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Original article

Virgin coconut oil supplementation attenuates acute chemotherapy hepatotoxicity induced by anticancer drug methotrexate via inhibition of oxidative stress in rats

Ademola C. Famurewa[a,*], Odomero G. Ufebe[a], Chima A. Egedigwe[b], Onyebuchi E. Nwankwo[c], Godwin S. Obaje[d]

[a] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria
[b] Department of Chemistry/Biochemistry/Molecular Biology, Faculty of Science, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria
[c] Department of Biological Sciences, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
[d] Department of Anatomy, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria

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ABSTRACT

Background: The emerging health benefit of virgin coconut oil (VCO) has been associated with its potent natural antioxidants; however, the antioxidant and hepatoprotective effect of VCO against methotrexate-induced liver damage and oxidative stress remains unexplored. The study explored the antioxidant and hepatoprotective effects of VCO against oxidative stress and liver damage induced by anticancer drug methotrexate (MTX) in rats.

Methods: Liver damage was induced in Wistar rats pretreated with dietary supplementation of VCO (5% and 15%) by intraperitoneal administration of MTX (20 mg/kg bw) on day 10 only. After 12 days of treatment, assays for serum liver biomarkers (aminotransferases), alkaline phosphatase, albumin and total protein as well as hepatic content of malondialdehyde, reduced glutathione and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) were carried out. Liver was used to examine histopathological changes.

Results: MTX administration induced significant increase in serum liver enzymes along with marked decrease in albumin and total protein compared to control group. Hepatic activities of antioxidant enzymes were significantly decreased, while malondialdehyde increased significantly. Treatment with VCO supplemented diet prior to MTX administration attenuated MTX-induced liver injury and oxidative stress evidenced by significant improvements in serum liver markers, hepatic antioxidant enzymes and malondialdehyde comparable to control group. Histopathological alterations were prevented and correlated well with the biochemical indices.

Conclusion: The study suggests antioxidant and hepatoprotective effects of VCO supplementation against hepatotoxicity and oxidative damage via improving antioxidant defense system in rats. Our findings may have beneficial application in the management of hepatotoxicity associated with MTX cancer chemotherapy.

1. Introduction

Chemotherapy has emerged as an effective treatment for different cancer types with robust evidence base for decades. However, its clinical use is associated with undesirable side effects which constitute a major concern for physicians. The major side effect is chemotherapy-induced hepatotoxicity produced due to long-term use, direct and/or indirect insult on hepatocytes [1]. The central role of the liver in xenobiotics metabolism is well established, and therefore liver is prone to toxic attack [2].

Methotrexate (MTX) has remained a widely used antimetabolite anticancer agent with clinical benefits and an important therapeutic alternative in the treatment of severe psoriasis and rheumatoid arthritis. It is a classical antifolate that induces apoptosis in cells with high division rate and therefore has a strong cytotoxic effect on rapidly dividing cancer cells [3]. It inhibits folate-driven enzymatic processes, particularly dihydrofolate reductase and thymidylate synthase involved in

* Corresponding author.
E-mail address: ademola.famurewa@funai.edu.ng (A.C. Famurewa).

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purine nucleotide and thymidylate synthetic pathways, and their inhibition ultimately interferes with DNA synthesis, repair, and cellular replication during the S-phase of cell cycle [4]. Therefore, MTX not only affects tumor cells but also rapidly dividing cells such epithelial stem cells and crypts of gastrointestinal mucosa [5]. Hepatic injury is the major potential side effect that limits MTX long-term clinical use; it may occur following chronic high dose administration of MTX. The underlying mechanism of MTX-induced liver toxicity is not clearly defined in the literature [6,7]; however, some studies have demonstrated the contribution of oxidative stress to the mechanism of MTX-induced liver damage [8,9]. Overproduction of reactive oxygen species (ROS) implicated in MTX hepatotoxicity depletes mitochondria enzymatic and non-enzymatic antioxidant defense systems [10,11]. Recent evidences demonstrate that MTX administration triggers reduction in NADPH, GSH and up-regulates ROS and hepatotoxic manifestations associated with decreased cellular antioxidant defense system resulting in hepatic oxidative damage [6,9,12]. The use of natural non-toxic cytoprotective agents as adjuvants may play an important role to decrease the incidence of side effects of MTX chemotherapy with preservation of chemotherapeutic efficacy. Unrefined oil is becoming of great interest today, and increasing evidence supports potential health benefits of plant oils. They are reservoir of phytochemical antioxidants and constitute sources of dietary supplements for favourable health effects [10,13,14]

