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Virgin coconut oil protects against liver damage in albino rats challenged with the anti-folate combination, trimethoprim-sulfamethoxazole

Abstract

Background: Trimethoprim-sulfamethoxazole (TMP-SMX) is a broad-spectrum antibiotic. However, its use is associated with toxic reactions. Virgin coconut oil (VCO), derived from coconut, has been widely used throughout history for its medicinal value. The aim of this study was to investigate the beneficial actions of VCO against TMP-SMX-induced alterations in serum biochemical end points.

Methods: Twenty rats were divided into four groups. Group 1 (control) received no drug, whereas group 2 received TMP-SMX (8/40 mg/kg) twice daily for 7 days. Group 3 was administered coconut oil at a dose of 600 mg/kg body weight per day. The last group was treated with TMP-SMX (8/40 mg/kg) and coconut oil (600 mg/kg) simultaneously. Blood samples were collected from all groups on the 8th day of the experiment for measurement of serum biochemical parameters. Organ weights and coefficients were also evaluated.

Results: TMP-SMX caused a significant ($p<0.05$) increase in the levels of serum total bilirubin, lactate dehydrogenase, and alkaline phosphatase by 192%, 67%, and 41%, respectively, relative to controls. This was followed by a significant reduction in triglyceride and relative kidney weight by 40% and 7%, respectively. There were no significant differences ($p>0.05$) in the activities of serum aminotransferases, total acid phosphatase, γ-glutamyl transferase, uric acid, cholesterol, albumin, and urea levels. Supplementation of VCO ameliorated TMP-SMX-induced effects by restoring the levels of total bilirubin, alkaline phospahatase, and lactate dehydrogenase.

Conclusions: The results of this study demonstrate that the active components of coconut oil had protective effects against the toxic effects induced by TMP-SMX administration, especially in the liver of rats.

Keywords: anti-folate; coconut oil; kidney; liver; serum biochemistry; testis; trimethoprim-sulfamethoxazole.

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Introduction

Trimethoprim-sulfamethoxazole (TMP-SMX) is a commonly used antibiotic for respiratory, gastrointestinal, and urinary tract infections caused by a range of aerobic Gram-positive and Gram-negative bacteria. It also has activity against *Listeria monocytogenes*, *Nocardia*, and *Pneuomcystis jiroveci* [1]. Daily prophylaxis with TMP-SMX has been shown to reduce morbidity and improve survival in HIV-infected patients [2]. TMP-SMX inhibits folate production in bacteria by blocking bacterial dihydrofolic acid reductase, which causes reduced production of purines and subsequently DNA [3]. Earlier, rashes and gastrointestinal distress were believed to be the most frequent side effects of the drug until a reported case of hyperkalemia [4]. Furthermore, researchers have demonstrated the hepatotoxic, nephrotoxic, and genotoxic effects of the antibiotic [5–7].

Coconut oil, derived from coconut (*Cocos nucifera* L), has been widely used throughout history for its medicinal value and as food, food ingredient, and functional food [8]. It consists of a mixture of triglycerides containing a high proportion of medium-chain saturated fatty acids, especially myristic acid [9]. Virgin coconut oil (VCO) is obtained from fresh, mature coconut kernel without the use of heat and without undergoing refining process [10]. This retains the important biologically active components in the oil such as antioxidant vitamins and phenolic compounds. The potential benefits of this oil in preventing or ameliorating different biological conditions due to its active polyphenol components has been demonstrated. Supplementation of the diet with VCO has been shown

to reduce the cholesterol and triglyceride levels, maintain blood coagulation factors, and prevent oxidation of low-density lipoprotein lipids [11, 12]. Also, VCO has been reported to have anticancer, antimicrobial, anti-inflammatory, and hepatoprotective properties [13–16]. Furthermore, Hayatullina and coworkers [17] reported that VCO effectively improved bone structure and prevented bone loss in an animal model of osteoporosis.

Serum biochemical parameters, when employed accurately, can provide important and useful information in assessing not only the extent and severity of liver damage but also the type of liver damage and can differentiate between membrane injury vs. cholestasis and hepatic function [18]. In this study, we researched the effects of VCO on serum biochemical parameters and organ weights of Wistar rats treated with TMP-SMX.

