Journal of Ethnopharmacology 142 (2012) 331–336

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Polyphenols in madhumega chooranam, a Siddha medicine, ameliorates carbohydrate metabolism and oxidative stress in type II diabetic rats

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ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form
12 March 2012
Accepted 3 April 2012
Available online 23 May 2012

Keywords:
Madhumega chooranam
Polyphenolic
Anti-diabetic
Carbohydrate metabolism
Oxidative stress
GLUT 4

ABSTRACT

Ethnopharmacological relevance: Present study was undertaken to demonstrate the mode of anti-diabetic action of a polyherbal Siddha Medicine, Madhumega chooranam (MMC).
Materials and methods: MMC was fractionated into phenolic (PMMC) and non-phenolic (NPMMC) portions in order to identify bioactive fraction. Study was performed in type II diabetic rats. Role of PMMC and NPMMC on liver glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucokinase and glycogen content were determined. Their role on superoxide dismutase, reduced glutathione and lipid peroxidation were investigated. In addition, their effects on GLUT4 and PPARγ gene expression were studied. Pancreas and liver histopathology was studied using hematoxylin and eosin stain.
Results: PMMC improved carbohydrate metabolism by decreasing glucose-6-phosphatase and fructose-1,6-bisphosphatase and increasing glucokinase and glycogen contents in diabetic rats liver. It alleviated oxidative stress by increasing superoxide dismutase, glutathione and decreasing lipid peroxidation content. PMMC up-regulated liver GLUT4 and PPARγ mRNA expression in comparison to the vehicle or NPMMC rats.
Conclusion: Madhumega chooranam mediates its anti-diabetic action through the inhibition of gluconeogenesis and activation of glycolytic pathways in type II diabetic rats. Increased GLUT4 and PPARγ expressions provide additional information on its glucose uptake/sensitising and hypolipidemic potential. Phenolic components of MMC were found to be the bioactive principles.
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1. Introduction

According to International Diabetes Federation, there were 40 million diabetics in India in 2007 and this number is predicted to rise to almost 70 million by 2025. Countries with largest number of diabetics will be India, China and USA by 2030 (King et al., 1998), which is alarming and needs immediate attention for the development of safer therapeutic regimens. World over there is a resurgence of traditional medical systems, based on the holistic nature of their approach to healing.

Siddha system of medicine is one of the major traditional medical systems of India, which is prevalent mainly in Tamilnadu. In Siddha, diabetes mellitus (DM) is termed as "Madhumegam" (Madhu means "sweet" and megam means "excessive urination"). Madhumega chooranam (MMC), a polyherbal Siddha medicine, is in vogue for the treatment of DM for more than five decades in Tamilnadu. It contains crude powders of *Muraya koeenigi*, *Terminalia chebula*, *Phyllanthus emblica*, *Tinospora cardifolia*, *Syzgium cumini*, *Cyprus rotundus* and *Phyllanthus amarus* which were mixed in equal parts. Literary evidences on the therapeutic

Abbreviations: MMC—Madhumega Chooranam, PMMC—phenolic portion of Madhumega Chooranam; NPMMC—non-phenolic portion of Madhumega Chooranam, OECD—Organisation for Economic and Cooperation Development; PG—Plasma glucose, PTC—Plasma Total; PTG—Plasma Triglycerides cholesterol, PI—Plasma Insulin; SOD—Superoxide dismutase, GSH—reduced glutathione; LPO—lipid peroxidation, GLUT4—Glucose transporter 4; PPARγ—Peroxisome Proliferator—Activated Receptor γ, STZ—Streptozotocin; CM—Carboxyl methyl cellulose, TCA—Trichloro acetic acid; ANSA—Ammonium molybdate, 1-amino 2-naphthol 4-sulphonic acid, BSA—Bovine serum albumin; PMS—Phenazonium methosulphate, NBT—Nitro blue tetrazolium chloride; NADH—Nicotinamide adenine dinucleotide reduced, DTNB—5,5′-dithiobis 2-nitro benzoic acid; BHT—butylated hydroxy toluene, TBA—2-thiobarbituric acid; SSA—5-sulphosalicylic acid, IAEC—Institutional Animal Ethical Committee; LD_{50}—Lethal Dose 50, HPTLC—High Performance Thin Layer Chromatography; NPD—Normal pellet diet, HFD—High fat diet; RT-PCR—Reverse Transcriptase-Polymerase Chain Reaction, ROS—Reactive Oxygen Species

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http://dx.doi.org/10.1016/j.jep.2012.04.003

benefits of MMC reveal it has no or lesser side effects. Present study was undertaken to investigate role of MMC on carbohydrate metabolism and oxidative stress in a rat model of type II diabetes and also to identify its bioactive principles.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid and streptozotocin were purchased from Sigma-Aldrich chemical (USA). CMC, glucose-6-phosphate, TCA, ANSA, fructose 1,6-bisphosphate, BSA, PMS, NBT, NADH, GSH, DTNB, BHT, sulphanilamide, o-phosphoric acid, napthyl ethylene diamine dihydrochloride were purchased from M/s. SISCO Research Laboratories, Mumbai, India. Tetra sodium pyrophosphate, TBA, SSA were supplied by M/s. Himedia laboratories, Mumbai, India. Glucose, triglycerides and cholesterol kits were procured from M/s. Accurex Biomedical Pvt. Ltd., India. Rat insulin ELISA kit was purchased from Crystal Chem Inc., USA. All other chemicals and reagents used were of analytical grade.

