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Beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats☆

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ABSTRACT

Methotrexate (MTX) is a commonly used antineoplastic and anti-rheumatoid agent whose efficacy is limited by marked organ toxicities associated with oxidative stress. The study investigated beneficial effect of virgin coconut oil (VCO) supplementation on MTX-induced oxidative stress and inflammation in rats. Rats were divided into 4 groups (n = 6): Control, MTX (20 mg/kg bw), VCO (5%) + MTX and VCO (15%) + MTX. The pre-treatment with VCO for 14 days was followed by single intraperitoneal injection of MTX and the rats were sacrificed after 3 days. Serum activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and levels of reduced glutathione (GSH) and malondialdehyde (MDA) were determined. Interleukin-6 (IL-6), C-reactive protein (CRP) and nitric oxide (NO) levels were also evaluated. MTX induced a distinct diminution in serum activities of oxidative stress markers (SOD, CAT, GPx and GSH), while lipid peroxidation considerably increased demonstrated by MDA level. Similarly, levels of IL-6, CRP and NO increased prominently in MTX control rats. The VCO supplementation markedly enhanced resistance to the

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MTX-induced biochemical alterations in rats. VCO can be a useful adjuvant natural product in MTX chemotherapy by reducing oxidative stress and pro-inflammatory responses.

1. Introduction

Cancer patients undergoing chemotherapy may incur a debilitating side effect that may prompt dose reductions or discontinuation of the treatment regimen, ultimately leading to reduction in treatment efficacy.[1] Methotrexate (MTX) is an antimetabolite anticancer agent that has also shown efficacy in the treatment of rheumatoid disorders. Methotrexate alone or in combination with other anticancer agents is used in the treatment of breast cancer, leukemias, lymphomas, osteosarcoma, and several malignant brain tumors.[2] It is prescribed as a single dose in the management of ectopic pregnancy without adverse outcomes associated with ovary or *in vitro* fertilization.[3] Although MTX is well tolerated, however, its long-term use is often associated with major organ toxicity, and thus limits clinical applications. The toxicity of MTX in various organs such as the intestine, liver, kidney, testis and central nervous system has been reported.[4] Recent findings have shown that MTX-induced toxicity is associated with pro-inflammatory responses in animal models.[5,6] The action mechanism of MTX inhibits dihydrofolate reductase and thymidylate synthase involved in the synthesis of DNA precursors such as thymidylates and purines.[7] The inhibition impairs DNA synthesis, DNA repair mechanism and replication during the S-phase of cell cycle in rapidly dividing cells including cancer cells and some normal dividing cells.[6]

The underlying mechanism of MTX toxicity is unclear in the existing literature.[8] However, a number of mechanisms have been reported, including antioxidant defense deregulation and the activation of transcription factor, nuclear factor kappa B, to upregulate some genes responsible for the production of pro-inflammatory mediators.[6,8] Accumulating evidence has implicated contribution of oxidative stress to MTX-induced organ toxicity.[3] Animal studies have shown that generation of reactive oxygen species (ROS) by MTX depletes first-line antioxidant enzyme systems, including reduced glutathione.[4,9–12] The resulting oxidative damage leads to lipid peroxidation and cell membrane damage in tissues. The intriguing evidence that synthetic antioxidants may play important role in pathogenesis has triggered a paradigm shift favoring natural products with antioxidant efficacy to reduce oxidative stress consequences in biological processes.[13]

Virgin coconut oil (VCO) is a nutritional and medicinal food in the traditional coconut growing areas. It is an unrefined kernel oil obtained from fresh and mature coconut (*Cocos nucifera*) by mechanical or natural means, with or without the use of heat and without chemical bleaching and deodorizing.[14] It is being known for many beneficial health effects associated with its phenolic acids and flavonoid contents.[15] Phytochemical analysis found that *p*-coumaric and ferulic acids are the major potent phenolics in the VCO.[16] Previous studies found VCO to mitigate oxidative stress, dyslipidemia and inflammation in organs of animal models via antioxidant activities.[17–19] Although some agents have demonstrated antioxidant potential against MTX-mediated toxicity, the possible role of functional oils such as VCO that is affordable and amenable to daily diet need considerable attention. We sought to investigate whether dietary VCO supplementation has protective effects against MTX-induced oxidative stress and pro-inflammation in Wistar rats.

2. Materials and methods

2.1. *Chemicals*

Methotrexate was purchased from the Morningside Healthcare Ltd, Leicester, UK. The assays for biochemical parameters (antioxidant enzymes and reduced glutathione) were carried out using commercial kits purchased from Randox Laboratory Ltd., UK. Thiobarbituric acid (TBA) was purchased from Hi Media Laboratories Pvt. Ltd, Mumbai, India. Commercial kits for nitric oxide, interleukin-6 and C-reactive protein were purchased from R&D Systems, USA and TULIP DIAGNOSTICS, respectively. All other chemicals used were high-quality analytical-grade reagents.

2.2. *Animals*

Twenty-four adult male rats (about 7 weeks old) were purchased from the Veterinary Department, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were kept in the Animal House of the Department of Biochemistry, Ebonyi State University, Nigeria, in constant environmental conditions with 12 hr light/12 hr dark cycle and free access to clean water and commercial chow. All experimental protocols were in accordance with the guidelines and standards of animal's care approved by the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.[20]

2.3. *Virgin coconut oil*

VCO was obtained from a dealer in Badagry, Lagos State, Nigeria. Evaluation of the procedures of VCO production showed that the VCO was made from fresh mature coconuts via traditional wet processing as reported by Nevin and Rajamohan.[21] Briefly, the viscous slurry obtained from the ground coconut meat was dissolved in clean water. The milky solution obtained was filtered through cheesecloth and the milky filtrate was left standing for 48 h to separate the creamy top and water layers. The sticky top layer was carefully removed and subjected to mild heating (50 °C) to remove moisture. The floating oil was gently scooped out and filtered into an airtight container and used for the present study.

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