Biomedicine & Pharmacotherapy 90 (2017) 15–23

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Elsevier Masson France
EM|consulte
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Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histo-chemical study

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 4 March 2017
Accepted 7 March 2017

Keywords:
Royal jelly
Cyclophosphamide
Prostatic tissue

ABSTRACT

Almost all the chemotherapy treat many cancer types effectively, but it leads to severe side effects. Chemotherapy like cyclophosphamide (CP) not works only on the active cells, such as cancer cells, but also acts on the healthy cells. Royal jelly (RJ) was reported to have a lot of therapeutic effects besides being an anti-oxidant and anti-cancer agent. The purpose of this study was to assess the possible protective role of RJ in ameliorating the toxic effects of CP overdose in the rat prostatic tissue. The rats were separated into 4 groups; control group, RJ group, CP group and RJ with CP group. Prostatic specimens were processed for biochemical, histological and immune-histo-chemical studies. The mean area fractions of eNOS and Bax expression were measured in all groups, and statistical analysis was carried out. The results showed that in CP treated group, there were marked biological changes in the form of significant increase in prostatic malondialdehyde (MDA) and C – reactive protein (CRP). Additionally there was a significant decrease in glutathione peroxidase (GPx) in prostatic tissue if compared with the control group. Furthermore, the histological changes showed marked acinar and stromal prostatic degeneration. Most prostatic acini showed less PAS reaction and more (eNOS and Bax) expression if compared with the control group. Concomitant administration of RJ with CP revealed a noticeable amelioration of these biochemical and histological changes. In conclusion, RJ provided biochemical and histo-pathological improvement in CP induced prostatic tissue toxicity. These findings revealed that this improvement was associated with a decrease in the tissue oxidative damage and apoptosis.

1. Introduction

Management of many cancers has been achieved by aggressive chemotherapy and radiotherapy. Unfortunately, chemotherapy is not specific to the tissue, and places both normal and cancer cells at risk by direct and indirect mechanisms [1]. Cyclophosphamide (CP) is the most common cytotoxic alkylating agent which is most commonly used as an anti-cancer agent [2]. This drug has a significant immunosuppressive activity and is widely used clinically in the treatment of autoimmune diseases and for renal and bone marrow transplantations [3]. It is very interesting to know that CP uptake into healthy cells is higher than in cancer cells, rendering healthy cells more susceptible to damage [4]. As with all alkylating agents, CP may cause significant side effects even when given at low doses [5]. Some reports reveal that intra-peritoneal administration of CP caused severe tissue oxidative stress [6] and massive cellular damage [7], consequently triggering apoptosis [8], and death of cancer and healthy cells [9]. CP mediated a lot of changes in the male reproductive system; these changes were in the form of impairing gonadotropins secretion from the testis, induction of hormonal changes, increasing the sensitivity of cells to oxidative damage, depleting the cellular content of ATP and genotoxic effects [10]. This may result in impairment of patient's quality of life and reduced cancer control due to the inability to deliver adequate dose-intensive therapy against the cancer [11]. CP can alkylate DNA, which prevents the duplication of the genome in dividing cells as it arrests the S-phase of the cell cycle, and also induces apoptosis in embryonic neural progenitor cells of the telencephalon 6–12 h after administration [12]. Not only the cancer patients who are exposed to the hazards

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http://dx.doi.org/10.1016/j.biopha.2017.03.020
0753-3322/

of CP but also the pharmacists and nurses also occupationally exposed to the drug during its production or distribution [13].

Pharmacotherapy which uses natural substances can be currently regarded as a very promising future alternative to conventional therapy [14]. Royal jelly (RJ) is a honey bee product secreted from the mandibular glands and hypo pharyngeal glands of the worker honeybees and serves as a main primary food for the queen and larvae during their first few days of life [15]. Moreover, this substance contains major proteins with high levels of peptides and more essential amino acids [16]. It has also a suppression effect on the oxidative and nitrosative stress [17].

The aim of this current study was to evaluate the possible protective effect of RJon the biochemical, histo-pathological and immune-histo-chemical alterations in the rat prostatic tissue induced by CP and also to study the possible mechanisms through which RJ produces its protective effect.

2. Material and methods

2.1. Animals

Adult male Wister rats, weighting 180–200 g were obtained from the National Research Center, Cairo, Egypt. Animals were kept at standard living conditions (temperature (25 ± 2 °C), 45 ± 5% humidity, and 12-h light/dark cycles) and were allowed free access to tap water and standard rodent chow (El-Nile Company, Egypt). The rats were divided randomly into four groups of 6 animals each after 2 weeks of acclimatization. Animals and their care were conducted according to the animal care committee of Minia University, Egypt and coincide with international guidelines.

