Available online at www.sciencedirect.com

Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

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

Ademola C. Famurewa[a,*], *Abiola M. Folawiyo*[b], *Elizabeth B. Enohnyaket*[b], *Sharon O. Azubuike-Osu*[c], *Innocent Abi*[d], *Sunday G. Obaje*[e], *Opeyemi A. Famurewa*[f]

[a] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria*
[b] *Department of Physiology, College of Medicine, Ebonyi State University, Abakaliki, Nigeria*
[c] *Department of Physiology, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria*
[d] *Department of Physiology, College of Health Sciences, Benue State University, Makurdi, Nigeria*
[e] *Department of Anatomy, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria*
[f] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Science, University of Jos, Jos, Nigeria*

ARTICLE INFO

Article history:
Received 30 January 2018
Revised 13 April 2018
Accepted 4 May 2018

Keywords:
Virgin coconut oil
Methotrexate
Supplementation
Oxidative stress
Inflammation

ABSTRACT

Background: 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.

Methods: 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.

Results: 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 MTX-induced biochemical alterations in rats.

* Corresponding author at: Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria.
E-mail address: ademola.famurewa@funai.edu.ng (A.C. Famurewa).

https://doi.org/10.1016/j.imr.2018.05.001

Conclusion: 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. 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.

2.4. Experimental design

Following 2 weeks of acclimatization, rats were randomized and grouped into 4 (n = 6 per group) and the treatment design was as follows:

Group 1 (normal control): received clean water and commercial chow for 17 days.

Group 2 (MTX control): received MTX (20 mg/kg body weight, ip) on day 14 only

Group 3 (5% VCO diet + MTX): received 5% VCO (w/w) supplemented diet for 17 days + MTX (20 mg/kg body weight, ip) on day 14 only.

Group 4 (15% VCO diet): received 15% VCO (w/w) supplemented diet for 17 days + MTX (20 mg/kg body weight, ip) on day 14 only.

The body weight of each rat was determined and recorded on day 1, 14 and 17 using an electronic balance. At the end of the experimental period, blood samples were collected from all animals (fasted) via retro-orbital venous plexus by capillary tubes into plain sample bottles and allowed to clot. The clotted blood samples were centrifuged (3,000 g for 15 min) for separation of serum used for analysis of antioxidant enzymes, reduced glutathione, malondialdehyde and pro-inflammatory markers.

2.5. Biochemical analyses

Evaluation of biochemical parameters was carried out using standard commercially available kits. The antioxidant enzymes — superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) were analysed in serum samples with RANDOX kits following manufacturer's instructions. Lipid peroxidation marker, malondialdehyde (MDA) was estimated by the method of Wallin *et al.*[22] using Thiobarbituric acid (TBA) purchased from Hi-Media Laboratories, Mumbai, India. Nitric oxide (NO) was analysed by commercial kit that contained Griess reagents (R&D Systems Inc., USA) according to the method of Green *et al.*[23] The level of interleukin-6 (IL-6) was estimated by ELISA method with assay kit from R&D Systems Inc., USA. Level of C-reactive protein (CRP) was estimated according to the method of Andersen and McCarthy[24] as described in TULIP DIAGNOSTICS kit for C-reactive protein.

Fig. 1

2.6. Statistical analyses

Data were analysed using SPSS software (Window version 22). The differences between groups were compared using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Data were represented as arithmetic mean ± standard error of mean (SEM). $P < 0.05$ was considered significant.

3. Results

3.1. Effect of VCO supplementation on body weight of rats administered MTX

Table 1 presents the effect of VCO supplementation on body weight at the commencement of the supplementation (Day 1), on Day 14 and at sacrifice (Day 17). At the commencement of the study, the body weights of rats in the four groups were not significantly different ($p > 0.05$). Similarly, VCO supplementation for 14 days induced insignificant body weight change in rats. However, on day 17, it was observed that MTX administration induced marked decrease in body weight of rats in MTX control group compared to normal control.

Table 2

3.2. Effect of VCO supplementation on oxidative stress markers in MTX-induced toxicity in rats

Methotrexate administration produces significant adverse effect on the serum redox status which was evidenced by biochemical analyses. The activities of SOD, CAT and GPx were significantly ($p < 0.01$, $p < 0.05$) depressed in MTX-intoxicated rats. Reduced glutathione (GSH), a non-enzymatic tripetide antioxidant, markedly decreased in MTX-treated rats compared to normal control ($p < 0.01$). Similarly, malondialdehyde (MDA), a toxic product of lipid peroxidation appreciably increased in comparison to normal control ($p < 0.01$). However, the pretreatment of dietary supplementation with VCO to rats administered MTX attenuated the MTX-induced alterations in oxidative stress markers. It was observed that the restorative effect of VCO was higher in rats pretreated with 15% VCO supplementation.

