Original Research

Exploring the trichloroacetic acid-induced toxicity on the hepato-renal system and intervention by virgin coconut oil-rich diet

Ajeigbe, Kazeem Olasunkanmi [1*], Oladokun, Olayemi Olutobi [2]

Abstract

Virgin coconut oil (VCO) is known for many beneficial health effects associated with its phenolic acids and flavonoid contents. We investigated the mechanisms underlying the antioxidative, anti-inflammatory, and anti-apoptotic mechanisms of Virgin Coconut oil-rich diet in treating trichloroacetic acid (TCA)-induced hepatic and renal damage in rats. Rats received TCA (250 mg/Kg b.wt, p.o) for ten days, followed by 5%, 10% or 15% VCO per gram feed for twenty-one days. Serum liver enzymes, urea, creatinine, tissue oxidative stress parameters, and inflammatory and apoptotic markers were then evaluated along with histological examination. TCA raised serum transaminases (ALT, AST), alkaline phosphatase (ALP), total bilirubin, urea and creatinine levels, which were abrogated by a VCO-rich diet dose-dependently. The activity of superoxide dismutase, catalase, glutathione peroxidase and nuclear factor erythroid 2-related factor 2 in the liver and kidney were enhanced, while malondialdehyde, tumour necrosis factor-α, interleukin-1β, nuclear factor-kB level hitherto increased by TCA were quashed by the VCO- rich diet ($p<0.05$). Similarly, the augmented level of Caspase-3 in the organs exposed to TCA was downregulated in favour of significantly increased BCl-2. Further, histomorphometry data validated the biochemical findings observed for the anti-inflammatory and anti-apoptotic potentials of VCO. Hepatocyte ballooning, pleomorphism and vascular congestion in the liver, loss of tubular architecture, tubular congestion and leukocyte infiltration in the kidney, all occasioned by TCA-intoxication, were evidently mitigated. Virgin coconut oil-rich diet could ameliorate liver and renal injury associated with trichloroacetic acid exposure via antioxidative, anti-inflammatory and anti-apoptotic mechanisms.

Keywords

Virgin coconut oil, diet, oxidative stress, anti-inflammation, anti-apoptosis

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Raziskovanje toksičnosti, ki jo povzroča trikloroocetna kislina na hepato-renalni sistem, in posredovanje prehrane, bogate z deviškim kokosovim oljem

Izvleček

Deviško kokosovo olje (VCO) je znano po številnih blagodejnih učinkih na zdravje, povezanih z vsebnostjo fenolnih kislin in flavonoidov. Raziskali smo mehanizme, na katerih temeljijo antioksidativni, protivnetni in anti-apoptotični mehanizmi prehrane, bogate z deviškim kokosovim oljem, pri zdravljenju s trikloroocetno kislino (TCA) povzročene poškodbe jeter in ledvic pri podganah. Podgane so prejemale TCA (250 mg/kg telesne teže, p.o.) deset dni, čemur je sledil 5 %, 10 % ali 15 % VCO na gram krme enaindvajset dni. Nato smo skupaj s histološkim pregledom ocenili serumske jetrne encime, sečnino, kreatinin, parametre tkivnega oksidativnega stresa, vnetne in apoptotične markerje. TCA je zvišala serumske transminaze (ALT, AST), alkalno fosfatazo (ALP), skupni bilirubin, sečnino in raven kreatinina, ki jih je prehrana, bogata z VCO, odpravila odvisno od odmerka. Aktivnost superoksid dismutaze, katalaze, glutation peroksidaze in faktorja 2, povezanega z jedrnim faktorjem eritroid 2, v jetrih in ledvicah je bila okrepljena, medtem ko so bili malondialdehid, faktor tumorske nekroze-α, interlevkin-1β, raven jedrnega faktorja-kB, ki je bila doslej povečana s TCA, zmanjšani s prehrano, bogato z VCO ($p<0,05$). Podobno je bila povečana raven kaspaze-3 v organih, izpostavljenih TCA, znižana v korist znatno povečanega BCl-2. Poleg tega so histomorfometrijski podatki potrdili biokemične ugotovitve, opažene za protivnetni in anti-apoptotični potencial VCO. Baloniranje hepatocitov, pleomorfizem in vaskularna kongestija v jetrih ter izguba tubularne arhitekture, tubularna kongestija in infiltracija levkocitov v ledvicah, ki so bili posledica zastrupitve s TCA, so bili očitno omilili. Prehrana, bogata z deviškim kokosovim oljem, bi lahko izboljšala poškodbe jeter in ledvic, povezane z izpostavljenostjo trikloroocetni kislini, prek antioksidativnih, protivnetnih in anti-apoptotičnih mehanizmov.