2.2. Animals and husbandry

Female Sprague Dawley rats weighing 120–130 g (initial weight) were housed in groups (3–5 animals/cage) in polypropylene cages in a well ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of 23 ± 2 °C and 40%–65% relative humidity, with a 12 h light/dark artificial light cycle. They were provided with rodent feed (M/s. Provimi Animal Nutrition India Pvt. Ltd, Bengaluru) and purified water ad libitum, prior to dietary manipulation. Guidelines of "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India approved the study (IAEC/XVIII/SRU/130/2010).

2.3. Madhumega chooranam

Madhumega chooranam was procured commercially from M/s. Arogya Healthcare Pvt. Ltd., Chennai and stored according to the manufacturer's instruction (at room temperature).

2.4. Preparation of phenolic and non-phenolic fractions

Phenolic fraction was separated by refluxing MMC in 80% ethanol (pH 4.0) for 30 min in a reflux condenser. The solution was cooled and centrifuged at 3500 rpm for 10 min. Supernatant containing phenolic (PMMC) and the residue which contains non-phenolic (NPMMC) components were concentrated separately and stored at room temperature. Chemical stability of PMMC and NPMMC was ensured by periodical HPTLC analysis (once in 30 days for 3 months; data not shown).

2.5. Standardisation and acute oral toxicity of MMC, PMMC and NPMMC

MMC was subjected to preliminary phytochemical, tannins, flavonoids and total phenols analysis. Total phenolic content in PMMC and NPMMC was determined by Folin–Ciocalteu method (McDonald et al., 2001). MMC was standardised for gallic acid content by HPTLC (Sawant et al., 2010). Acute oral toxicity test of MMC, PMMC and NPMMC was performed following OECD 423 guideline.

2.6. Induction of type II diabetes in rats

Type II diabetes was induced by following Srinivasan et al. (2005) method with minor modifications. Rats were allocated to two dietary regimens consisting of 10 and 80 animals by feeding either normal pellet diet (NPD) or high fat diet (HFD) ad libitum, respectively, for a period of four weeks. After 4 weeks of dietary manipulation, HFD rats with increased PTC ($\sim$3 fold) and PTG ($\sim$4 fold) levels were demarcated as hyperlipidemic and were injected with low dose (35 mg/kg, i.p) of streptozotocin. NPD animals were injected with citrate buffer (1 ml/kg, i.p). 72 h after STZ injection, rats with fasting glucose level ≥ 250 mg/dl were selected for study. Animals were kept on their respective diet till the end of the study.

2.6.1. Groups and treatment

Following confirmation of diabetes, rats were randomized into 7 groups of 6 in each group and scheduled to treatment protocol for 21 days. Weekly body weight, PG, PTC, PTG and PI were determined in the experimental rats.

- Group-1: Normoglycemic (0.3% CMC as vehicle; 5 ml/kg/day, p.o)
- Group-2: Hyperglycemic (0.3% CMC as vehicle; 5 ml/kg/day, p.o)
- Group-3: PMMC (50 mg/kg/day, p.o)
- Group-4: PMMC (100 mg/kg/day p.o)
- Group-5: NPMMC (50 mg/kg/day, p.o)
- Group-6: NPMMC (100 mg/kg/day, p.o)
- Group-7: Metformin (250 mg/kg, p.o)

2.6.2. Plasma clinical chemistry

PG, PTC and PTG were analysed using commercial diagnostic kits (M/s. Accurex diagnostic kit, India) in a semi-automated biochemical analyser (Star 21^{Plus}, India). PI was measured using rat insulin kit (Crystal Chem Inc., USA) in a Multiskan® Spectrum, Thermo Scientific, USA.

2.6.3. Key markers of carbohydrate metabolism

Glucose-6-Phosphatase (Swanson, 1955); Fructose 1, 6-bisphosphatase (Gancedo and Gancedo, 1971); Glucokinase (Newgard et al., 1983) and Glycogen content (Sadasivam and Manickam, 1996) were estimated in liver tissues.

2.6.4. Key markers of oxidative stress

Superoxide dismutase (Kakkar et al., 1984); reduced glutathione (Jollow et al., 1974); lipid peroxidation content (Ohkawa et al., 1979) and total protein (Lowry et al., 1951) were measured in liver tissues.

2.6.5. Gene expression of GLUT4 and PPARγ by reverse transcriptase PCR

GLUT4 and PPARγ mRNA expression were studied in liver tissues. Primers sequence used were as follows. PPAR-γ: sense, 5′–CAT GCT TGT GAA GGA TGC AAG–3′; antisense, 5′–TTC TGA AAC CGA CAG TAC TGA CAT–3′. GLUT4: sense, 5′–GGA GGT GAA ACC CAG TAC AGA ACT–3′; antisense 5′–GGT GGC TCT CCC ACC ATT TT–3′ and β-actin: sense, 5′–TGC TGT CCC TGT ATG CCT CT–3′; antisense, 5′–AGG TCT TTA CGG ATG TCA ACG–3′. RT-PCR was carried out as described earlier (Hall et al., 1998).

2.6.6. Histopathology

At the end of treatment, animals were euthanized to collect liver and pancreas. Organs were blotted and freed from blood, fixed in 10% neutral buffered formalin for 48 h, trimmed and

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