3.3. Effect of VCO supplementation on pro-inflammatory markers in MTX-induced toxicity in rats

Regarding pro-inflammatory markers, administration of MTX caused significantly increased ($p < 0.01$, $p < 0.05$) serum levels of IL-6, CRP and NO in rats. The pretreatment with dietary supplementation of VCO prominently ($p < 0.01$, $p < 0.05$) attenuated the increase in levels of IL-6, CRP and NO induced by MTX. However, it was observed that NO reduced insignificantly in rats fed 5% VCO supplemented diet compared to MTX control. Also, decrease in levels of these markers was more pronounced in rats fed 15% VCO supplemented diet.

4. Discussion

There is currently an unmet need for adjuvant therapy to modulate anticancer drug-induced toxicity during chemotherapy. Clinicians face the challenge of adverse effects emanating from treating patients with cancer therapies.[25] Methotrexate is efficacious and unique in that it can be administered safely in a multitude of dosing strategies. However, the limitation of MTX in clinical use is its side effects associated with oxidative stress and inflammatory responses.[4] Pharmacological interventions to mitigate MTX toxicities, including leucovorin rescue, have not substantially yielded desired results.[26] Abundant evidence suggests that the antioxidant phytochemicals in natural products may abrogate oxidative toxicity as found in MTX chemotherapy.[4–6] The present study aimed at investigating the protective effect of dietary VCO supplementation against the development of MTX-induced toxicity in rats.

In the present communication, acute-single dose of MTX considerably reduced the rat weight after 3 days of its

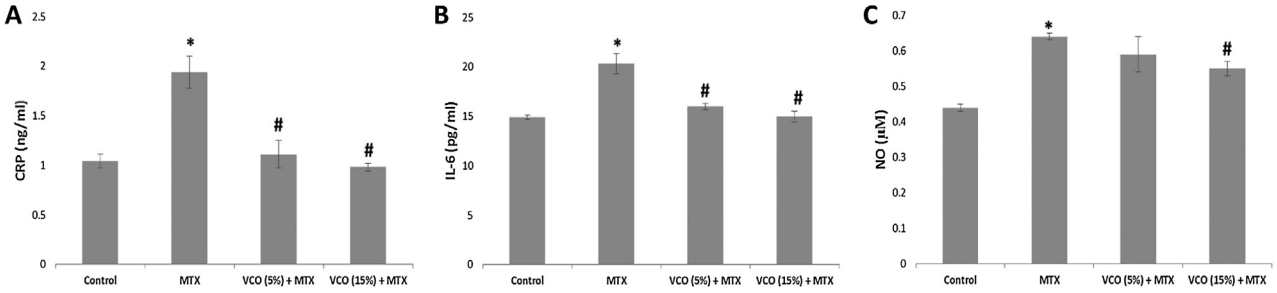

Figure 1 – (A) Effect of VCO supplementation on serum level of C-reactive protein (CRP) in MTX-administered rats. VCO = virgin coconut oil; MTX = Methotrexate. Values are expressed as mean ± SEM (n = 6). *Significant when compared to control ($p < 0.05$); #significant compared to MTX control ($p < 0.05$), (B) Effect of VCO supplementation on serum level of interleukin-6 (IL-6) in MTX-administered rats. VCO = virgin coconut oil; MTX = Methotrexate. Values are expressed as mean ± SEM (n = 6). *Significant when compared to control ($p < 0.01$); #significant compared to MTX control ($p < 0.01$), (C) Effect of VCO supplementation on serum level of nitric oxide (NO) in MTX-administered rats. VCO = virgin coconut oil; MTX = Methotrexate. Values are expressed as mean ± SEM (n = 6). *Significant when compared to control ($p < 0.01$); #significant compared to MTX control ($p < 0.05$).

administration. A number of systematic investigations on MTX toxicity found that MTX reduces body weight and organ weight insignificantly.[9,27] According to Miyazono et al., rat body weight decreased at 24 h after the MTX administration and after 48 to 72 h increased.[28] In this study, significant decrease in body weight was found after 3 days of MTX-induced toxicity corroborating the recent experimental report by Abd-allah and Sharaf el-din.[29] However, the reduction in weight may be associated with oxidative stress-mediated necrosis, cirrhosis and fibrosis in major organs, including the liver.[30,31]

