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Chrysin attenuates testosterone-induced benign prostate hyperplasia in rats

Sherif M. Shoieb, Ahmed Esmat, Amani E. Khalifa, Ashraf B. Abdel-Naim*

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt

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ABSTRACT

Benign prostate hyperplasia (BPH) is a common age-related health problem affecting almost 3 out of 4 men in their sixties. Chrysin is a dietary phytoestrogen found naturally in bee propolis and various plant extracts. It possesses antioxidant, anti-inflammatory and anti-proliferative properties. The current study was conducted to explore the role chrysin plays in protection against testosterone-induced BPH in rats. On grounds of a preliminary experiment, a dose of chrysin (50 mg/kg) was chosen for further investigation. Testosterone significantly depleted glutathione, suppressed superoxide dismutase and catalase activities, and elevated lipid peroxidation. Moreover, it markedly scaled down the level of cleaved caspase-3 enzyme, reduced Bax/Bcl-2 ratio and mRNA expression of p53 and p21; conversely, protein expression of proliferating cell nuclear antigen was enhanced. Chrysin alleviated testosterone-induced oxidative stress and restored cleaved caspase-3 level, Bax/Bcl-2 ratio and mRNA expression of p53 and p21 to almost control levels. Chrysin prevented the increase in binding activity of nuclear factor kappa B (NF-κB) p65 subunit, mRNA expression of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R). These data highlight the protective role of chrysin against experimentally-induced BPH. This is attributed - at least partly - to its antioxidant, antiproliferative and proapoptotic properties.

1. Introduction

Benign prostatic hyperplasia (BPH) could be defined as uncontrolled, albeit non-malignant proliferation of prostatic glandular and stromal cells (Aaron et al., 2016). It is a chronic age-related condition affecting almost 3 out of 4 men in their sixties (Wei et al., 2008). The pathophysiology of this disease remains poorly understood. However, numerous studies reported that oxidative stress, suppression of apoptosis and proliferation surge play crucial roles in its development and progression. A compensatory cellular proliferation might be precipitated by oxidative stress resulting in hyperplastic growth in prostatic tissue (Minciullo et al., 2015). Moreover, oxidative stress can activate transcription factor NF-κB which in turn regulates inflammatory and cellular proliferation pathways (Hamid et al., 2011). Furthermore, the suppression of cell apoptosis, which effects a rise in the total number of both stromal and epithelial cells, has been highly associated with the development of BPH. Subsequently, enhancing cell apoptosis has been proposed as a promising strategy for the evolution of anti-BPH agents (Hong et al., 2013; Mosli et al., 2015). The IGF-1/IGF-1R axis is one of disparate growth factors which, along with their corresponding receptors, are involved in prostatic tissue proliferation. Androgenic activation induces production of IGF-1 in prostatic stromal cells and stimulates the surrounding prostatic epithelium to proliferate via IGF-1R (Bogdanos et al., 2003; Garrison and Kyprianou, 2004; Zheng et al., 2013). Consequently, an emerging attractive strategy for treating BPH is targeting IGF-1/IGF-1R axis genes in the prostatic stroma (Reynolds and Kyprianou, 2006).

Natural products, with limited unfavorable effects, have become highly crucial in BPH management (Lin et al., 2013). Chrysin (5,7-dihydroxyflavone) (Fig. 1) is a naturally-occurring flavonoid in bee propolis, honey and various plant extracts (Barbarić et al., 2011; Pichichero et al., 2010). A 50 mg/kg chrysin dose was reported to protect against doxorubicin-induced oxidative stress and elevation of NF-κB protein expression in rats (Mantawy et al., 2014). Additionally, chrysin possesses pro-apoptotic properties evinced by its activation of caspase-3 and -9 and a raised Bax/Bcl-2 ratio in A549 lung cancer cells (Samarghandian et al., 2014). Moreover, chrysin has anti-proliferative properties where it inhibits growth by down-regulating the expression of proliferating cell nuclear antigen (PCNA) in HeLa cells (Zhang et al., 2004). Based on the aforementioned attributes, this study was carried out to evaluate the potential protective effect of chrysin in an animal model of BPH induced by testosterone (Fig. 1) in rats.

* Corresponding author. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt.
E-mail address: abnaim@pharma.asu.edu.eg (A.B. Abdel-Naim).

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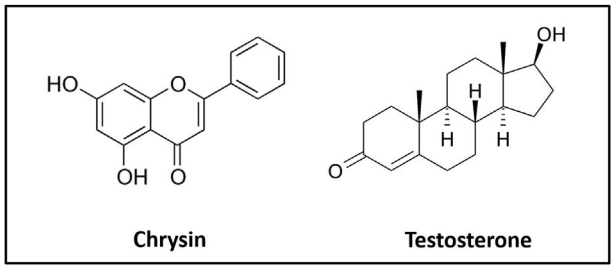

Fig. 1. The chemical structures of chrysin and testosterone.

2. Materials and methods

2.1. Drugs and chemicals

Chrysin, thiobarbituric acid (TBA) and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Testosterone enanthate; (Steroid S.P.A, Cologno Monzese, Italy) was compassionately provided by the Chemical Development Industries Co. (CID), Cairo, Egypt. NF-κB (p65) Transcription Factor Assay Kit (ab133112) was purchased from Abcam, UK. EpiQuik™ Nuclear extraction Kit (OP-0002) was obtained from Epigentek, NY, USA. The remaining chemicals were of the highest commercially available analytical grade.

