Food and Chemical Toxicology 47 (2009) 2631–2639

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Effects of *guggulsterone* isolated from *Commiphora mukul* in high fat diet induced diabetic rats

Bhavna Sharma [a], Rajani Salunke [a], Swati Srivastava [a], Chandrajeetbalo Majumder [b], Partha Roy [a,*]

[a] Molecular Endocrinology Laboratory, Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee 247 667, Uttarakhand, India
[b] Fluid Particle Research Laboratory, Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee 247 667, Uttarakhand, India

ARTICLE INFO

Article history:
Received 20 April 2009
Accepted 21 July 2009

Keywords:
Commiphora mukul
Guggulsterone
Adipocytes
High fat diet
Serum lipids

ABSTRACT

Sedentary lifestyle, consumption of energy-rich diet, obesity and longer lifespan are some of the major reasons for the rise of metabolic disorders like type II diabetes, obesity, hypertension and dyslipidemia among people of various age groups. High fat diet induced diabetic rodent models resembling type II diabetic condition in human population were used to assess the anti-diabetic and hypolipidemic activity of *guggulsterone* (isolated from *Commiphora mukul* resin). Four groups of rats were fed high fat diet, for 16 weeks. On feeding the normal rats with fat rich diet they showed increased serum glucose, cholesterol and triglyceride levels along with increase in insulin resistance significantly ($p < 0.05$) in comparison to control animals. Different biochemical parameters like GTT, glycogen content, glucose homeostatic enzymes (like glucose-6-phosphatase, hexokinase), insulin release in vivo and expression profiles of various genes involved in carbohydrate and lipid metabolism clearly demonstrated the hypoglycemic effect of this extract. *Guggulsterone* demonstrated a differential effect with a significantly improved PPARγ expression and activity in in vivo and in vitro conditions, respectively. However, it inhibited 3T3-L1 pre-adipocytes differentiation in vitro. The results presented here suggest that the *guggulsterone* has both hypoglycemic and hypolipidemic effect which can help to cure type II diabetes.

1. Introduction

Diabetes is defined as a state in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose level. Diabetes is the world's largest endocrine disorder and is one of the major killers in recent times (Bhat et al., 2008). According to World Health Organization (WHO), the world wide global population is in the midst of a diabetes epidemic with people in Southeast Asia and Western Pacific being mostly at risk. The number of cases for diabetes which is currently at 171 million is predicted to reach 366 million by the end of 2030 (WHO, 2006). Therefore, it is necessary to search for new drugs and interventions that can be used to manage this metabolic disorder. The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (type 2).

Plants have always been an exemplary source of drugs and many currently available drugs have been derived directly or indirectly from them. India is a country with a vast reserve of natural resources and has a rich history of traditional medicines (Grover et al., 2002). In spite of having enormous potential to cure many diseases, traditional medicines could not achieve the popularity among the scientific community due to the lack of authentication, identification and qualitative as well as quantitative standardization of these medicines. At the same time, the presence of heavy metal contaminants and undesired compounds which impose hazardous effects on the physiological system makes the issue more complicated (Samy et al., 2008). The only way to surmount these problems is to purify the medicinal products so as to eliminate any kind of detrimental components before using them in herbal preparations, which is described as "*shodhan*" in ancient medicinal literature (Tiwari, 2005). This can be achieved following a sequential activity based analysis along with the identification as well as standardization of active principles using certain modern analytical procedures.

The strategy stated above was adopted to study the anti-diabetic potential of a well known herbal resin known as gum resin, gum guggul or oleo resin from *Commiphora mukul* (Family: Burseraceae); commonly known as "guggul". The gum resin has been used in Ayurvedic medicine for centuries to treat a variety of ailments, including obesity, bone fractures, arthritis, inflammation, cardiovascular disease, and lipid disorders (Satyavati, 1991; Sinal and Gonzalez, 2002; Urizar and Moore, 2003).

Two stereoisomers, *E*- and *Z*-*guggulsterone* (*cis*- and *trans*-4,17(20)-pregnadiene-3,16-dione, respectively), are the most

* Corresponding author. Tel.: +91 1332 285686; fax: +91 1332 273560.
E-mail address: paroyfbs@iitr.ernet.in (P. Roy).