Virgin coconut oil from *Cocos nucifera* is emerging as functional food oil with high antioxidant capacity that may have beneficial health effects against oxidative damage and side effects of chemotherapy. It is oil obtained from fresh, mature kernel of the coconut by mechanical or natural means, with or without the use of heat and without chemical refining, bleaching and deodorizing. [15]. The phytochemical analysis suggests that dietary consumption of VCO may significantly improve health due to high antioxidant phenolic compounds, including protocatechuic, vanillic, caffeic, ferulic and *p*-coumaric acids [15]. Studies indicate antioxidant, antihyperlipidemic, antinociceptive, anti-inflammatory, and antimicrobial activities of VCO [16–20], along with several other bioactivities associated with its antioxidant properties. In other studies, VCO demonstrates hepatoprotective potential against liver damage induced by antibiotic Trimethoprim-sulfamethoxazole [21] and cyclophosphamide [22]. To our knowledge, Nair et al. [22] is the only existing report to highlight the potential beneficial role of VCO against side effects of cyclophosphamide chemotherapy. Currently, it is not known whether dietary consumption of VCO may alleviate or protect liver in MTX chemotherapy. Therefore, the present study aimed at evaluating the possible antioxidant and hepatoprotective effect of VCO against methotrexate-induced hepatotoxicity in rats.

2. Material and methods

2.1. Drug and chemicals

Methotrexate was purchased from the Morningside Healthcare Ltd, Leicester, UK. The kits used for biochemical assays of liver function parameters were obtained from Randox Laboratory Ltd., UK. Thiobarbituric acid (TBA) was purchased from Hi Media Laboratories Pvt. Ltd, India. All other reagent used were obtained commercially and of analytical grade.

2.2. Animals

Male Wistar rats weighing 100 to 120 g were purchased from a private Animal Breeding House, near University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were maintained under standard environmental condition (25 ± 1 °C and 12 h dark/12 h light cycle) and were allowed free access to pelleted commercial growers mash (Vital Feeds Nigeria Ltd, Jos, Nigeria) and clean water *ad libitum*. The rats were acclimatized one week preceding treatment and were handled in humane manner according to the approved animal experimental procedures given by the NIH Publication on Guide for the Care and Use of Laboratory Animals.

2.3. Preparation of virgin coconut oil and diet

The VCO was extracted using wet extraction method according to Nevin and Rajamohan [23]. Mature coconuts purchased from commercial sellers in Abakaliki, Ebonyi State were used for the extraction of VCO. The coconut meat (solid endosperm) from mature coconuts (*Cocos nucifera L.*) was grind and made into viscous slurry. About 400 ml of water was added to the slurry obtained and sieved through cheesecloth to obtain creamy coconut milk, which was left standing for 48 h to separate the creamy top and water layers. The top layer was carefully removed and subjected to mild heating (50 °C) to remove moisture. The heating thus separates the oil which was gently scooped out and filtered into an air-tight container. This oil thus prepared without refining, bleaching and deodorizing was used for the current study. The VCO supplemented diets, 5% w/w VCO and 15% w/w VCO, were separately prepared from the fresh oil.

2.4. Experimental design

Following 1 week of acclimatization, rats were divided into 4 different groups (6 rats/group) and fed with the following diets: Group 1 (control) rats fed on commercial rat pellet (basal diet); Group 2 (MTX) fed with basal diet + MTX (20 mg/kg bw) administered ip on day 10 only for induction of hepatotoxicity [11]; Group 3 fed with 5% VCO supplemented basal diet + MTX (20 mg/kg) administered ip on day 10 only; Group 4 fed with 15% VCO supplemented basal diet + MTX (20 mg/kg bw) administered ip on day 10 only. The control rats received physiological saline (drug vehicle) on day 10 only. At the end of the treatment period (12 consecutive days), 48 h after MTX administration, fasted animals were sacrificed; blood collected via cardiac puncture into plain bottles and centrifuged (3000*g* for 15 min) for separation of serum. The liver was dissected out, washed using cold saline solution, dried with tissue paper and weighed. The tissue was minced and homogenized in phosphate buffered saline (5% w/v, pH 6.4) and centrifuged (3500*g* for 15 min). The supernatant obtained was used for analyses of malondialdehyde and antioxidant enzyme activities. The remaining liver sample was fixed in 10% buffered formalin for histopathological examination.

2.5. Biochemical analyses

The liver function markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB) and total protein (TP) were analysed in serum stored at 4 °C using commercial kits by RANDOX following manufacturer's instructions. Supernatant obtained from the liver homogenate was used for oxidative stress markers. Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund [24], catalase (CAT) activity by the method of Aebi [25], glutathione peroxidase (GPx) was determined according to the method of Paglia and Valentine [26], while reduced glutathione (GSH) content was analysed using Beutler method [27]. Lipid peroxidation in the liver tissue was estimated by measuring thiobarbituric acid reactive substances (TBARS) expressed in terms of malondialdehyde (MDA) content using the method of Ohkawa et al. [28].