Several studies have implicated increased generation of reactive oxygen and nitrogen species and the consequent impairment in antioxidant defense systems in drug-induced toxicity.[32] Oxidative stress and lipid peroxidation has emerged to play a critical role in the pathogenesis of MTX-induced toxicity. Interestingly, the single administration of MTX (20 mg/kg) to rats induced prominent alterations in antioxidant defense mechanisms. It was found that MTX markedly depressed serum activities of SOD, CAT and GPx. Further, it significantly reduced the level of GSH, a non-enzymatic antioxidant that detoxifies H_2O_2 and peroxides in association with GPx, glutathione reductase and glutathione-S-transferase.[9] SOD is an endogenous antioxidant enzyme that catalyzes dismutation of superoxide radical to H_2O_2. The antiradical activity of CAT is of great significance as it prevents the conversion of H_2O_2 to the most damaging oxygen radical, hydroxyl radical (•OH), and concomitantly breakdown H_2O_2 to water and molecular oxygen. GPx uses thiol-reducing power of GSH to reduce oxidized lipids, modulate hydroperoxides generation and protein targets of ROS.[33,34] It is well known that damage to these antioxidant molecules exacerbate oxidative stress and lipid peroxidation confirmed in this study by distinct rise in MDA level. The MTX mechanism for reducing antioxidant defense is unclear; current literature suggests that it reduces GSH and cellular NADPH leading to amplified depression in other antioxidant enzymes.[35] Clinical manifestations consistent with oxidative stress and lipid peroxidation have been confirmed in humans.[36] Our findings are in consonance with previous reports that MTX administration triggers oxidative stress in various tissues.[2–5,8] In this study, serum activities of SOD, CAT and GPx, and GSH level were remarkably elevated in rats pretreated with VCO supplemented diet (5% and 15%) compared to MTX control. In corollary, lipid peroxidation was prevented as demonstrated by marked decrease in MDA levels. A growing body of evidence indicates VCO as emerging functional food oil due to reported beneficial health effects associated with its antioxidant potentials.[37] Recent reports have shown the antioxidant capacity of VCO via *in vivo* and *in vitro* studies.[12,16] Although VCO is a saturated natural oil, its phytochemistry indicates the presence of potent natural antioxidants that could be responsible for the improvement in antioxidant defenses in the present study.[38]

Moreover, there is a robust body of evidence that free radical generation and oxidative stress is capable of eliciting systemic inflammatory responses.[3,39] A previous study reports that MTX induced small intestinal damage via mechanism of increased ROS and inflammation.[29] In the study, the inflamed intestine demonstrates oxidative stress and the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin 1β (IL-1β) were markedly elevated by MTX toxicity. In this study, the oxidative stress status induced by MTX significantly elevated the cytokine IL-6, the acute phase protein CRP and NO compared to normal control group. However, MTX has been implicated in the recruitment of immunocytes such as neutrophils and macrophages to tissues. In support, Alamir et al found that IL-1β and cytokine-induced neutrophil chemoattractant were markedly increased in MTX-treated rats.[40] NF-κB in macrophages is a redox-sensitive transcription factor such that the increased oxidative stress induced by MTX might have activated it for the release of proinflammatory cytokine IL-6 and consequent production of CRP found elevated in the current study.[6,8,39,40] Nitric oxide (NO) is a free radical gas with several pathophysiological functions.[18] It has been shown that MTX increases activity of inducible nitric oxide synthase (iNOS) resulting in increased NO pro-

Table 1 – Effect of VCO supplementation on initial and final body weight of rats administered MTX

Group	Body weight of rats (g)		
	Before supple (Day 1)	After suppl (Day 14)	After suppl (Day 17)
Control	128.4 ± 3.0	158.3 ± 3.2	161.8 ± 4.3
MTX	121.2 ± 2.9	151.4 ± 3.5	136.2 ± 2.3*
VCO (5%) + MTX	114.2 ± 2.1	142.7 ± 3.7	147.2 ± 2.9
VCO (15%) + MTX	115.4 ± 4.4	142.1 ± 6.9	138.9 ± 3.2

VCO: virgin coconut oil; MTX: Methotrexate; suppl: supplementation. Values are expressed as mean ± SEM (6 rats/group).
* Significant when compared to control group in the same column ($p < 0.05$).

