Acta Histochemica 118 (2016) 152–163

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Mallotus roxburghianus modulates antioxidant responses in pancreas of diabetic rats

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ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form
15 December 2015
Accepted 17 December 2015

Keywords:
Mallotus roxburghianus
Diabetes
Antioxidant enzymes
PCNA
Visfatin
Histopathology

ABSTRACT

Mallotus roxburghianus has long been used by Mizo tribal people for the treatment of diabetes. Scientific validation at known doses may provide information about its safety and efficacy. Methanolic leaf extract of *M. roxburghianus* (MRME 100 and 400 mg/kg) was tested in comparison with normal and alloxan diabetic rats for 28 days p.o. in terms of body and pancreatic weight, blood glucose level, antioxidant enzymes, expression of visfatin and PCNA, histopathology and histomorphometric measurements of pancreas. The results were evaluated statistically using ANOVA, correlation and regression and Principal component analysis (PCO). MRME (100 and 400 mg/kg) treatment significantly ($p < 0.0001$) decreased the body weight, blood glucose level, improved the mass and size of pancreas, elevated the levels of antioxidant enzymes and up regulate the expression of visfatin and PCNA. PCO analysis was good to fitness and prediction distinguishes the therapeutic effects of *M. roxburghianus* from the alloxan induced diabetic rats. MRME has significant role in protecting animals from alloxan-induced diabetic oxidative stress in pancreas and exhibited promising antihyperglycaemic and antioxidant activities along with significant reversal of disturbed antioxidant status and lipid peroxidative damage. Pancreatic architecture and physiology under diabetic oxidative stress have been significantly modulated by MRME and validated as a drug candidate for antidiabetic treatment. *M. roxburghianus* treatment restores the antioxidant enzyme system and rejuvenates the islets mass in alloxanized rat by accelerating visfatin and PCNA expression in pancreatic tissue.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors. Hyperglycaemia caused by damaged β-cells of the pancreas (Type I DM) or resistance to the action of insulin at the cellular level (Type II DM), or possibly a combination of both factors (American Diabetes Association, 2009). The global prevalence of diabetes was estimated to be 9% (387 million people) among adults, 175 million cases undiagnosed and the intensity may set to rise beyond 592 million by 2035 and in India more than 66 million people currently diagnosed with the disease (International Diabetes Federation, 2014). All types of DM are on the increase and one fifth of all adults with diabetes in the world live in the South-east Asia (Central Intelligence Agency, 2013).

Nowadays research focus has been emphasized more towards the discovery of anti-diabetic drugs from plant sources that have many beneficial effects than oral hypoglycaemic agents (Grover et al., 2002a). In the developing countries more than 80% of the people are relied on medicinal plants for diabetes (WHO, 2008). The use of medicinal plants for the management of DM has also been authenticated by world health organization and traditional medicine strategy (2014–2030) has been restructured recently for the promotion of safe and effective use of medicinal plants (WHO, 2013). More than 1000 medicinal plants have been reported for the treatment of DM but only 115 plants are supported by *in vitro* or *in vivo* experimental evidence (Ezuruike and Prieto, 2014). Taking all this information into account, further research is required to validate the anti-diabetic effects of medicinal plants that are being used at the regional level.

Hypoglycemic potential of medicinal plants has been examined in alloxan induced diabetic animals which is a cost effective method and frequently used appropriate model worldwide (Frode and Medeiros, 2008). Alloxan (1,3-diazinane-2,4,5,6-tetrone) is a toxic glucose analogue selectively destroys β-cells in the pancreas generating reactive oxygen species (ROS) causing Type I DM (Szkudelski,

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http://dx.doi.org/10.1016/j.acthis.2015.12.007
0065-1281/

2001). Scientific reports indicate that diabetic condition is associated with surplus production of free radicals and accumulation of lipid peroxidation by-products which play a relevant role in the etiology and pathogenesis of both experimental and human diabetes mellitus (Soto et al., 2004). High blood glucose levels (BGLs) not only enhance the production of ROS but also affect antioxidant activities of ROS scavenging enzymes (Palanduz et al., 2001). Free radicals are formed excessively during diabetic condition by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (Maritim et al., 2003). It is well known that pancreatic β-cells are very sensitive to oxidative stress, which is accelerated by glucolipotoxicity in the diabetic state (Kaneto et al., 1999; Robertson, 2004). Visfatin, an adipokine molecule is shown to be involved in multiple aspects of pancreatic β-cell biology, including a role in the modulation of both insulin sensitivity and β-cell function and regulation of insulin secretion and receptor signaling (Chen et al., 2006; Li et al., 2006; Revollo et al., 2007; Brown et al., 2010). Proliferative cell nuclear antigen (PCNA), marker of cell proliferation, used to detect the number of proliferating cells in pancreatic β-cell and PCNA is decreased during diabetic condition (Kanda et al., 2010).