2.2. Chemicals and antibodies

Cyclophosphamide was purchased from (AstaMedica, Germany). The royal jelly used was purchased from Santa Cruz Biotechnology Inc, USA; secreted by worker honeybees [(mixture of protein, glucose, lipid, vitamins, and minerals), (CAS number 8031-67-2)]. Kits for total antioxidant capacity (TAC) (Biodiagnostics, Egypt), glutathione peroxidase (GPx) (Sigma Chemical Company, USA), tumor necrosis factor (TNFα) ELISA kit (IDlabsT-Minc. Biotechnology, Canada), C-reactive protein (CRP) (Agappe Diagnostic LTD, India), high density lipoprotein (HDL) kit (Spectrum, Egypt), Cholesterol kit (Biomed diagnostic, Egypt), Triglycerides (human, Germany), eNOS, and Bax (Thermo scientific. USA) were purchased. All other chemicals were of analytical grade and were obtained from commercial sources.

2.3. Experimental design

Animals were randomly divided into four groups as following:

1. Control group (group I): It consists of 6 rats which received 5 ml saline orally by gastric tube for 14 days.
2. Royal jelly [RJ (group II)]: It consists of 6 rats which received 300 mg/kg/day RJ orally for 14days by gastric tube [18].
3. Cyclophosphamide group [CP (group III)]: It consists of 6 rats in which rats were received normal saline for 14days followed by a single dose of CP on the 15th day in a dose of 150 mg/kg intraperitoneally [19].
4. Royal jelly and Cyclophosphamide group (group IV): It consists of 6 rats in which rats were received a daily oral dose of royal jelly in a dose of 300 mg for 14days by gastric tube days followed by a single dose of CP 150 mg/kg/day intraperitoneal at the 15th day

Treatments were done between 9.00 and 10.00 a.m. to minimize possible diurnal effects. At 48 h after CP injection for all groups, animals were anesthetized with ether and sacrificed. Blood samples were collected from each rat for biochemical analysis. Prostatic specimens were taken. Parts of each specimen (dorsal parts) were excised for histological and immune-histochemical studies; the remaining parts were used for tissue homogenates preparation.

2.4. Biochemical analysis

Blood samples were collected from each rat and centrifuged (centrifuge Jantezki, T30, Germany), at 5000 rpm for 10 min for serum collection. Sera were separated and kept in refrigerator at −80 °C until assessment of various parameters.

2.5. Preparation of tissue homogenates

Specimens from prostate were weighed and homogenized separately in potassium phosphate buffer 10 mM pH (7.4). The ratio of tissue weight to homogenization buffer was 1:5. The homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was kept at −80 °C until assessment of malondialdehyde (MDA), glutathione peroxidase (GPx), nitric oxide (NO) and cholesterol levels.

Serum levels of total antioxidant capacity (TAC), C reactive protein (CRP), tumor necrosis factor (TNFα), high density lipoprotein (HDL), triglycerides, cholesterol, prostatic GPx and prostatic cholesterol were detected according to the manufacturers' guidelines using commercially available kits.

Prostatic MDA, an index of lipid peroxidation, was determined by using 1, 1, 3, 3-tetramethoxypropane as standard [20]. Total nitrite/nitrate, the stable oxidation end products of nitric oxide, served as an index of nitric oxide level and was measured by reduction of nitrate into nitrite using activated cadmium granules, followed by color development with Griess reagent in acidic medium [21].

2.6. Histological examination

Specimens were fixed in 10% neutral-buffered formalin, dehydrated, cleared, and embedded in paraffin wax. Tissue sections of 5–6 μm thickness were obtained and deparaffinized. Some sections were stained with hematoxylin and eosin [22] to verify the histological structures and with Periodic acid Schiff method (PAS) for demonstration of neutral muco-substances [23]. For immunohistochemical detection of inducible nitric oxide synthase (eNOS) and (Bax), we used eNOS, and Bax monoclonal mouse antibodies according to the manufacturer's protocol. The slides were then counterstained, dehydrated, and mounted. The eNOS and BAX immunoreactive sites stained brown and nuclei stained blue. The positive control for anti-eNOS antibody was heart tissue while positive control for the anti-BAX antibody was human lymphoma tissue. For negative control slides, the same steps, but without the 1ry antibody (not included).

2.7. Photography

An Olympus (U.TV0.5XC-3) light microscopy was used. Slides were photographed using an Olympus digital camera. Images were processed using Adobe Photoshop

The mean area fraction of eNOS and Bax expression was quantified in 6 fields for each group using image J 22 program.