Table 2 – Effect of VCO supplemented diet on serum oxidative stress markers- SOD, CAT, and GPx (U/mg protein), GSH (mg/g protein) and MDA (nmol/mg protein) of MTX-treated rats.

Group	SOD	CAT	GPx	GSH	MDA
Control	0.22 ± 0.01	9.83 ± 0.20	44.3 ± 1.70	4.50 ± 0.16	1.25 ± 0.30
MTX	0.18 ± 0.01*	7.46 ± 0.22*	19.6 ± 2.00*	3.36 ± 0.24*	2.18 ± 0.20*
VCO (5%) + MTX	0.22 ± 0.01#	8.67 ± 0.19#	32.2 ± 0.66#	3.91 ± 0.02#	0.93 ± 0.23#
VCO (15%) + MTX	0.23 ± 0.01#	10.2 ± 0.28#	34.5 ± 1.28#	4.29 ± 0.10#	1.02 ± 0.03#

VCO: Virgin coconut oil; MTX: Methotrexate; Values are mean + SEM (6 rats/group).
* $p < 0.01$: significant when compared to control group in the same column.
# $p < 0.01$: significant when compared to MTX group in the same column.

duction implicated in oxidative damage.[41] Our finding were in line with earlier studies that demonstrated proinflammatory activity of MTX.[29,40,41] Here, we have shown for the first time that VCO attenuates the proinflammation induced by MTX, as it significantly decreased IL-6, CRP and NO in rats administered MTX and pretreated with VCO (5% and 15%). The mechanism underlying the anti-inflammatory effect may be due to the VCO ability to reverse MTX-induced oxidative stress as indicated by increase in SOD, CAT, and GPx activities and GSH as well as evident decrease in MDA level in the current study. Secondly, dietary polyphenols are well reported to be modulators of inflammatory cascades to avert the cause of pathogenesis and demonstrate beneficial health effects.[42] Particularly, literature indicates that polyphenols triggers indirect mechanisms for the release of anti-inflammatory signaling molecules, such as IL-10 and decrease IL-1β-induced NF-??B p65 DNA binding activity counteracting inflammation.[42,43] Perceptibly, the bioactive polyphenols in VCO may modulate inflammatory networks resulting into decrease in inflammatory mediators found in this study. However, Hamsi et al had earlier reported that repeatedly heated VCO may not possess the anti-inflammatory effect.[17] The current beneficial health effects are in line with the previously reported antioxidant and anti-inflammatory effect of VCO against arthritis, cyclophosphamide, cardiovascular risk, insulin resistance and hepatic steatosis.[18,19,44–46] Further clinical studies would be necessary following the currently growing number of evidences supporting health benefits of VCO in preclinical models. There is a potential for VCO supplementation to be considered as a part of adjuvant strategies against side effects of MTX chemotherapy.

In conclusion, the present investigation demonstrated that the toxicity of MTX may be associated with impairment in antioxidant defense mechanism in addition to a perturbation of pro-inflammatory mediators which could be reversed by dietary supplementation of VCO. VCO supplementation may be beneficial in MTX chemotherapy by reducing oxidative stress and pro-inflammatory responses.

Conflict of interest

The authors declare no conflict of interest

References

1. Tran CD, Sundar S, Howarth GS. Dietary zinc supplementation and methotrexate-induced small intestinal mucositis in metallothionein-knockout and wild-type mice. *Cancer Biol Therapy* 2009;8(17):1662–7.
2. Daggulli M, Dede O, Utangac MM, Bodakci MN, Hatipoglu NK, Penbegul N, et al. Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. *Int J Clin Exp Med* 2014;7(12):5511–6.
3. Kelleni MT, Ibrahim SA, Abdelrahman AM. Effect of captopril and telmisartan on methotrexate-induced hepatotoxicity in rats: impact of oxidative stress, inflammation and apoptosis. *Toxicol Mech Meth* 2016;26(5):371–7.
4. Yuksel Y, Yuksel R, Yagmurca M, Haltas H, Erdamar H, Toktas M, Ozcan O. Effects of quercetin on methotrexate-induced nephrotoxicity in rats. *Human Exp Toxicol* 2017;36(1):51–61.
5. El-Sheikh AA, Morsy MA, Abdalla AM, Hamouda AZ, Alhaider IA. Mechanisms of thymoquinone hepatorenal protection in methotrexate-induced toxicity in rats. *Mediators Inflamm* 2015, http://dx.doi.org/10.1155/2015/859383.
6. Rashid S, Ali N, Nafees S, Ahmad ST, Hasan SK, Sultana S. Abrogation of 5-flourouracil induced renal toxicity by bee

