Phytomedicine 23 (2016) 989–997

Contents lists available at ScienceDirect

Phytomedicine

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

Original Article

Rottlerin, a polyphenolic compound from the fruits of *Mallotus phillipensis* (Lam.) Müll.Arg., impedes oxalate/calcium oxalate induced pathways of oxidative stress in male wistar rats

Nirlep Chhiber[a], Tanzeer Kaur[b], Surinder Singla[a,*]

[a] Department of Biochemistry, Panjab University, Chandigarh-160015, India
[b] Department of Biophysics, Panjab University, Chandigarh-160015, India

ARTICLE INFO

Article history:
Received 15 February 2016
Revised 2 June 2016
Accepted 8 June 2016

Keywords:
Hyperoxaluria
PKCδ
Rottlerin
NADPH oxidase
Renal function

ABSTRACT

Background: Oxalate and/or calcium oxalate, is known to induce free radical production, subsequently leading to renal epithelial injury. Oxidative stress and mitochondrial dysfunction have emerged as new targets for managing oxalate induced renal injury.
Hypothesis: Plant products and antioxidants have gained tremendous attention in the prevention of lithiatic disease. Rottlerin, a polyphenolic compound from the fruits of *Mallotus phillipensis* (Lam.) Müll.Arg., has shown free radical scavenging, antioxidant activity and has been reported to interfere in signaling pathways leading to inflammation and apoptosis. In this study, the potential role of rottlerin, in rats exposed to hyperoxaluric environment was explored.
Methods: Hyperoxaluria was induced by administering 0.4% ethylene glycol and 1% ammonium chloride in drinking water to male wistar rats for 9 days. Rottlerin was administered intraperitoneally at 1 mg/kg/day along with the hyperoxaluric agent. Prophylactic efficacy of rottlerin to diminish hyperoxaluria induced renal dysfunctionality and crystal load was examined along with its effect on free radicals generating pathways in hyperoxaluric rats.
Results: 0.4% ethylene glycol and 1% ammonium chloride led to induction of hyperoxaluria, oxiadtive stress and mitochondrial damage in rats. Rottlerin treatment reduced NADPH oxidase activity, prevented mitochondrial dysfunction and maintained antioxidant environment. It also refurbished renal functioning, tissue integrity and diminished urinary crystal load in hyperoxaluric rats treated with rottlerin.
Conclusions: Thus, the present investigation suggests that rottlerin evidently reduced hyperoxaluric consequences and the probable mechanism of action of this drug could be attributed to its ability to quench free radicals by itself and interrupting signaling pathways involved in pathogenesis of stone formation.
© 2016 Elsevier GmbH. All rights reserved.

Introduction

Renal stones are believed to be a form of pathological calcification, which is triggered by reactive oxygen species (ROS) and development of oxidative stress (Khan, 2014). High oxalate, as well as crystals of calcium oxalate and calcium phosphate generates ROS, leading to injury, inflammation, alteration in gene expression and mitochondrial dysfunction in renal epithelial cells (Jonassen et al., 2005). There is sufficient clinical and experimental data to support that ROS is involved in the formation of calcium oxalate kidney stones (Khan, 2014). In case of calcium oxalate nephrolithiasis, an increased production of a variety of crystallization modulating macromolecules takes place. Presence of such macromolecules and ROS induced damage to proteins, lipids, carbohydrates and nucleotides, lead to renal injury and inflammation during stone formation (Khan, 2004).

The main contributors of ROS at cellular levels are mitochondria and NADPH oxidase (Meimaridou et al., 2006; Khan et al., 2010). NADPH oxidase is considered as a major source of ROS in kidneys (Geiszt et al., 2000). Oxalate and/or CaOx crystals induced ROS in renal tissue is also primarily produced by NADPH oxidase (Joshi et al., 2013) and mitochondria (Khand et al., 2002). In the view of oxidative imbalance caused by calcium oxalate crystals, a number of studies have pointed out the potential role of antioxidants towards its management. Since, targeting the contributors of ROS is

Abbreviation: ROS, reactive oxygen species; CaOx, calcium oxalate; PKCδ, protein kinase C δ; EG, ethylene glycol; ALP, Alkaline phosphatase; LDH, lactate dehydrogenase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; ETC, electron transport chain; GSH, reduced glutathione; GSSG, oxidised glutathione.

* Corresponding author.
E-mail address: singlask6@gmail.com (S. Singla).

http://dx.doi.org/10.1016/j.phymed.2016.06.005
0944-7113/© 2016 Elsevier GmbH. All rights reserved.

the best strategy, therefore many studies have explored potential of apocynin (inhibitor of NADPH oxidase) (Li et al., 2009) and N-acetyl cysteine (Sharma et al., 2015) to impart protection against hyperoxaluria. Recently PKCδ activation is also suggested to be an important contributor of ROS and rottlerin being its direct/indirect inhibitor showed to protect LLC-PK1 cells from oxalate induced oxidative stress (Thamilselvan et al., 2009).

The plant *Mallotus phillipensis* (Lam.) Müll.Arg. is well recognised to be an antiurolithiatic plant and is being traditionally used in kidney stone treatment in Indian folklore (Gillespie and Stapleton, 2004). In a study by Mohandas et al. (2015), the vidangadi churna, which contains *Mallotus phillipensis* (Lam.) Müll.Arg. as one of its constituents, has shown to possess significant antiurolithiatic property. Rottlerin is the major phytochemical isolated from the fruit coverings of this plant and has shown prophylactic properties in other pathological conditions (Maioli et al., 2012). Although initially suggested as PKCδ inhibitor, lately it was revealed that rottlerin is a mitochondrial uncoupler as it depolarizes the mitochondrial membrane potential and reduces cellular ATP levels. Being an uncoupler of mitochondria, rottlerin affects ROS production and interestingly it has both pro-apoptotic and anti-apoptotic abilities (Soltoff, 2007). Moreover, rottlerin inhibits ROS synthesis imposed by various NADPH oxidase isoforms irrespective of directly blocking PKCδ activity (Jagnandan et al., 2007). The antioxidant potential of rottlerin was established in MCF-7 cells as a result of its hydrogen donating ability and free radical scavenger activity (Maioli et al., 2009). Being itself an antioxidant, it is imperative to explore the effect of rottlerin in animals exposed to hyperoxaluria. The present study was designed to investigate the efficacy of rottlerin in combating oxalate induced renal injury and pathways of ROS generation in rats.