2.2. Animals

All animal procedures were approved by the Bioethical and Research Committee, Ain Shams University, Cairo, Egypt. Ten-week-old male Sprague–Dawley rats (220–250 g) were obtained from the Nile Co. for Pharmaceutical and Chemical industries, Cairo, Egypt. All animals were maintained in an air-conditioned room on a 12-h light/dark cycle. Animals were kept on water and standard food pellets *ad libitum*. After 7 days of acclimatization, animals were randomly segregated into the experimental groups. Throughout the time course of the experiments, changes in food consumption or behavioral changes have not been observed in the different groups.

2.3. Experimental design

2.3.1. Acute oral toxicity study

The acute oral toxicity of chrysin was evaluated in female Sprague-Dawley rats according to OECD guideline No.423 (OECD, 2001). Based on previous pilot study in our laboratories, limit test was performed. Animals were fasted overnight and chrysin was administered orally using gastric feeding needle at a dose of 2000 mg/kg (10 ml/kg dosing volume).

2.3.2. Dose-response study

Rats were randomly alienated into five groups (8 animals/group) and treated for the first five consecutive days/week for 2 successive weeks as follows: Group 1: rats were administrated a mixture of pure DMSO and corn oil (1:9 v/v, 2.5 ml/kg p.o.) 1 h before olive oil (1 ml/kg, s.c.) injection. Group 2: rats were administrated a mixture of pure DMSO and corn oil (1:9 v/v, 2.5 ml/kg p.o.) 1 h before injection of testosterone (3 mg/kg; in olive oil), subcutaneously. Groups 3, 4 and 5: rats were administrated chrysin dissolved in mixture of pure DMSO and corn oil (1:9 v/v p.o.) at doses of (25, 50 or 100 mg/kg), respectively, 1 h before injection of testosterone (3 mg/kg; in olive oil), subcutaneously. The used dosing regimen was based on preliminary experiments and data available in the literature (Thiyagarajan et al., 2002; Alonso-Magdalena et al., 2009; Atawia et al., 2013). At 72 h, following the last testosterone dose, samples of blood were obtained from the retro-orbital plexus and permitted to clot. Serum was separated by centrifugation at 3000*g* for 10 min and used for determination of prostatic-specific antigen (PSA). After sacrificing rats, prostates were dissected and weighed. Thereafter, prostate indexes were calculated. In order to conduct histological examination, samples from the ventral lobe of the prostate were kept in 10% formalin (neutral and buffered) for preparation of paraffin blocks. Based on the result of the dose-response study, an optimal dose of chrysin was chosen for the subsequent single-dose study.

2.3.3. Single-dose study

Rats were randomly alienated into 4 groups (10 rats/group) and treated for 5 consecutive days/week for 2 successive weeks as follows: group 1 (control group) was orally given (2.5 ml/kg) chrysin vehicle and (1 ml/kg) olive oil S.C. Group 2 was orally given chrysin vehicle and 3 mg/kg testosterone S.C. for induction of BPH. Group 3 was orally given 50 mg/kg chrysin, 1 h before testosterone administration, and group 4 was given 50 mg/kg chrysin orally and olive oil S.C. Rats were sacrificed and the prostatic tissues were dissected out seventy-two hours following last testosterone administration. Some of the ventral lobes were kept in 10% formalin (neutral and buffered) for the immunohistochemical examination. One half of the remaining prostate tissues were stored for real-time polymerase chain reaction (RT-PCR) as provided in RNeasy Mini Kit (Qiagen, Hilden, Germany) and the other half was stored at −80 °C for subsequent biochemical analyses.

2.4. Prostate weight and the prostate index

Prostate gland from each rat was collected and weighed. Prostate index for each rat was the ratio of prostate weight to body weight (mg/g).

2.5. Assay for level of serum PSA

Rat PSA ELISA kit (Shenzhen New Industries, Biomedical Engineering Co., Ltd., China) was used to assess level of PSA in serum. The experiment was conducted in line with the manufacturer's instructions, where a 96-well plate, coated with the appropriate anti-Rat antibody was used. 50 µl of the standards and the samples were added in the plate, each; 200 µl/well of enzyme conjugate reagent were added and incubated at room temperature for 45 min. The solution was then discarded, followed by washing 4 times, using the wash buffer provided. 100 µl/well of substrate-chromogen solution were added and left to incubate for 15 min at room temperature. An aliquot of 100 µl of 1N H_2SO_4 was added to the wells and mixed thoroughly. Finally, the plate was read spectrophotometrically at 450 nm posthaste.

2.6. Histopathological examination

Prostate tissues were histopathologically examined. Ventral prostates were briefly maintained in 10% formalin and embedded in paraffin for producing 4 µm-thick sections. This was followed by de-paraffinising, rehydration and staining with hematoxylin and eosin (H&E).

2.7. Immunohistochemical detection of PCNA

The paraffin-embedded tissue sections (4 µm) were autoclave-dried,