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Original article

Pharmacological effects of Vitamin C & E on Diclofenac Sodium intoxicated Rats

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ABSTRACT

Objective: The aim of this study was to evaluate the probable protective effect of vitamin C and vitamin E on diclofenac-induced acute nephrotoxicity using biochemical, molecular and histopathological examination in rats following administration of diclofenac sodium (50 mg/kg, I.M).
Methods: Ninety male Wister rats were allotted in six equal groups. Rats in the 1st group (control group) were injected with physiological saline, while rats in the 2nd group (C-group) were given vitamin C (100 mg/kg orally via stomach tube) for 5 successive days. The 3rd group (E-group) was given vitamin E (250 mg/kg orally in diet) for 5 successive days. Rats in the 4th group (D-group) were injected by diclofenac sodium (50 mg/kg, I.M) for 5 successive days. The 5th group (DvC-group) was given diclofenac sodium (50 mg/kg, I.M) and vitamin C (100 mg/kg orally via stomach tube) for 5 successive days. Rats in the 6th group (DvE-group) were given diclofenac sodium (50 mg/kg, I.M) and vitamin E (250 mg/kg orally in diet) for 5 successive days. Blood samples were collected two days post treatment (1st week of experiment), 2nd and 4th week of the experiment for assessment of urea, creatinine, malondialdehyde, nitric oxide and superoxide dismutase activities. At the end of 4th week, rats were sacrificed and kidneys were excised for biochemical analyses, histopathological evaluation and determination of kidney interleukin-1β, interleukin-18, demsin and nepherin expressions in by reverse transcriptase–polymerase chain reaction (RT-PCR).
Results: The results showed that, diclofenac induced severe kidney damage as indicated by histopathological changes and increased serum oxidative stress parameters. Behavioral changes were monitored; a significant increase in uremia in intoxicated animals was also noted indicating that diclofenac sodium provoked kidney damage in rats. Application of vitamin C (DvC-group) and vitamin E (DvE-group) were found to improve the abovementioned abnormalities.
Conclusion: The present data suggest that, vitamin C and vitamin E might play an important role in reducing oxidative stress and kidney damage induced by diclofenac sodium.

1. Introduction

Acute kidney injury (AKI) is generally defined as a drop in kidney function resulting in piling up of waste products in the bloodstream. There are many causes of AKI includes: nephrotoxins, oxytetracycline, aminoglycosides and nonsteroidal anti-inflammatory drugs (NSAIDs) [1].

Non-steroidal anti-inflammatory drugs (NSAIDs), are regularly used to relief non-specific fever [2]· These agents are the most extremely used for treatment of many inflammatory diseases [3] and still have an effective role in the relieving of pain and in decreasing inflammation and palliation of fever [4]. Diclofenac is a phenylacetic acid derivative that was developed specifically as a non-steroidal anti-inflammatory (NSAID) drug [5].

Diclofenac is often prescribed in both human and veterinary practices as an anti-inflammatory, analgesic as well as antipyretic agent. Diclofenac acts by inhibiting cycloxygenase-1 (COX-1) and cycloxygenase-2 (COX-2) enzymes, thus preventing prostaglandin synthesis from arachidonic acid. Renal physiology is a partially COX-dependent system as COX-1 and COX-2 are

Abbreviations: Akt1, protein kinase B; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CCl4, carbon tetrachloride; FA, folic acid; Fas, programmed cell death-receptor; GSH, glutathione; GSH-PX, glutathione peroxidase; HDL, high density lipoprotein; IL, interleukin; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; SD, standard deviation; SOD, superoxide dismutase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-alpha.

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continuously expre4ssed in the kidney and its vessels [6]. Although NSAIDs are effective in treatment of pain related to arthritis, they have severe side effects, mostly ulceration of the gastrointestinal (GI) tract [7]. Moreover, Diclofenac also causing severe gastrointestinal problems like bleeding of gastric mucosa, decreased gastric blood flow and mucosal apoptosis [8] The kidney is another organ affected by the toxic effects of NSAIDs [9]. Diclofenac like other NSAIDs, may cause stomach-related side effects and nephrotoxicity through blocking the synthesis of prostaglandin [10]. To date, there is no specific agent used to protect against Diclofenac sodium-induced nephrotoxicity.

Normally, oxidants and antioxidants are in balance in the body. However, oxidative stress occurs when reactive oxygen radicals are produced excessively and/or antioxidants are insufficient. As a result, oxidative stress causes lipid peroxidation. Malondialdehyde (MDA) is the world-wide accepted biological marker of lipid peroxidation [11]. The effects of NSAIDs in therapeutic doses on oxidative stress have been confirmed [12], but there is little knowledge on the effects of high doses of these drugs [13]. Er et al. [14] concluded that, NSAIDs affect oxidative balance, some have antioxidant effect while others have oxidant effect.

