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Original article

Protective effects of phloridzin against methotrexate-induced liver toxicity in rats

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A R T I C L E I N F O

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A B S T R A C T

Background: Liver is the largest internal organ concerning with metabolism, hormonal balance and clarifying of the toxins. One of the main complications of methotrexate (MTX) therapy was the hepatic injury.
Objective: This study was conducted to elucidate the possible protective effects of phloridzin (PHL) against MTX-induced hepatotoxicity as compared to standard agent N-acetylcysteine (NAC).
Materials and methods: Rats were randomly divided into a normal control group, a respective group (PHL 40 mg/kg/day orally (p.o.) for 10 consecutive days), a hepatotoxicity control group (MTX 20 mg/kg, i.p., once), and three treated groups received NAC (150 mg/kg/day; a reference standard), PHL (40 mg/kg/day) and PHL (80 mg/kg/day) p.o. for 10 consecutive days, at the end of the day 3 of the experiment rats were administered MTX. Assessed biomarkers included serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) as liver function parameters, serum tumor necrosis factor-α (TNF-α) and cyclooxygenase-II (COX-II), as inflammatory biomarkers, hepatic total antioxidant capacity (TAC), thiobarbituric acid reactive substances (TBARS), glutathione reduced (GSH), nitrite (NO_2^-), catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) as oxidative stress biomarkers. Furthermore, hepatic caspase-3 expression was assessed. Biochemical and molecular estimations reinforced by histopathological findings.
Results: Rats pre-treated with PHL significantly reduced hepatic injury, evidenced by significant reductions in ALT, AST and LDH, TNF-α and COX-II levels, significant reductions in hepatic NO_2^- and TBARS levels, and significant elevations in hepatic TAC, GSH, GST, CAT and SOD levels. Additionally, downregulation of hepatic caspase-3 expression. Finally, histopathological results consistent with our previous findings.
Conclusion: PHL protects against hepatic injury in rats mainly through mitigation of oxidative stress, inflammation and apoptosis in hepatic tissues and may be promising to alleviate and early treatment of MTX-induced hepatotoxicity in man.

1. Introduction

Liver is the main vital organ in the body. However, the drug-induced hepatic injury could be classified as a major problem that challenges the course of the drug therapy and limits its beneficial role [1]. Drugs-induced liver injury through different pathways comprises an immunological reaction, direct toxic effect, and active metabolite formation [2]. Methotrexate (MTX), is a folic acid antagonist widely used for treatment and prophylaxis of several disorders, including autoimmune diseases, malignant tumours, and inflammatory disorders [3,4]. The molecular mechanism by which MTX induces hepatotoxicity is not fully understood, but clinical and experimental studies suggest oxidative stress-mediated injury has a role in this toxicity, mainly by depleting folate species and this enhances several biochemical pathway alterations, including purine metabolism [5]. These biochemical alterations have been mainly reported for both the therapeutic and the toxic effects of MTX mainly through the propagation of the oxidative stress and initiation of inflammatory pathways [6,7]. Furthermore, a reduction in an intracellular NADPH levels, which in consequence depletes reduced glutathione (GSH), which is an important cytosolic antioxidant agent [8]. Finally, MTX disrupts oxidant/antioxidant status and these effects can be attenuated by treatment with scavengers of reactive oxygen species (ROS). It is reported that this approach has promise for preventing and early treatment of MTX-induced liver damage [9].

Phloridzin (PHL) is a dihydrochalcone glycoside, which is a kind of flavonoid glycoside mainly found in apples and strawberries with a chemical structure shown in Fig. 1 [10]. PHL is a competitive inhibitor

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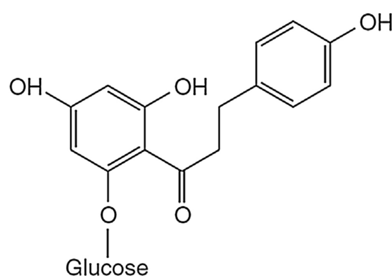

Fig. 1. Chemical structure of PHL [10].

of sodium-glucose linked transporters (SGLTs) [11], a potent antioxidant [12], anti-inflammatory [13], inhibition of platelet activation [14] and recently used in digestive diseases as liver disorders [15].

N-acetylcysteine (NAC) is a well-documented as a powerful antioxidant, anti-inflammatory, hepatoprotective, sulfhydryl group donor and a precursor for GSH synthesis [16]. NAC plays a vital role as cell survival promotion, downregulation of apoptosis and scavenging of ROS [17]. NAC is used clinically in the treatment of cancer [18], heart disease [19], colitis [20] and pancreatitis [21].

Based on the previous background, the aim of this experimental study was to evaluate the possible hepatoprotective effects of PHL against MTX-induced hepatotoxicity when compared to standard agent NAC.