propolis via targeting oxidative stress and inflammation in Wistar rats. *J Pharm Res* 2013;7:189–94.
7. Gu S, Wu Y, Yang J. Screening of cytoprotectors against methotrexate-induced cytogenotoxicity from bioactive phytochemicals. *Peer J* 2016;4:e1983, http://dx.doi.org/10.7717/peerj.1983.
8. Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, Yazdi M, Bonyadi F, Mohajeri D, et al. Preadministration of turmeric prevents methotrexate-induced liver toxicity and oxidative stress. *BMC Complement Altern Med* 2015;15:246–58.
9. Famurewa AC, Ufebe OG, Egedigwe CA, Nwankwo OE, Obaje GS. Virgin coconut oil supplementation attenuates acute chemotherapy hepatotoxicity induced by anticancer drug methotrexate via inhibition of oxidative stress in rats. *Biomed. Pharmacother* 2017;87:437–42.
10. Erboga M, Aktas C, Erboga ZF, Donmez YB, Gurel A. Quercetin ameliorates methotrexate-induced renal damage, apoptosis and oxidative stress in rats. *Ren Fail* 2015;37(9):1492–7.
11. Tag HM. Hepatoprotective effect of mulberry (*Morus nigra*) leaves extract against methotrexate induced hepatotoxicity in male albino rats. *BMC Complement Altern Med* 2015;15:252.
12. Famurewa AC, Ekeleme-Egedigwe CA, Nwali SC, Agbo NN, Obi JN, Ezechukwu GC. Dietary supplementation with virgin coconut oil improves lipid profile and hepatic antioxidant status and has potential benefits on cardiovascular risk indices in normal rats. *J Diet Suppl* 2017, http://dx.doi.org/10.1080/19390211.2017.1346031.
13. Kumar Y, Yadav DN, Ahmad T, Narsaiah K. Recent trends in the use of natural antioxidants for meat and meat products. *Compr Rev Food Sci Food Saf* 2015;14(6):796–812.
14. Marina AM, Chem-Man YB, Amin I. Virgin coconut oil: emerging functional food oil Trends. *Food Sci Tech* 2009;20:481–7.
15. Srivastava Y, Semwal AD, Majumdar A, Yildiz F. Quantitative and qualitative analysis of bioactive components present in virgin coconut oil. *Cogent Food Agric* 2016;2(1):1164929, http://dx.doi.org/10.1080/23311932.2016.1164929.
16. Illam SP, Narayanankutty A, Raghavamenon AC. Polyphenols of virgin coconut oil prevent pro-oxidant mediated cell death. *Toxicol Mech Meth* 2017;27(6):442–50.
17. Hamsi MA, OthmanF, Das S, KamisahY, Thent ZC, Qodriyah HM, et al. Effect of consumption of fresh and heated virgin coconut oil on the blood pressure and inflammatory biomarkers: an experimental study in Sprague Dawley rats. *Alexandria J Med* 2015;51:53–63.
18. Vysakh A, Ratheesh M, Rajmohanan TP, Pramod C, Premlal S, Girish kumar B, et al. Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. *Int Immunopharmacol* 2014;20:124–30.
19. Nair SS, Manalil JJ, Ramavarma SK, Suseela IM, Thekkepatt A, Raghavamenon AC. Virgin coconut oil supplementation ameliorates cyclophosphamide-induced systemic toxicity in mice. *Human Exp Toxicol* 2016;35:205–12.
20. National Institutes of Health (NIH). *Guide for the care and use of laboratory animals*. Bethesda: National Institutes of Health; 1985.
21. Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clin Biochem* 2004;37:830–5.
22. Wallin B, Rosengren B, Shetzer HG, Cameja G. Lipid oxidation and measurement of thiobarbituric acid reacting substances (TBARS) formation in a single microtitre plate: its use for evaluation of antioxidants. *Anal Biochem* 1993;208: 10–5.
23. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids. *Anal Biochem* 1982;126:131–8.
24. Andersen HC, McCarthy M. Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever. *Am J Med* 1950;8:445–55.
25. Curigliano G, Cardinale D, Dent S, Criscitiello C, Aseyev O, Lenihan D, et al. *CA Cancer J Clin* 2016;66:309–25.