0278-6915/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2009.07.021

important constituents studied in detail for their therapeutic potential from *C. mukul* (Fig. 1). Various studies have been conducted to understand and illustrate the mechanism of action and potential of *guggulsterone* as a therapeutic agent using synthetic *E* and *Z* isomers (Urizar and Moore, 2003; Burris et al., 2005; Ding and Staudinger, 2005; Ichikawa and Aggarwal, 2006). Although numerous reports are available on the hypolipidemic nature and its plausible relation with type II diabetes treatment, but in most of the cases in place of natural *guggulsterone* their synthetic counterparts have been used. The synthetic products mainly consist of either *E* or *Z* forms instead of the combination of them, as they exist naturally in guggul lipids along with other components as mentioned and recommended in traditional Ayurvedic literature.

The aim of the present study was to understand the probable mechanism of the hypoglycemic and hypolipidemic nature of the *guggulsterone E/Z* rich purified fraction from *C. mukul* in type 2 diabetic rat models. Glucose tolerance test (GTT), lipid profile, in vivo insulin secretion, glycogen content and enzyme activities were analyzed to evaluate its effect on biochemical parameters. Further its actions at cellular and molecular levels were analyzed by determining the expression patterns of various target genes like glucokinase (GK), glucose-6-phosphatase (G6Pase), phosphoenol pyruvate carboxykinase (PEPCK), glucose transporter-4 (Glut-4) and aldose reductase (AR) in different target tissues like liver, muscle, adipocyte and kidney. On the basis of results obtained from a broad range of experiments it is evident that *guggulsterone E/Z* is not only effective as lipid lowering agent but at the same time has the ability to combat different targets of insulin resistance and type 2 diabetes. Elucidation of exact pathway at the molecular level is the next target in order to establish *guggulsterone* as an effective medicine for the treatment of diabetes.

2. Materials and methods

2.1. Plant material

The guggul resin was purchased from a local vendor of Roorkee, India. The plant material was identified as per the literature of Ayurveda and by local experts of herbal gardens and further confirmed by Dr. H.S. Dhaliwal, Professor of Plant Biotechnology, Indian Institute of Technology, Roorkee.

2.2. Purification of guggulsterone

Guggulsterone was purified following the method of Bajaj and Dev (1982) with slight modifications as per our laboratory conditions. Briefly, the resin was soaked in ethyl acetate (EtOAc) at room temperature for 24 h with continuous stirring and the filtrate was then concentrated using rotavapour. Concentrated EtOAc extract was then further washed with 3 N hydrogen chloride and 10% sodium bicarbonate to get a neutral fraction. Neutral fraction was washed thrice with brine and again concentrated on rotatory vacuum evaporator and a dark brown gummy neutral fraction was obtained. Mixture of the earlier obtained neutral fraction along with 10% semicarbazide on silica and toluene were stirred and heated at 60–65 °C for 14 h. The mixture was then cooled at room temperature and filtered. Silica was then washed with toluene thrice and refluxed with 10% oxalic acid and toluene for 2.5 h and then filtered. Silica gel was then extracted several times with EtOAc and the combined extracts were then washed with water and brine followed by removal of the solvent to collect the required ketonic fraction.

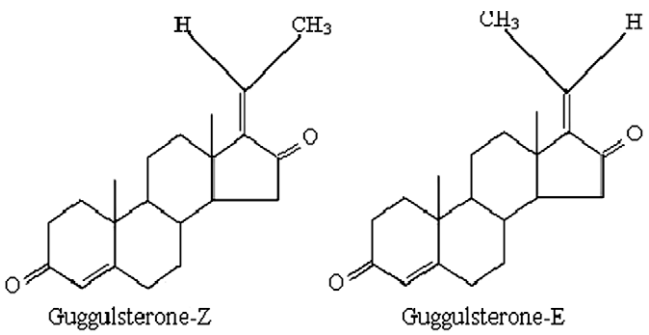

Fig. 1. Isomers of *guggulsterone*.

