.com (G. Kumar), priya.shail19@gmail.com (P.S. Kushwaha), bhardwaj.monika09@gmail.com (M. Bhardwaj), pranesh.psit@gmail.com (P. Kumar), atulcbmr@gmail.com (A. Rawat), dineshcbmr@gmail.com (D. Kumar), anandlohia@gmail.com (A. Prakash), balaram@hyderabad.bits-pilani.ac.in (B. Ghosh), sudiptapharm@gmail.com (S. Saha).
[1] These are main authors who contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2016.02.004
0378-8741/

Association, 2010). Hyperglycemia and hyperlipidemia are the common features for diabetes mellitus with alteration of glucose and lipid metabolism and modification of liver enzyme levels (Taskinen, 2002).

Type II diabetes mellitus (non-insulin-dependent diabetes mellitus) is the fourth leading cause of death in worldwide which is also responsible for disease prevalence up to 90% (Jenson et al. 1998; Moller 2004). Nowadays, synthetic drugs and insulin are used for diabetes treatment. However, these drugs have major side effects, such as drug resistance, dropsy, and weight gain (Tahrani et al., 2010). In contrary, several folk medicines (traditional) have demonstrated potential for diabetes treatment with less tolerability and side effects. Thus, the above fact warranted us to search for more natural antidiabetic agents from the traditional medicine (Tahrani et al., 2010).

Ficus racemosa (FR) (Family: Moraceae)is found mostly all parts of India, Southeast Asia and Australia (Joy et al., 2001). FR plant is used for thousands of years in Ayurvedic system of medicine in India and is very useful against obesity and diabetes.It is popularly known as gular (Joy et al., 2001). A vast and wide range of chemical compounds like polyphenols, friedelane-type triterpenes, norfriedelane type triterpene, eudesmane-type sesquiterpene including various glycosides had been isolated from this plant. FR have multi diversified pharmacological actions which include hypolipidemic, antidiabetic, antiinflammatory, antioxidant, antipyretic, antidiarroheal, antipyretic, antibacterial, antifungal, antifilarialactions (Chopra et al., 1986; Atal and Kapur, 1982). Previously, β-sitosterol isolated from leaves and stem bark which has potent antidiabetic activity (Chopra et al., 1986). Our groups also isolated three sterols from the leaves of FR and these sterols possessed antidiabetic properties in streptozotocin (STZ) induced diabetic rats (Kushwaha et al., 2015). On the other hand, previous investigation suggested that the stem bark of FR is rich with flavonoids (Choudhury and Yadhav, 2013). Flavonoids have multi diversified pharmacological responses which include anticancer, antioxidant, antihistaminic, antiinflammatory and hepatoprotective properties (Wang, 2000). Literature review demonstrated that flavonoids have dramatic role in glucose lowering properties also (Babu et al., 2013). However, detail studies have been necessary to establish their mechanism of action and side effects *in vivo*.

Again, no detailed antidiabetic studies had been previously performed with the isolated flavonoids form stem bark of FR in STZ induced diabetic rats. Therefore, the main objective was to investigate the role of isolated flavonoids from stem bark of FR in curing diabetes. To achieve this goal, four flavonoids had been isolated from FR stem bark and antidiabetic, hypolipidemic actions and toxicological responses of these materials were determined in STZ induced rats. Later, binding affinities to peroxisome proliferator activated receptor gamma ($PPAR_\gamma$) and glucose transporter-1(GLUT1) through docking studies were performed to investigate the mechanism of action of isolated materials. We hypothesized for the first time that the flavonoids produced its antidiabetic action via binding to $PPAR_\gamma$ and GLUT1.

2. Material and methods

2.1. Materials

Petroleum ether (60–80 °C), methanol, chloroform, n-butanol were purchased from SD Fine Chemicals, Mumbai, India. Pyrogallol, STZ, anthrone, guanidine hydrochloride were purchased from Loba Chemicals, New Delhi, India. Other chemicals were obtained from Himedia, Mumbai, India. All the solvents and chemicals were of analytical grades with 99% purity.

2.2. Plant materials

The fresh stem bark of FR were collected from Lucknow, Uttar Pradesh, India and authenticated by Department of Horticulture, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow. A voucher specimen has been deposited for future reference (45/SM/DAPS/BBAU/14). The plant materials (2 kg stem bark) were air dried under shade, powdered and defatted with petroleum ether (60–80 °C) using Soxhlet apparatus by successive solvent extraction method (Chakraborty et al., 2009). Later, extraction was performed using methanol:water (90:10) and the extracted samples were evaporated to dryness using rotary vacuum evaporator (IKA, Germany). The final yield was 12.3%.

2.3. Isolation of flavonoids

The presence of flavonoids in the extract was determined using Pew's test (Anwal et al., 2014). 1.0 mL of aqueous extract (5% w/v) was mixed with 0.02 g of metallic zinc and 1.5 mL concentrated sulphuric acid. The formation of red color indicated the presence of flavonoids.

60 g of extract was dissolved in water and extracted successfully with n-hexane, chloroform, ethyl acetate and n-butanol to yield n-hexane (8.5 g), chloroform (17.3 g), ethyl acetate (8.5 g) and n-butanol (10.7 g). Chloroform soluble fraction was subjected to chromatogram using silica gel (60–120 mesh) and eluted with n-hexane and ethyl acetate with various ratios of 70:30 (FR6, 305 mg, 0.508%), 50:50 (FR7, 280 mg, 0.467%) and 40:60 (FR9, 340 mg, 0.567%) and recrystalized with methanol. In another chromatography, ethyl acetate fraction was further chromatogrammed using ethyl acetate and methanol in a ratio of 90:10 to yield FR8 (275 mg, 0.458%) (Hossain and Rahman, 2015). This procedure was repeated for three times to get more flavonoids.

2.4. General experimental procedures for characterization of flavonoids

Infrared spectrum (IR) data were obtained on anNicolet™ iS™50 FT-IR Spectrometer (Waltham, MA, USA). Nuclear magnetic resonance spectroscopy (NMR) spectra were measured on Bruker 800 MHz NMR spectrometer (^{1}H 800 MHz, ^{13}C 200 MHz) NMR spectrometers (Rheinstetten, Germany)processed in Topspin-2.1. Direct-infusion mass spectroscopy (MS) data were acquired using a hybrid triple quadrupole linear ion trap MS (QTRAP MS) equipped with an electrospray ionization (ESI) source (2000 QTRAP, Applied Biosystems, Foster City, CA, USA).

2.5. Experimental animals

Albino Wistar rats (110–130 g) were used for this experiment and Institutional Animal Ethical Committee (IAEC) approved the protocol before starting the experiment (Approval no. UIP/IAEC/2014/FEB/10/R1). Rats were kept in standard temperature (25 ± 5 °C), relative humidity (55 ± 10%). They were acclimatized in laboratory condition for 7 days.

2.6. Induction of hyperglycemia

The hyperglycemic condition was developed by injecting intraperitonially single dose of STZ (50 mg/kg), dissolved in normal saline after overnight fasting. On 5th day, blood sample was collected through tail vain and glucose level was measured through one touch select Glucometer (Johnson & Johnson, India) strips. Hypoglycemic condition was considered with rats having 250 mg/dL blood glucose level (Arunachalam and Parimelazhagan, 2013).