Several *Mallotus* species exist, which often exhibit different pharmaceutical activities (Nguyen Hoai et al., 2009). Leaf and twig decoction of *Mallotus roxburghianus* Muell., (MR) a shrub belonging to Euphorbiaceae family, is being used by the tribal people of Mizoram, India for the treatment of DM. This medicinal plant is locally known as Zawngtenawhlung, widely distributed in the eastern Himalayan region of Bangladesh, Myanmar and Northeast India especially in Mizoram (Lalhlenmawia et al., 2007). MRME is also claimed to have antidiabetic activity by local healers in the South-East Asia (Nguyen Hoai et al., 2009). The earlier studies on methanolic extract of *M. roxburghianus* (MRME) have shown its non-toxic nature, antioxidant activity and antidiabetic potential in diabetic rats (Rana et al., 2005; Lalhlenmawia et al., 2007). Its major constituents including two phenolic compounds (betulinic acid, bergenin) have been isolated and characterized (Rana et al., 2005; Roy et al., 2015). Though MR has antioxidant and antihyperglycaemic potential, an in depth study of its physiological and molecular mechanisms are yet to be investigated. Considering the antioxidant effect of MR, we hypothesize that MR can act as a protectant against alloxan-induced pancreatic oxidative damage. The present study was designed to evaluate anti-lipid peroxidative and antioxidant effects of MRME in pancreas of alloxan induced diabetic rats.

2. Materials and methods

2.1. Collection of plant material

Fresh mature leaves of MR were collected from Ngopa village, Champai district, Mizoram in September, 2014. The land accessed for the collection of plant materials is privately owned and all necessary permits were obtained from the landlord. The plant specimen was identified and authenticated by the Department of Forestry, Mizoram University, Aizawl, Mizoram, India. A voucher specimen (MZU/ZOO/4568) was deposited in the Department of Zoology, Mizoram University, Aizawl, Mizoram.

2.2. Preparation of methanolic plant extract

After the collection the plant leaves were shade dried at room temperature (28 ± 2 °C) for 28 days. The dried leaves were powdered in a mixer grinder and kept in an air tight plastic container prior to the extraction. Cold percolation procedure was followed for the preparation of methanolic extract of mallotus plant [20]. Dried leaf powder (3 kg) was placed in a rectangular polypropylene container and macerated with 6 L of methanol (99.8%, Merck grade) for 7 days. The container was sealed tightly with cello tape and kept in the shaker incubator (model: SLM-INC-OS-250, Shalimar Specialty Chemical Ltd., Bangalore, Karnataka, India) at 28 ± 2 °C and 500 rpm. After filtering the extract twice through Whatman No.1 filter paper, the solvent was removed using a rotary evaporator (Buchi, Germany) under reduced pressure at 40 °C for 4 h. The percentage yield residue after extraction with methanol was 23.9 g w/w) and was stored at 4 °C in an airtight bottle until further use.

2.3. Experimental animals

Forty two adult Wistar albino adult rats (3 months old, normoglycemic) weighing 160 ± 20 g were used for this study. The animals were bred in polypropylene cages (47 × 34 × 20 cm) and pathogen free condition in the Animal Care Facility at the Department of Zoology, Mizoram University, Aizawl, Mizoram, India. They were maintained under environmentally controlled conditions (temperature: 25 ± 2 °C; light/dark cycle: 12:12 h) with free access to water and food *ad libitum* (standard pellet diet; Pranav Agro Industries, Maharashtra, India). All the ethical protocols and guidelines for animal handling and treatment were followed and approved by the Committee on the Ethics of Animal Experiments of the Mizoram University Animal Ethical Committee (MZUAEC), Mizoram University, Aizawl, Mizoram, India (Permit Number: MZU/IAEC/14-15/12).

2.4. Induction of diabetes by alloxan

DM was induced by a single intraperitoneal injection of freshly prepared alloxan (cat# 52011; SD Fine Chemicals, Kolkata, West Bengal, India) at a dose of 150 mg/kg of body weight freshly dissolved in distilled water to overnight fasted rats ($n = 7$ per treatment group) (Ryle et al., 1984; Gurusubramanian and Roy, 2014). Control rats were injected with saline only. Eight days after alloxan treatment, DM induction in the experimental animals was confirmed by measuring blood glucose level (BGL) in blood samples from the tail vein of overnight fasted rats. BGL was measured on 0, 7, 14, 21 and 28 days by One Touch uses glucose oxidase test strips (Life Scan Johnson and Johnson, Mumbai, Maharashtra, India). The blood glucose level was >250 mg/dL considered to be diabetic and those rats were included in the experiment. This day was considered the first day of the experiment.

2.5. Experimental design

After acclimatization, 42 rats (14 normal and 28 alloxan-induced diabetic) were used. Rats were weighed and divided in six groups of seven rats each as follows: (i) Control group (C), normal rats treated with distilled water, (ii) MR control group (M), normal rats treated with MRME 400 mg/kg, p.o., (iii) Alloxan group (A), diabetic control rats treated with distilled water, (iv) MRME 100-alloxan group (M100), diabetic rats treated with MRME 100 mg/kg, p.o., (v) MRME400-alloxan group (M400), diabetic rats treated with MRME 400 mg/kg, p.o., and (vi) glibenclamide-alloxan Group (G), diabetic rats treated with glibenclamide 0.1 mg/kg, p.o., (Daonil, 5 mg TAB, Sanofi Aventis Pharma India). Treatment with MRME extract and glibenclamide was administered every 24 h by oral gavage to the test rats for a total of 28 days.

Fasting BGLs of blood samples were measured from the tail vein of overnight fasted rats. Before alloxan treatment the body weight and BGL levels ranged between 166.52–169.88 g and 91.84–99.25 mg/dL, respectively (Table 1). After 8 days of alloxan treatment, rats exhibiting BGL of >350 mg/dL were considered to be diabetic and were included in the present study. This day was considered the first day of the experiment. Rats from each treatment