Biomedicine & Pharmacotherapy 86 (2017) 127–135

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Lithium induced, oxidative stress and related damages in testes and heart in male rats: The protective effects of *Malva sylvestris* extract

Anouar Ben Saad[a,b,*], Ilhem Rjeibi[a], Hichem Alimi[a], Sana Ncib[d], Amani Smida[a,b], Nacim Zouari[c], Lazhar Zourgui[a,c]

[a] Research of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa, 2112, University of Gafsa, Tunisie
[b] Laboratory of Active Biomolecules Valorisation, Higher Institute of Applied Biology of Medenine, University of Gabes, 4119 Medenine, Tunisie
[c] Higher Institute of Applied Biology ISBAM Medenine, 4119, University of Gabes, Tunisie
[d] Unit of Common Services, Faculty of Sciences Gafsa, 2112, University of Gafsa, Tunisie

ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form 27 November 2016
Accepted 1 December 2016

Keywords:
Lithium toxicity
Malva sylvestris
Antioxidant
Oxidative stress
Polysaccharide

ABSTRACT

Malva sylvestris is widely used in Mediterranean and European traditional medicine and ethnoveterinary for the treatment of various diseases. This study, carried out on male Wistar rats, evaluates the beneficial effects of *Malva sylvestris* extract upon lithium carbonate-induced damages in testes and heart. For this purpose, *Malva sylvestris* extract at a dose of 0.2 g/kg was orally administrated, followed by 25 mg/kg lithium carbonate (intraperitoneal injection, twice daily). Lithium carbonate treatment significantly ($p < 0.01$) decreased the weight of testes, accessory sex organ and heart, sperm count and motility, and serum testosterone level. In addition, exposure to lithium carbonate significantly ($p < 0.01$) increased lipid peroxidation level (LPO) and decreased superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in testes and heart. Treatment with *M. sylvestris* extract affords substantial protection in testes and heart by altering all the parameters to near normal levels that were further confirmed by histological examination. The beneficial effect of *M. Sylvestris* extract in several organs could be attributed to the interaction of antioxidant components, such as complex polysaccharides, as confirmed by phytochemical analysis.

1. Introduction

Lithium has been used in medicine for more then sixty years. As its beneficial effect has been revealed in the cases of psychiatric and neurological diseases [1,2]. Prolonged treatment with therapeutic levels of lithium causes toxic side effects [3]. The most important ones include disturbances of the heart, liver, kidney, testes, glands and gastrointestinal system functions [4–6]. In the past few years, much interest has been centered on the role of naturally occurring dietary substances for the control and management of various chronic diseases [7,8]. With this background, it is important to find a suitable preventive/therapeutic agent for lithium treatment, which could be cheap, easily available, effective in low doses, less toxic, with a convenient mode of administration and having a high content of powerful antioxidant compounds that could prove beneficial in chelating lithium from the intracellular and extracellular sites. The use of medicinal herbs and plants has proved successful different organs against oxidative stress in various experimental models [9]. *Malva sylvestris* L. (Malvaceae), usually known as common mallow, is an annual plant with shallowly lobed leaves and purple flowers which bloom in late spring. This plant is native to Europe, North Africa and Southwest Asia. It is widely used in traditional medicine for the treatment of various ailments, including abdominal disorders, bacterial infections, cough, skin diseases, and gastric ulcers [10]. Many pharmacological activities were reported of *M. sylvestris* extract including anti-inflammatory [11], antidiabtic [12], antioxidant [13], hepatoprotective and nephroprotective [9,14]. This plant is sources of diverse secondary metabolites, including, terpenoids, flavonoids, tannins and other phenolic compounds [15–17]. These points made us interested to do research on *M. sylvestris* against lithium carbonate-induced toxicity. However, no studies have been performed to study whether *M. sylvestris* have beneficial effects on lithium carbonate-induced toxicity of organ injury. Therefore, the present paper describes the ameliorative effects of a *M. sylvestris* extract on lithium carbonate-induced oxidative stress in rat testes

* Corresponding author at: Anouar Ben Saad, Research of Macromolecular Biochemistry an Genetics, Faculty of Sciences of Gafsa, University of Gafsa, 2112, Tunisia.
E-mail address: anouarsaad75@gmail.com (A.B. Saad).

http://dx.doi.org/10.1016/j.biopha.2016.12.004
0753-3322/

and heart. Examined parameters were: (1) testes, genital tract (seminal vesicles, epididymis, prostate) and heart weight, (2) spermatozoa count and motility and serum testosterone level, (3) lipids peroxidation level (LPO) and (4) antioxidant enzymes (catalase, superoxide-dismutase and glutathione peroxidase) activities in testes and heart. It is also of interest to study the chemical compounds and the *in vitro* antioxidants activity of the *M. sylvestris* extract.

2. Materials and methods

2.1. Plant material

The leaves of *M. sylvestris* were collected from rural areas in the region of Gafsa located in the south west of Tunisia. A voucher specimen (MSE 0730) was identified by a botanist, Dr, Lefi El Kadri, at the Faculty of Sciences of Gafsa, Tunisia. The plant leaves were cleaned under shade, grounded to a fine powder and extracted (30 g) with 500 ml of methanol (50%), for 24 h at room temperature with magnetic stirring. The extract was centrifuged at 4500*g* for 10 min and lyophilized [18]. The yielded 19.02 g of green residue was stored at −21 °C until use. The yielded MS is totally soluble when dissolved in distilled water and gives a slightly viscous solution.