Materials and methods

Chemicals

TMP-SMX (Bactrim) was obtained from the Redeemer's University Pharmacy, located at Mowe, Ogun State, Nigeria. The serum biochemical parameters, namely, albumin, urea, bilirubin, uric acid, γ-glutamyl transferase (GGT), triglyceride, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and acid phosphatase (AcP) were measured using diagnostic kits.

Virgin coconut oil

VCO by the trade name Aquila® was obtained from a dealer in Redemption City, Mowe, Ogun State, Nigeria. According to the producers, Aquila® is made from fresh mature coconuts through the process of cold press without additives. At room temperature, pure coconut oil is liquid in nature but solidifies at temperatures below 24°C. This serves as a confirmatory test of the purity of the oil.

Animals and treatment

Twenty male adult albino rats of the Wistar strain, weighing between 120 and 160 g, were used in this study. Animals were obtained from the primate colony of the Department of Veterinary Anatomy, University of Ibadan. Rats were fed a commercial pelleted diet (Ladokun Feeds Ibadan, Nigeria) and drinking water ad libitum. They were also maintained under standard laboratory conditions and subjected to a natural photoperiod of 12 h light/12 h dark cycle. Rats were randomly assigned into four groups of five rats each. Rats in group A served as controls and received distilled water. Group B rats were treated with TMP-SMX at the dose of 8/40 mg/kg body weight twice daily, representing the human therapeutic dose. Rats in group C were treated with VCO at a dose of 600 mg/kg body weight per day [19]. The last group was treated with TMP-SMX (8/40 mg/kg) and coconut oil (600 mg/kg) simultaneously. All treatments were given via the oral route twice daily for 7 consecutive days. The animals were sacrificed 24 h after the last treatment. All experiments conformed to guidelines governing the handling of laboratory animals as outlined by the Redeemer's University Committee on Ethics for Scientific Research.

Preparation of samples

Rats were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture into clean, dry centrifuge tubes. Blood samples were centrifuged at 3000 *g* for 10 min on a laboratory centrifuge (Heraeus Labofuge 300, Thermo Scientific, Hampshire, UK). Serum was carefully separated out and stored frozen until required for analysis. The liver, lungs, testes, and kidney tissues were excised, transferred into ice-cold 0.25 M sucrose solution, blotted with clean tissue paper, and weighed.

Serum biochemical analysis

Biochemical analyses were carried out to determine the serum concentrations of albumin, urea, bilirubin, uric acid, triglyceride, cholesterol, AST, ALT, ALP using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, UK), GGT, LDH, and AcP using Cypress Diagnostic kits (Cypress Diagnostics, Leuven, Belgium) according to the protocols described in the manufacturers' manual.

Data analysis

All data were expressed as mean±standard error of the mean (SEM). Differences between the groups were determined by one-way analysis of variance (ANOVA), and post hoc testing was performed for intergroup comparisons using Tukey's test (Graph Pad Prism version 3.00; Graph Pad Software Inc., San Diego, CA, USA). Values were regarded as significantly different at $p<0.05$.

Results

Effect of VCO on relative organ weights and liver function markers in TMP-SMX-treated rats

Administration of TMP-SMX did not significantly ($p>0.05$) alter the relative weights of the liver, lungs, and testes of the animals when compared with controls. However, TMP-SMX alone and TMP-SMX plus VCO administration caused a significant ($p<0.05$) decrease in the relative weights of kidneys when compared with controls (Table 1). The results presented in Table 2 show insignificant ($p>0.05$) differences in the activities of AST, ALT, GGT, and total acid phoshatase (TAP), as well as levels of

Table 1 Effect of coconut oil on organ weights and their coefficients in rats treated with TMP-SMX.

	KW, g	Kidney coefficient	LW, g	Liver coefficient	LGW, g	Lung coefficient	TW, g	Testes coefficient
A	0.74±0.01	0.77±0.01	3.86±0.26	3.97±0.23	0.80±0.08	0.84±0.09	0.84±0.09	0.85±0.11
B	0.82±0.02	0.72±0.01[a]	4.35±0.17	3.83±0.15	0.85±0.07	0.76±0.06	1.04±0.14	0.92±0.13
C	0.80±0.02	0.67±0.02[a]	4.56±0.29	3.77±0.24	1.02±0.35	0.85±0.29	1.28±0.34	1.06±0.28
D	0.74±0.02	0.60±0.02[a]	4.35±0.31	3.96±0.17	1.05±0.07	0.84±0.06	1.07±0.19	0.86±0.16

Values are expressed as mean±SEM, n=5. [a]$p<0.05$, significantly different from the control.

albumin in TMP-SMX-treated rats relative to controls. In contrast, TMP-SMX intoxication increased total bilirubin and ALP values by 192% and 41%, respectively, when compared with the control. Coadministration with VCO restored these serum parameters to near-normal values.