Materials and methods

Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, USA), Merck (Mumbai, India) and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Rottlerin (Pubchem CID: 5281847) was purchased from Merck Biosciences (Germany). Primary antibody for PKCδ was purchased from Sigma-Aldrich (St. Louis, MO).

In-vitro redox ability assay

Total redox capability by Fe^{3+}-Fe^{2+} transformation at different concentrations of rottlerin and hydrogen peroxide scavenging capacity of rottlerin was measured (Aggarwal et al., 2014). Briefly, the ability to reduce potassium ferricyanide [$K_3Fe(CN)_6$] was measured at 700 nm. Higher absorbance at 700 nm of the reaction mixture indicated greater reducing power. The hydrogen peroxide scavenging ability was estimated by measuring hydrogen peroxide concentration at 230 nm using the molar extinction coefficient for H_2O_2 (81 $mol^{-1}cm^{-1}$). The hydrogen peroxide scavenging ability was calculated by the formula: % scavenging $= (1–A_e/A_o) \times 100$, where A_o = absorbance without sample, and A_e = absorbance with sample. As a positive control, ascorbic acid was used.

Animals and treatment schedule

Healthy male wistar rats weighing between 150 and 200 g of equivalent age groups were obtained from central animal house of Panjab University, Chandigarh, India. The procedures followed were approved by the Institutional Animal Ethics Committee and were in accordance with the Guidelines for Humane Use and Care of Laboratory Animals (PU/IAEC/S/14/41).

To induce CaOx crystal formation, rats were exposed to 0.4% ethylene glycol (EG) with 1.0% ammonium chloride (NH_4Cl) in drinking water for 9 days. All rats were randomly divided into the groups having 5–7 rats each. Normal **(NRM)** rats were provided with standard animal feed and water ad libitum for 9 days. Hyperoxaluric group **(HYO)** of rats were given 0.4% EG (v/v) with 1.0% NH_4Cl (w/v) in drinking water for 9 days. Rottlerin treated **(HYR1)** rats were administered an intraperitoneal dose of 1 mg/kg/day in addition to hyperoxaluric dose of 0.4% EG with 1.0% NH_4Cl in their drinking water for 9 days. **ROT1** group rats were given intraperitoneal dose of 1 mg/kg/day alone for 9 days. The standardization of the hyperoxaluric rat model was already done in the lab from previous studies (Aggarwal et al., 2014).

Sample collection

At the end of treatment period, rats were placed in metabolic cages and urine was collected for 24 h period having 20 μl of 20% sodium azide as preservative. A drop of freshly obtained urine was spread on a glass slide and visualized under polarized light using Leica DM 3000 light microscope. Rats were anaesthetized with diethyl ether and sacrificed by decapitation on day 10. Before sacrificing, the blood was taken from orbital sinus into a centrifuge tube to collect serum. After dissection both kidneys were removed and transverse sections were fixed in formaldehyde for histological analysis. The paraffin embedded sections were cut and stained in Delafield's Hematoxylin and eosin staining and viewed using Leica DM 3000 light microscope.

Biochemical assays in urine and serum

Urinary oxalate level was quantified by the colorimetric method (Hodgkinsons and Williams, 1972). Concentration of creatinine was estimated by commercially available kit using manufacturer's instructions (Erba diagnostics Mannheim, Germany). Alkaline phosphatase (ALP) in serum was determined using commercially available kit (Recombigen laboratories). Urinary lactate dehydrogenase (LDH) was measured by decrease in absorbance at 340 nm resulting from the oxidation of NADH. Creatinine clearance was calculated according to standard clearance formula $C = U/S \times V$, where U = urinary concentration of creatinine, S = concentration of creatinine in the serum and V = urinary volume.

Isolation of mitochondria

The kidney was washed in normal saline at 4 °C, trimmed of adipose and connective tissues, weighed, and homogenized, (10% w/v) in buffer containing 0.25 M sucrose, 5 mM HEPES, 1 mM EDTA, and 0.1% bovine serum albumin pH 7.2. The homogenate was centrifuged at 1000 x *g* for 5 min to remove the nuclear fraction and cell debris. Mitochondrial pellet was obtained by centrifuging the post-nuclear supernatant at 14,000 x *g* for 20 min. The pellet was washed thrice with 1.15% potassium chloride solution and finally suspended in 0.25 M sucrose solution. The purity of mitochondrial preparation was checked by measuring the activity of citrate synthase (Spinazzi et al., 2012).

Measurement of oxidant/antioxidant status in renal tissue and renal mitochondria

Oxidant/antioxidant status in whole renal tissue was determined by assaying malondialdehyde (MDA) content, superoxide dismutase (SOD), catalase (CAT) and redox ratio; and in renal mitochondria by measuring redox ratio, glutathione peroxidase (GPx) and glutathione reductase (GR) by the methods as described previously (Veena et al., 2008; Aggarwal et al., 2014)

Download English Version:

https://daneshyari.com/en/article/2496281

Download Persian Version:

https://daneshyari.com/article/2496281

Daneshyari.com