Vitamin C is considered as an antioxidant agent that scavenges free oxygen radicals, including superoxide, hydrogen peroxide, peroxyl, and singlet oxygen. Thus, vitamin C may minimize some types of lipid peroxidation. Insufficient levels of vitamin C inhibit synthesis of collagen in cellular basal membranes and can destruct mucosal epithelium [15] .Vitamin C has an antitoxic effect against stress caused by pesticide [16] and potentiate the nonspecific immune responses [17]. It has been reported that, Heteropneustes fossilis can counter stress induced by cypermethrin through increasing tissue reserves of amino acids when fed on Vitamin C dietary supplement [18]. Vitamin C also has anti-inflammatory effect, prevents endothelial dysfunction and decreases the risk of cardiovascular diseases [19]. In addition, it has been shown that vitamin C treatments can improve kidney function in renal allograft recipients Williams et al. [20] decrease renal inflammation, and improve impaired renal function in salt-sensitive hypertensive rats[21].

Vitamin E is considered as an antioxidant agent, plays specific roles beyond that of its antioxidant function, it has ability to prevent chronic diseases, especially those believed to have an oxidative stress component such as cardiovascular diseases, atherosclerosis, and cancer [22]. It is possible that antioxidant deficiency may lead to increased lipid peroxidation and cell death due to mitochondrial compromise, meanwhile, vitamin E is an antioxidant which may act as scavengers of hydroxyl, peroxyl, and superoxide radicals and protect against plasma lipid and low-density lipoprotein peroxidation [23].

This paper seeks to describe the effect of Vitamin C and E as antioxidant factors in ameliorating the kidney damage caused by Diclofenac Sodium in rats.

2. Materials and methods

2.1. Drugs

Diclofenac sodium: Diclofenac sodium (Voltaren) ampoules each one contain 75 mg diclofenac sodium produced by Novartis.

Vitamin C: Vitamin C 20%, each 1 kg contains 200 gm Vitamin C, Lactose up to 1000 gm. Produced by Uni Company, ARE.

Vitamin E: Vitamin E, soft gelatin capsules, each capsule contain 400 mg Vitamin E, Produced by Pharco Phrmaceuticals Company, ARE.

2.2. Animals

Ninety healthy male albino rats initially weighting between 200 and 220 g were used in this experiment. The animals were purchased from animal house of Helwan, Faculty of Medicine. Animals were left for one week to acclimatize the place and to ensure normal growth and behavior. Rats were kept in cages, fifteen per cage, in a controlled environment and maintained under a 12 h light:dark cycle, 24 °C (±3 °C) and 50–70% humidity. Rats were provided with standard diet and water *ad-libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. Kits for biochemical analysis

All the diagnostic kits assaying hepatic and renal function tests were obtained from Bio-diagnostic Company, Cairo, Egypt. Other chemicals were obtained from EL-Gomhoria Company, Cairo, Egypt.

Prepared frozen serum samples were analyzed for, aspartate aminotransferase (AST), alkalin phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin, cholesterol, triglyceride, nitric oxide (NO) and superoxide dismutase (SOD) with semi-automatic spectrophotometer (BM-Germany,5010) using commercial test kits from Randox Laboratories Ltd (Crumlin, County Antrim, UK) according to enclosed pamphlets.

Serm concentrations of malondialdehyde (MDA), total antioxidant capacity (TAC) concentration, erythrocytic reduced glutathione (G-SH) content, erythrocytic SOD activity and Catalase activity in whole blood (CAT) and Nitric oxide (NO) in the kidney homogenate were determined using commercially available kits (Biodiagnostic Co, Cairo, Egypt).

2.4. Experimental protocol

Rats were randomly divided into six equal groups 15 rats each. Rats in the 1st group (control group) injected with 0.9% saline intraperitoneally (IP) for 5 successive days, while rats in the 2nd group (C-group) were received vitamin C (100 mg/kg orally via stomach tube) for 5 successive days[23]. The 3rd group (E-group) was given vitamin E (250 mg/kg orally in diet) for 5 successive days [24]. Rats in the 4th group (D-group) were injected with diclofenac sodium (50 mg/kg, I.M) for 5 successive days [25]. The 5th group (DvC-group) was given diclofenac sodium (50 mg/kg, I.M) and vitamin C (100 mg/kg orally via stomach tube) for 5 successive days [23]. Rats in the 6th group (DvE-group) were given diclofenac sodium (50 mg/kg, I.M) and vitamin E (250 mg/kg orally in diet) for 5 successive days[24].

Two days post treatment (at the end of 1st week), at the end of 2nd and 4th week of the experiment, blood samples were drawn from all animals via retro-orbital bleeding. Each blood sample was left in a plain test tube at room temperature for 1 h and then centrifuged for 10 min at 3000 rpm to obtain the serum. Serum