2.2. Metal (Fe^{2+}) chelating activity M. sylvestris extract

The metal (Fe^{2+}) chelating of *M. sylvestris* extract was measured as previously described [19]. Metal (Fe^{2+}) chelating activity was presented by IC_{50} value, defined as the extract concentration (mg/ml) required to chelate 50% of ferrous iron present in the test solution. Therefore, IC_{50} values were calculated from the graph of chelating percentages against extract concentration. Lower IC_{50} values reflected better chelating activity. Ethylenediamine tetra-acetic acid (EDTA) was used as positive control for metal chelating activity. All determinations were carried out in triplicate.

2.3. β-carotene/linoleic acid assay

The method described by Koleva et al. [20] was used with a slight modification. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 μl of linoleic acid and 200 mg Tween 40. Then, 100 ml of distilled water was added and the resulting mixture was vigorously stirred. Aliquots (2.5 ml) of the β-carotene/linoleic acid emulsion were transferred to test tubes containing different extract concentrations diluted in methanol and the systems were incubated for 2 h at 50 °C. The same procedure was repeated with BHA used as positive standard. Finally, absorbance's of mixtures was measured at 470 nm and the relative antioxidant capacities (AA) was calculated according to the formula:

$$AA\% = [1-(A_0-A_t)/A_{00}-A_{0t}] \times 100$$

Were A_0 is the absorbance at the beginning of the incubation with the extract; A_t is the absorbance after incubation with the extract; A_{00} is the absorbance at the beginning of the incubation without the extract and A_{0t} is the absorbance after incubation without the extract. Tests were carried out in triplicate. Extract concentration providing 50% inhibition (IC_{50}) was obtained plotting inhibition percentage versus extract solutions concentrations.

2.4. Extraction of MS polysaccharides

Powdered fenugreek seeds (5 g) were dissolved in distilled water (50 ml) and heated at 80 °C under magnetic stirring for 3 h. The whole extract was filtered and centrifuged at 12000*g* for 15 min at 4 °C. The obtained supernatant was overnight precipitated at 4 °C by adding ethanol (four times the volume of extract solution), followed by centrifugation at 4500*g* for 10 min. The precipitate was dissolved in distilled water (20 ml) and deproteinized by Sevag reagent (chloroform/butanol 4:1, v/v) as described by Navarini et al. [21]. The resulting aqueous fraction was again precipitated by ethanol as described above. After centrifugation, the precipitate was washed with anhydrous ethanol, dissolved in distilled water and extensively dialyzed against double-distilled water for three days. The purified *M. sylvestris* polysaccharides (MS) were then precipitated by adding ethanol and lyophilized.

2.5. Fourier transformation infra red (FT-IR) spectral analysis of polysaccharides

The IR spectrum reveals structural information about major and minor constituents. Dried powder of MS (10 mg) was encapsulated in 100 mg of KBr pellet in order to prepare translucent sample disks. Each powdered sample was loaded in FTIR spectroscope (Shimadzu, FTIR-8400S) with a scan range from 4000 to 500 cm^{-1} and a resolution of 6 cm^{-1}.

2.6. Experimental design

24 male Wistar rats from the local Central Pharmacy, Tunisia, weighing 120 ± 10 g were used in this study. The animals were put in metabolic cages and kept at a temperature 23 ± 2 °C, a humidity of 55 ± 5% and a circadian rhythm (12 h light/dark cycle). The animals were fed commercial tablets (Industrielle de Concentrés SICO; Sfax, Tunisia) and tap water ad libitum. The experimental protocols were according to the guide of care and use of laboratory animals used by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics. After the adaptation period, animals were divided into 4 groups of 6 rats each and treated as follows (Fig. 1):

Group (C): Control rats received distilled water (0.5 ml/100 g of b.w.) (i.p).

Group (Li): rats administered intraperitoneally (i.p.) with 25 mg/kg of b.w of lithium carbonate twice daily for 30 days [18].

Group (MS): rats given MS at 0.2 g/kg of b.w for 60 days and then injected with distilled water (0.5 ml/100 g b.w; i.p) during the last 30 days of MS treatment.

Group (MS + Li): rats given MS at 0.2 g/kg of b.w. for 60 days and then injected with lithium carbonate at a dose 25 mg/kg of b.w. (i.p.) during the last 30 days of MS treatment (Fig. 1). After the end of 60 days experimental period, the animals were sacrificed by decapitation in order to minimize the handling stress. The blood serum was obtained by centrifugation (1,500*g*, 15 min, 4 °C) and the testes, prostate, seminal vesicles, epididymis and heart, cleaned and weighed. All these samples were stored at −80 °C until use. About 1 g of testis or heart was sliced into small pieces and homogenized into a 2-ml ice-cold lysis buffer [tris-buffered saline (TBS), pH 7.4], the mixtures were homogenized on ice using an ultra-turrax homogenizer for 15 min, then filtered and centrifuged (5000*g*, 30 min, 4 °C). Supernatants (S1) were collected and stored at −80 °C until use.

2.7. Bench work

About 1 g of testis or heart was sliced into small pieces and homogenized into a 2-ml ice-cold lysis buffer [tris-buffered saline (TBS), pH 7.4], the mixtures were homogenized on ice using an ultra-turrax homogenizer for 15 min, then filtered and centrifuged (5000*g*, 30 min, 4 °C). Supernatants (S1) were collected and stored at −80 °C until use. Sperm motility was assessed by methods