Journal of Traditional and Complementary Medicine xxx (2017) 1–6

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Co-administration effects of aqueous extract of turnip leaf and metformin in diabetic rats

Moammadmehdi Hassanzadeh-Taheri [a], Mohammad Hassanpour-Fard [b], Mohammadreza Doostabadi [a], Hesam Moodi [a], Khadijeh Vazifeshenas-Darmiyan [c], Mehran Hosseini [d, *]

[a] Department of Anatomy, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[b] Department of Pharmacology, Faculty of Medicine, Berberis & Jujube Research Center, Birjand University of Medical Sciences, Birjand, Iran
[c] Department of Clinical Biochemistry, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[d] Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form
17 May 2017
Accepted 18 May 2017
Available online xxx

Keywords:
Turnip
Brassica rapa
Metformin
Interaction
Liver
Rat

ABSTRACT

Background: There is a variety of experimentally proven medicinal plants having antidiabetic properties but data on herb-drug interaction are very limited. Earlier studies indicated that aqueous extract of turnip leaf (AETL) has hypoglycemic potential in diabetic animals. The present study was conducted to evaluate co-administration effects of AETL and metformin, a commonly used antidiabetic drug, in diabetic rats.

Methods: Metformin at the two different doses (50,100 mg/kg) and AETL at the dose of 400 mg/kg (separately or concurrent with metformin) were orally given to streptozotocin-induced diabetic rats for 4 weeks daily. Fasting blood glucose (FBG) was measured at the times 0, 7, 14, 21 and 28 days after investigation. At the end of study, liver enzymes activity [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] as well as liver histopathology were evaluated.

Results: Both treatments could significantly decrease FBG levels when they administrated separately. Interestingly, co-administration of AETL and metformin in a dose dependent manner significantly improved hypoglycemic activity of metformin. While neither metformin nor AETL could ameliorate liver alterations alone, but in concomitant therapy they efficiently attenuated liver enzymes elevation and histological damages.

Conclusion: The results of the present study demonstrate that combination of metformin with AETL enhance the prior effectiveness and reduced the latter adverse effects by a synergistic interaction.

1. Introduction

Nowadays, due to the global rise in the aging population and obesity, the incidence and prevalence of diabetes are increasing. Since 1980, the number of diabetic patients has quadrupled in the world.[1] According to recent WHO report, in 2014, 422 million people with diabetes were estimated and the prevalence of the disease will steadily increase everywhere, particularly in the middle-income countries.[2] Based on cost evaluation from a recent systematic review study, it has been projected that the direct cost of diabetes to the world is more than US$ 827 billion, annually.[3]

There are many herbal medicines suggesting for the treatment of diabetes and some of them have been approved in experimental studies.[4,5] Lots of people thought that herbal products are inherently safe, owing to their natural origin not based on experimentally approved evidence.[6] Moreover, despite the fact that there are limited studies in the field of herb-drug interactions, some of patients prefer to use conventional drugs with herbal medicine, concurrently.[7,8] In fact, concomitant use of drug and herbal medicine may sometimes produce interaction effects. Interactions between herbs and drugs may increase or decrease the

* Corresponding author. Department of Public Health, Deputy of Research and Technology, Birjand University of Medical Sciences, Birjand, Iran. Fax: +98 56 32433004.
E-mail address: mehranhosseiny@yahoo.co.in (M. Hosseini).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2017.05.010

Please cite this article in press as: Hassanzadeh-Taheri M, et al., Co-administration effects of aqueous extract of turnip leaf and metformin in diabetic rats, Journal of Traditional and Complementary Medicine (2017), http://dx.doi.org/10.1016/j.jtcme.2017.05.010

pharmacological or toxicological effects of either components.[9] Therefore, it is imperative to promote credible research on safety and possibility interaction of herbal medicine with synthetic drugs.

Brassica rapa (turnip) has been cultivated and consumed for many centuries across Europe and expanding eventually to central and East of Asia.[10] The results of our previous studies showed that turnip root had hypolipidemic potential and its leaf exerted hypoglycemic and renoprotective efficiencies in diabetic rats.[11–14] On the other hand, metformin is a widely consumed oral antdiabetic drug in the world.[15] Evidence shows it is most prescribed antidiabetic drug amongst hospital prescriptions. Moreover, metformin plays a pivotal role in most of two-drug, three-drug and four-drug combination therapy in diabetic patients.[16] Metformin toxicity is rare, but in all reported cases it was used concurrently with other drugs or herbs.[17] It should be noted that metformin also plays a pivotal roles in most of marketed combination antidiabetic drugs like Glucovance, Metaglip, Benformin, Calformin etc.[18] Hence, metformin was used as a reference antidiabetic drug to study its concurrent effects with turnip leaf extract in diabetic rats.