2. Materials and methods

2.1. Animals

Sixty-six healthy male Swiss albino rats (weighing 200 ± 10 g) were obtained from the laboratory animal colony (Faculty of Medicine, Assiut University, Egypt). Rats were divided into 12 cages at a regulated environment (12-h dark/light cycle, 22 ± 2 °C temperature and 50 ± 5% humidity). Rats were fed with standard diet (El-Nasr Company, Abou Zaabal, Cairo, Egypt) with free access to water ad libitum. Experimental ethics and procedure in accordance with the international ethical guidelines for animal care and approved by the ethical committee, Faculty of Pharmacy, Minia University, Egypt.

2.2. Drugs, chemicals and reagent kits

Methotrexate was purchased from T3A Company (Cairo, Egypt), phloridzin dihydrate was purchased from Sigma-Aldrich chemical company (St Louis, MO, USA). NAC was obtained from SEDICO (6th October, Giza, Egypt). Thiobarbituric acid, GSH, Pyrogallol, N-(1-Naphthyl) ethylenediamine dihydrochloride and 5,5'-dithio-bis-(2- nitrobenzoic acid; DTNB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were obtained from local sources with highest analytical grade.

2.3. Experimental design

After the acclimatization period, sixty-six male Swiss albino rats were divided into six groups; 11 rats in each group as follows:

1. Group I: control group which received normal saline only.
2. Group II: Administered PHL 40 mg/kg/day, p.o., for 10 consecutive days, at the end of the 3rd day, rats were injected with normal saline i.p. once.
3. Group III: Injected i.p. once with MTX 20 mg/kg bw [22].
4. Group IV: Administered NAC 150 mg/kg/day, p.o., for 10 consecutive days, at the end of the 3rd day, rats were injected i.p. once with MTX 20 mg/kg bw [23].
5. Group V: Administered PHL 40 mg/kg/day, p.o., for 10 consecutive days, at the end of the 3rd day, rats were injected i.p. once with MTX 20 mg/kg bw [15].
6. Group VI: Administered PHL 80 mg/kg/day, p.o., for 10 consecutive days, at the end of the 3rd day, rats were injected i.p. once with MTX 20 mg/kg bw [15].

2.4. Methods

2.4.1. Induction of liver toxicity

Administration of single dose of methotrexate to all groups (except control and PHL40) for developing liver injury was done. Each animal received 20 mg/kg methotrexate in normal saline i.p. once [22].

2.4.2. Serum preparation

At the end of the experiment, rats were anasthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) by i.p. injection [24], blood samples were withdrawn by a direct cardiac puncture. Sera were separated and immediately stored at −40 °C till the time of assay.

2.4.3. Preparation of tissue homogenate

Liver of each animal was excised being careful to remove adhering fat and connective tissues and washed in ice-cold isotonic saline and divided into three portions. The first portion was stored in 10% neutral buffered formalin solution and was subjected to histopathological examinations. The second portion was homogenized (20%) with (Cole-Parmer instrument company, USA) in cold phosphate buffered saline (PBS). Tissue homogenates were centrifuged at 3000 rpm for 15 min at 4 °C. The supernatant was collected, divided into aliquots and stored at − 70 °C for evaluation of oxidative stress parameters. The third portion was frozen in liquid nitrogen and was stored separately at −70 °C for subsequent reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

2.4.4. Assessment of biochemical parameters

Using commercially available kits, ALT, AST were analyzed according to the method described by Reitman and Frankel [25]. LDH was analyzed according to the method described by Izquierdo and Dias [26]. Serum and tissue total proteins were determined according to the method described by lowery et al. [27]. Serum COX-II level was determined using ELISA kit according to the manufacturing instruction based on the principle described by Van Weemen and Schuurs [28]. Serum TNF-α was determined using ELISA kit according to the manufacturing instruction based on the principle described by Wolters et al. [29]. Hepatic GSH was assayed according to the method described by Ellman [30]. Hepatic TBARS were assayed according to the method described by Uchiyama and Mihara [31]. NO_2^- was assayed according to the method described by Montgomery and Dymock [32]. Hepatic TAC was assayed according to the method described by Koracevic et al. [33]. Antioxidant enzyme GST, CAT and SOD were assayed according to the methods described by Keen et al. [34], Claiborne [35] and Marklund [36] respectively.

2.4.5. Determination of hepatic Caspase-3 expression level by reverse transcriptase-polymerase chain reaction

Total RNA was extracted using Thermoscript™ reverse transcriptase-polymerase chain reaction (RT-PCR) system (Promega, Madison, WI, USA) according to the manufacturer's protocol, cDNA synthesis was done by reverse transcriptase (RT) reaction using 1 μg of total RNA according to manufacture instruction with random primers. RT-PCR technique using available kits and according to the method described by Dahiya et al. [37], using specifically designed primers as follow: The caspase-3 gene was analyzed with the primers 5′-CGGCAGGCCTGATGAAG-3′(sense) and 5′GGACAGCAGTTCAAAATGGATTA-3′ (antisense). The β-actin gene was analyzed with the primers 5′-CCACCATGTACCCAGGCATT-3′ (sense) and 5′ACGCAGCTCAGTAACAGTCC-3′ (antisense).

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