For silica gel column chromatography, silica gel (mesh size 150–200) was packed in a glass column and equilibrated with n-butanol. Concentrated ketonic fraction was loaded on the column. The column was then initially washed with *n*-butanol, followed by elution with increasing amount of EtOAc in benzene (C_6H_6). Fractions rich in *guggulsterone E* and *Z* were then eluted with 15–25% EtOAc in C_6H_6. The extract was then further concentrated, freed of solvent and stored at −20 °C prior to use. Purity of the phytochemical was checked by HPLC using nova pack column (150 mm × 4.6 mm, i.d., 5-μm particle) (Waters, USA). The HPLC system consisted of Agilent (Palo Alto, CA, USA) G1312A binary gradient pump with dual λ absorbance detector. For elution, methanol and milli Q water was used in 46:54 ratio at a flow rate of 1.2 ml/min and UV detection at 245 nm.

2.3. Animal groups and treatment

All the experiments were performed as per the guidelines of the Institutional Animal Ethics Committee (registration number: 563/02/a/CPCSEA) and had the prior approval of the project through the same committee. Experiments were carried out on pathogen-free male albino Wistar rats, *Rattus norvegicus*, of age group 3–4 weeks, purchased from the Animal House facility of All Indian Institute of Medical Sciences, New Delhi, India. They were housed in a well-ventilated animal house in polypropylene cages bedded with sterilized rice husks with 12 h light:12 h dark schedule. The animals were fed *ad libitum* with a balanced animal pellet diet (Ashirwad Animal Feed Industries, Punjab, India) or High Fat diet prepared in house according to DIO (Diet Induced Obesity) Diets, New Brunswick, NJ, USA, specifications for High Fat Diet (HFD, D12451) wherein Lard was used as the fat source and the diet contained approximately 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate. Briefly per Kg of the diet was composed of casein, 200 g; corn starch, 73 g; sucrose, 173 g; malto dextrin,100 g; cellulose, 50 g; soybean oil, 25 g; lard, 177.6 g; L-cystine, 3 g; mineral mix (S10026), 10 g; di-calcium phosphate, 13 g; calcium carbonate, 5.5 g; potassium citrate mono-hydrate, 16.5 g; vitamin mix (V10001), 10 g; choline bitartarate, 2 g. The animals had access to normal drinking water at all the times.

The animals were randomly divided into four groups ($n = 8$) as given below:

Group I – Normal pellet diet fed (control).
Group II – High fat diet fed rats (diabetic).
Group III – High fat diet fed rats treated with 75 mg/kg bw *guggulsterone E/Z* (treated).
Group IV – High fat diet fed rats treated with 20 mg/kg bw Metformin (metformin).

Rats from groups II, III and IV were fed with modified high fat diet for 16 weeks and rats form group I were fed normal pellet diet. On completion of 16 weeks, the rats from groups III an IV were gavaged with *guggulsterone* and metformin, respectively, for another 8 weeks. Rats from groups I and II received normal saline as vehicle control.

2.4. Effects on fasting blood glucose (FBG) and glucose tolerance test (GTT)

FBG and GTT were determined after 8 weeks of treatment with *guggulsterone*. On completion of the 8 weeks of treatment, GTT was performed by oral administration of glucose load of 1 g/kg bw in 0.1 ml water to overnight-fasted animals. Blood samples were collected from the tail vein at 30, 60, 90, 120 and 150 min after the oral glucose load and the blood glucose was measured using GOD–POD glucose estimation kit (Excel Diagnostics Pvt. Ltd., Mumbai, India). The results were expressed initially in terms of milligrams per deciliter of blood. Time-averaged mean glucose responses was also computed as positive incremental areas under the curves (AUC) above the baseline values using the trapezoidal rule, divided by the time interval (h) expressed as mg/dl.2.5 h as described earlier (Wolever et al., 1991; Purves, 1992; Allison et al., 1995; Potteiger et al., 2002). Positive incremental areas were considered so as to ignore the negative area i.e., the area below baseline if postprandial glucose values fall below basal fasting glucose (Wolever et al., 1991).

2.5. Estimation of plasma insulin

In order to analyze the effect of *guggulsterone* on insulin release, on completion of the treatment, plasma insulin levels were measured in all the groups of animals using enzyme immunoassay kits according to the manufacturer's instructions (Cayman Chemical, USA).

2.6. Estimation of lipid profile in blood samples

On completion of the treatment, blood samples were collected from all the four groups of animals and total cholesterol (TC), high density lipoprotein (HDL) cholesterol and triglyceride (TG) levels in plasma were determined using commercially available kits according to the instructions of the manufacturer (Transasia Bio Med-