2. Objective

As the medicinal plants with antidiabetic properties are widely use separately or in combination with conventional drugs among diabetic patients, this study aimed to evaluate the effects of co-administration of aqueous extract of turnip leaves (AETL) and metformin on blood glucose and liver function markers in diabetic rats.

3. Material and methods

3.1. Plant collection and extract preparation

The turnips (*Brassica rapa*) were collected during December 2011 from Birjand, South Khorasan province, Iran. The plant was identified by an expert botanist, and a voucher specimen (221) kept in the herbarium of Agricultural Faculty of Birjand University, Birjand, Iran.

Brassica rapa leaves were dried in shade and at the room temperature, then milled by an electric grinder. The powder was macerated in distilled water 1:10 (w/v) on a magnetic stirrer for 2 days at room temperature. Afterwards, the mixture was passed through filter paper (Blue Ribbon, Grade 589, Germany), and concentrated under vacuum evaporator. Subsequently, lyophilized by freeze dryer (Dena Vacuum Industry, model FD-5005-BT, Iran) to produce aqueous extract. The extraction yield was 15.7%.

3.2. Animals

Male Wistar albino rats, weighting 180–220 g procured from the Research Centre of Experimental Medicine at Birjand University of Medical Sciences, Brjand, Iran. The rats were maintained in controlled environment (12 h light/dark cycles and 21–25 °C temperature) and fed with standard laboratory animal pellet diet (Javanneh-Khorasan co, Iran) as well as tap water, *ad libitum*. The experimental procedures used in the present study were approved by the Ethic Committee of Laboratory Animals at Birjand University of Medical Sciences, Birjand, Iran.

3.3. Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemicals Company (St. Louis, MO, USA) and metformin tablets from Merck Sante' S.A.S.(Lyon, France). The STZ and metformin were freshly dissolved in citrate buffer, pH 4.5 or saline 0.9% for intraperitoneal (IP) and oral administration, respectively.

3.4. Induction and assessment of diabetes

The overnight fasted rats were made diabetic with a single IP injection of 45 mg/kg STZ[19] and control normal rats (NC group, n = 8) received vehicle (citrate buffer). Diabetes was confirmed in animals by measuring the fasting blood glucose (FBG) levels three days after the STZ injection. The rats with FBG levels above 350 mg/dl were considered as severe diabetic and used in this study.[20]

3.5. Experimental design

Based on previous studies, aqueous extract of turnip leaves (AETL) at the dose of 400 mg/kg, as an effective dose, was used in this study.[11,13] After two weeks of diabetes confirmation, the animals were randomly divided into six equal groups (n = 8). Group 1 received saline as diabetic model (DM), groups 2 and 3 received metformin at the doses of 50 mg/kg (MET50) and 100 mg/kg (MET100) respectively, group 4 treated with 400 mg/kg AETL alone (AETL400), groups 5 and 6 received 400 mg/kg AETL plus metformin at the dose of either 50 mg/kg or 100 mg/kg, respectively (AETL + MET50 or AETL+100 MET). Moreover, 8 healthy rats allocated as normal control (CON) group and treated with saline 0.9 %.

Drugs were dissolved in saline (0.9%) solution and orally administrated to the animals using intragastric tube for a period of four weeks. The same volume of saline (0.9%) was administrated to the NC group.

3.6. Measurement of blood glucose and biochemical liver function markers

Weekly measurement of FBG was done using a commercially available digital glucometer (Accu Chek, Germany). At the end of the experimental period, after 14 h fasting and under anesthesia with ketamine:xylazine (65:10 mg/kg), the blood collection was performed from the animals' heart. Plasma was collected to assess the liver function by measuring plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The AST and ALT levels were determined by the automatic biochemistry analyzer (Roche Hitachi 912, Japan) and commercially available kits (Pars Azmoon, Iran).

3.7. Histopathology

Following the anesthesia and blood collection, the animals' livers were removed and fixed in 10% formaldehyde in phosphate buffered saline (0.01 M). Tissue samples of the liver were processed for paraffin-embedding and serial sections were made for staining with hematoxilin and eosin dyes. For each rat, three random sections were analyzed under a light microscope (UPLAN FI, Japan). They were evaluated according to a check list semi quantitatively for the degree of histopathological changes (Table 2).

3.8. Statistical analysis

The data of FBG and liver enzymes concentrations were presented as means ± SD. Results were analyzed using one-way ANOVA, followed by Dunnett's post-hoc test to determine significant differences among the means. The level of statistical significance was set at $P < 0.05$. SPSS 22 for Windows was used to perform the total statistical analysis.

Please cite this article in press as: Hassanzadeh-Taheri M, et al., Co-administration effects of aqueous extract of turnip leaf and metformin in diabetic rats, Journal of Traditional and Complementary Medicine (2017), http://dx.doi.org/10.1016/j.jtcme.2017.05.010