Effect of VCO on some serum marker enzymes in TMP-SMX-treated rats

Table 2 reveals the effect of VCO on some other serum marker enzymes in TMP-SMX-treated rats. There was no significant change between the GGT and TAP activities in the control and TMP-SMX-treated groups and between the TMP-SMX-treated and the TMP-SMX plus VCO-treated groups. TMP-SMX, however, produced a significant ($p<0.05$) increase in LDH activity by 67% when compared with the control. VCO significantly ($p<0.01$) ameliorated this TMP-SMX-induced enzyme abnormality.

Effect of VCO on kidney function markers and lipid profile parameters in TMP-SMX-treated rats

Administration of TMP-SMX to rats produced a slight reduction in uric acid and urea levels when compared with the control group. However, supplementation with VCO did not attenuate these reductions. Furthermore, TMP-SMX treatment did not significantly alter the total cholesterol levels when compared with the control. Coadministration with VCO elicited a 26% increase in total cholesterol levels, although this was not significant when compared with the control. However, TMP-SMX significantly ($p<0.01$) reduced triglyceride levels when compared with the control. Supplementation with VCO did not significantly ($p>0.05$) attenuate the levels of triglycerides in comparison with the control (Table 2).

Discussion

TMP-SMX is a broad-spectrum antibiotic used to treat a variety of infections. Due to its efficacy, its ease of dosing, and relatively low expense, it has become a popular choice among many physicians. The aim of the present study was to demonstrate the efficacy of VCO in the modulation of altered serum biochemical variables in TMP-SMX-treated rats. Major findings of this study are that TMP-SMX induced liver damage in the treated animals as evidenced by elevated serum bilirubin levels and LDH and ALP activities. Furthermore, data revealed reduction in triglyceride levels in TMP-SMX-treated rats. Interestingly, our results indicate that administration of VCO at a dose of 600 mg/kg reduced

Table 2 Effect of coconut oil on some serum enzymes in rats treated with TMP-SMX.

Parameters	Control	TMP-SMX	VCO	TMP-SMX+VCO
AST, U/l	116±2.08	114.9±1.46	113.2±7.27	115.1±3.13
ALT, U/l	59.31±2.02	62.25±0.63	58.91±0.26	56±2.80
Albumin, g/L	2.63±−0.28	2.87±0.12	1.76±0.13	2.53±0.54
Total bilirubin, mg/dL	0.13±0.12	0.38±0.12[a]	0.38±0.05	0.09±0.05[b]
Uric acid, mg/dL	2.08±0.70	1.56±0.34	1.33±0.13	1.85±0.30
Triglyceride, mg/dL	203.8±19.98	122.6±3.70[a]	133±9.67	162.2±28.0
Cholesterol, mg/dL	153.7±12.95	131.9±27.67	83.50±9.96	167.1±4.79
Urea, mg/dL	474±94.39	453.3±22.41	288.9±3.65	364.2±55.0
ALP, U/l	131.8±16.5	185.6±23.41[a]	47.9±6.50[a]	165.6±31.9[a]
LDH, U/l	43.95±8.99	73.62±13.51[a]	19.78±6.60[b]	25.55±3.40[b]
TAP, U/l	1.37±0.34	1.71±0.22	0.85±0.0	1.92±1.07
GGT, U/l	3.13±0.95	3.43±0.66	2.80±0.42	2.98±0.60

Values are expressed as mean±SEM, n=5. [a]$p<0.05$, significantly different from the control. [b]$p<0.05$, significantly different from TMP-SMX.

the levels of bilirubin, LDH, and ALP as well as improved the triglyceride level of TMP-SMX-treated animals. These results demonstrate the possible hepatoprotective effect of VCO against TMP-SMX-induced liver damage with cholestasis in rats. The beneficial effect of this oil in preventing or ameliorating different biological conditions may be due to its active polyphenol components.