26. Wiczer T, Dotson E, Tuten A, Phillips G, Maddocks K. Evaluation of incidence and risk factors for high-dose methotrexate-induced nephrotoxicity. *J Oncol Pharm Practice* 2016;22(3):430–6.
27. Dalaklioglu S, Genc GE, Aksoy NH, Aksit F, Gumuslu S. Resveratrol ameliorates methotrexate-induced hepatotoxicity in rats via inhibition of lipid peroxidation. *Hum Exp Toxicol* 2013;32:662–71.
28. Miyazono Y, Gao F, Horie T. Oxidative stress contributes to methotrexate- induced small intestinal toxicity in rats. *Scand J Gastroenterol* 2004;39:1119–27.
29. Abd-Allah OM, Sharaf El-Din AA. The possible protective effect of ginger against intestinal damage induced by methotrexate in rats. *Med J Cairo Univ* 2013;81(1): 1073–84.
30. Jitendra V, Yagnik B, Shivkumar R. Drug induced hepatotoxicity: effect of polyherbal formulation. *Pharmacog Mag* 2009;4(19):232–7.
31. West SG. Methotrexate hepatoxicity. *Rheum Dis Clin North Am* 1997;24:883–915.
32. Fallahzadeh AR, Rezaei Z, Rahimi HR, Barmak MJ, Sadeghi H, Mehrabi S, et al. Evaluation of the effect of pentoxifylline on cisplatin-induced testicular toxicity in rats. *Toxicol Res* 2017;33(3):255–63.
33. Veskoukis AS, Tsatsakis AM, Kouretas D. Dietary oxidative stress and antioxidant defense with an emphasis on plant extract administration. *Cell Stress Chaperones* 2012;17:11–21.
34. Rizwan S, Naqshbandi A, Farooqui Z, Khan AA, Khan F. Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney. *Food Chem Toxicol* 2014;68:99–107.
35. Vardi N, Parlakpinar H, Cetin A, Erdogan A, Ozturk IC. Protective effect of beta-carotene on methotrexate-induced oxidative liver damage. *Toxicol Pathol* 2010;38:92–597.
36. Iqal S, Armaghani A, Aiyer R, Kazory A. Methotrexate nephrotoxicity: Novel treatment, new approach. *J Oncol Pharm Practice* 2013;19(4):373–6.
37. Marina AM, Chem-Man YB, Amin I. Virgin coconut oil: emerging functional food oil. *Trends Food Sci Tech* 2009;20:481–7.
38. Srivastava Y, Semwal AD, Majumdar A, Yildiz F. Quantitative and qualitative analysis of bioactive components present in virgin coconut oil. *Cogent Food Agric* 2016;2(1):1164929.
39. Afolabi OK, Oyewo EB, Adekunle AS, Adedosu OT, Adedeji AL. Impaired lipid levels and inflammatory response in rats exposed to cadmium. *EXCLI J* 2012;11:677–87.
40. Alamir I, Boukhettala N, Aziz M, Breuillí D, Déchelotte P, Coëffier M. Beneficial effects of cathepsin inhibition to prevent chemotherapy-induced intestinal mucositis. *Clin Exp Immunol* 2010;162:298–305.
41. Leitão RF, Brito GA, Oriá RB, Braga-Neto MB, Bellaguarda EA, Silva JV, et al. Role of inducible nitric oxide synthase pathway on methotrexate-induced intestinal mucositis in rodents. *BMC Gastroenterol* 2011;11:90–9.
42. Li Z, Geng Y-N, Jiang J-D, Kong W-J. Antioxidant and anti-inflammatory activities of berberine in the treatment of

diabetes mellitus. *Evid Based Complement and Alternat Med* 2014, http://dx.doi.org/10.1155/2014/289264.
43. Maraldi T, Vauzour D, Angeloni C. Dietary polyphenols and their effects on cell biochemistry and pathophysiology 2013. *Oxid Med Cell Longev* 2014, http://dx.doi.org/10.1155/2014/576363.
44. Jaarin K, Norliana M, Kamisah Y, Nursyafiza M, Qodriyah HM. Potential role of virgin coconut oil in reducing cardiovascular risk factors. *Exp Clin Cardiol* 2014;20(8):3399–410.
45. Narayanankutty A, Mukesh RK, Ayoob SK, Ramavarma SK, Suseela IM, Manalil JJ, et al. Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats. *J Food Sci Technol* 2016;53(1):895–901.
46. Narayanankutty A, Palliyil DM, Kuruvilla K, Raghavamenon AC. Virgin coconut oil reverses hepatic steatosis by restoring redox homeostasis and lipid metabolism in male Wistar rats. *J Sci Food Agric* 2017, http://dx.doi.org/10.1002/jsfa8650.