It has been reported that increase or decrease in either absolute or relative weight of an organ after administration of a chemical or drug is an indication of the toxic effect of that chemical [20]. Administration of VCO and TMP-SMX did not significantly alter the relative weights of liver, lungs, and testes of the animals when compared with controls. This is in contrast with a report that pretreatment with VCO significantly reversed the increased weight of the liver in paracetamol-treated rats relative to their controls [16]. Elevated ALP level may indicate cholestasis (partial or full blockade of the bile ducts). Because bile ducts bring bile from the liver into the gall bladder and intestine, inflammation or damage to the liver can cause spillage of ALP into the blood stream. Increase in ALP and GGT are indicative of intrahepatic cholestasis [21]. In our study, we found that TMP-SMX caused slight but insignificant increase in the activity of serum GGT. In addition, a significant increase in serum ALP activity monitored in the same animals was observed when compared with control animals. This is a confirmation that synthesis and release of ALP increases in association with impaired bile flow [22]. High serum ALP levels coupled with fairly normal ALT and AST levels, as observed in this study, is indicative of obstruction of the bile duct. This is consistent with reports describing TMP-SMX-induced liver damage to include hepatocellular and mixed hepatocellular cholestatic injury [1]. Significant reduction in ALP levels in the VCO-treated groups indicates that the oil was able to offer protection to the liver against TMP-SMX-induced hepatotoxicity.

Bilirubin is a yellow pigment produced when heme is catabolized. In this study, we observed a threefold increase in serum total bilirubin levels in the TMP-SMX-treated rats compared with the control rats. This consequent hyperbilirubinemia may result from ineffective erythropoiesis, impaired ability of the liver to excrete normal amounts of bilirubin, or obstruction of excretory ducts of the liver [23]. The significant reduction in the level of total bilirubin in the serum of TMP-SMX-pretreated rats suggested the hepatoprotective potential of VCO against TMP-SMX-induced hepatotoxicity.

LDH is widely distributed throughout the body; cellular damage causes an elevation of the total serum LDH. When disease or injury affects tissues containing LDH, the cells release LDH into the bloodstream, where it is identified in higher than normal level. TMP-SMX administration elicited a significantly increased LDH activity in the rat serum and this may be attributed to hepatocellular necrosis as a result of leakage of the enzyme into the blood stream [24]. Reduction in the level of LDH in the VCO-treated rats compared to the TMP-SMX-treated rats is indicative of the protective effect of the oil.

Clinically, decreased triglyceride and very low density lipoprotein (VLDL)-cholesterol levels signal the presence of parenchymal liver diseases [25]. In the present study, VCO and TMP-SMX administered separately caused decreases in the triglyceride and total cholesterol levels in rat serum. Animal studies have shown that coconut oil lowered total cholesterol, triglycerides, and phospholipids [11], the reason being that coconut oil is composed of medium chain fatty acids which are rapidly metabolized in the liver into energy and as such do not participate in the biosynthesis and transport of cholesterol [26]. Supplementation of TMP-SMX treatment with VCO did not significantly attenuate the levels of triglycerides and total cholesterol in comparison with the control.

Furthermore, it has been suggested that increase in the activity of AcP in the blood might be due to the necrosis of the liver, kidney, and lung [27]. Although there was a slight increase in TAP activity in TMP-SMX-treated rats, there was no significant change between the TAP activities in the control and TMP-SMX-treated groups and between the TMP-SMX-treated group and the TMP-SMX plus VCO-treated groups.

Although the relative kidney weight size was significantly decreased in all the test rats compared with that in the control groups, TMP-SMX administration did not elicit any statistically significant change in the mean serum urea and uric acid levels relative to the control group. This is consistent with a report that aminoglycoside antibiotics did not change uric acid level in rats [28]. It is plausible that the kidney might not be damaged to such an extent that it affected urea and uric acid levels, but the reduction in relative organ weight may indicate a mild nephrotoxic effect [6].

Conclusions

Our data have shown that TMP-SMX is capable of producing alterations in serum biochemical parameters indicative of liver damage with cholestasis in rats. Our findings also strongly suggest that VCO has potential as a preventive and therapeutic agent for cholestasis and deserves clinical trial in the near future to examine its efficacy on hepatotoxicity induced by commonly used antibiotics.

Conflict of interest statement

Author's conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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