Ključne besede

deviško kokosovo olje, prehrana, oksidativni stres, proti vnetju, proti apoptozi

Introduction

Natural products have always been used for the treatment and prevention of various diseases and illnesses. Coconut (Cocos nucifera) oil is another type of natural product derived from coconut, which is an edible plant with a wide range of benefits due to its components (Ibrahim et al., 2020). It can also serve as a food source (Kabara, 2000). Unlike conventional coconut oil, virgin coconut oil (VCO) is an unrefined, unbleached and undeodorized oil (Raghavendra and Raghavarao, 2010; Ibrahim et al., 2020). It is rich in medium-chain fatty acid (MCFA), vitamins (like riboflavin, niacin, vitamin E), carbohydrates, and etcetera (Marina et al., 2009). Researchers have shown that VCO has more nutritional value/benefit than the coconut oil produced conventionally (Buderwitz 2013). Several studies have expressed the anti-inflammatory and anti-nociceptive (Zakaria et al., 2011), anti-hypercholesterolemic (Zakaria et al., 2010), anti-stress (Yeap et al., 2015), antimicrobial, immunomodulatory and even antiviral effects of VCO (Hartono et al., 2022).

The liver is an important organ in the body due to its numerous functions, which include red blood cell (RBC) formation, digestion, clearance/detoxification, etc. These hepatocytes remain connected by adherents and desmosomes, intercellular tightness, and gap junctions (Spray et al., 2013). Disruptions to these cells destroy their integrity and affect the normal functioning of the organ and system (Lala et al., 2022). Likewise, the renal system is important in filtering and eliminating waste products, and it is the primary course of elimination of toxicants like TCA (Schultz, 1999; Yu et al., 2000). Both the liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and these functions are challenged by a wide array of toxicants due to environmental exposure, including trichloroacetic acid.

Trichloroacetic acid (C2HCl3O2), a deliquescent crystallized substance with a relative molecular mass of 163.39 with a slight characteristic odour (O'Neil et al., 2006), dissolves in water and most organic solvents like acetone, benzene, methanol, and o-xylene (Morris and Bost, 2002). Its spectroscopy data was earlier reported by West and Astle (1985). It is classified as a haloacetic acid and was introduced as a selective herbicide in the late 1940s (Zhang et al., 2019). It is a chemical substance that can be found in many things; hence, its inconspicuous ingestion is high. In dermatology, TCA is used as a photoaging treatment (Sitohang et al., 2021). It acts as a moderate-deep chemical peel, rejuvenating the skin (Fischer et al., 2010). Moreover, trichloroacetic acid is used in analytical methods. Its use is effective in preparing samples by denaturing proteins in samples (Huang et al., 2014). It is also seen as a convenient catalyst for some chemical reactions (Karimi-Jaberi and Moaddeli, 2012).

Like other haloacetic acids, TCA is a by-product of water chlorination (Singer et al., 1995), a significant metabolite for trichloroethylene and perchloroethylene (El Arem et al., 2013) and a wide-spectrum pesticide for agricultural practices (Culloch, 2002). Its toxicity damages the structural and functional aspects of organs in the body, which include but are not limited to the kidneys and the liver, have all been well enunciated (Acharya et al., 1997; Pereira et al., 2001; El Arem et al., 2013). Unfortunately, injury to one or more organs in the body can lead to alteration in the body's functions. TCA is hematotoxic (Celik et al., 2009), neurotoxic and immunotoxic (Celik et al., 2010). Goldberg et al., 1990 reported in a human study an association between gestational exposure to TCA and increased risk for congenital heart defects (CHD) in offspring. Similar cardiac malformations were observed during subsequent studies performed on rat embryos exposed to TCA (Johnson et al., 2003). Likewise, pre-neoplastic lesions and hepatocyte ballooning with extensive vacuolation have equally been associated with TCA-induced experimental hepatocarcinogenesis (Mokhamer et al., 2022).

Further epidemiological findings have shown that there is a link between ingestion of water containing 'disinfection by-products' (like TCA) and a high rate of abortions, birth defects, and cancers (Aslani et al., 2019). US Environmental Protection Agency has equally considered TCA to be a human carcinogen (EPA, 2011), even though most of the studies that alluded to the carcinogenic property of TCA are in mice and rats.

The underlying mechanisms of TCA intoxication include an increase in oxidative stress and inflammation (Abdel-Hamid et al., 2011), DNA hypomethylation, peroxisome proliferation, oncogene activation, cell proliferation, and inhibition of intercellular communication (Harmon et al., 2011).

This study investigated the mechanisms by which Virgin coconut oil-rich diet (with varied percentages) institutes its antioxidant, anti-inflammatory, and anti-apoptotic potentials against the toxic effect of trichloroacetic acid on the liver and kidney of the experimental animals.

Methods

Drugs, Chemicals and Virgin Coconut Oil

Cold-pressed Virgin coconut oil was procured from Rutzjah Oil, a local manufacturer duly registered by Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC). Trichloroacetic acid was purchased from Sigma Aldrich®, St Louis, MI, USA. All other laboratory chemicals and reagents used are of analytical grade and procured from the UK.

Experimental Animals and Design

Thirty-five (35) male rats were purchased and acclimatized for seven days. These animals were randomly grouped into five groups (n=7), and a negative control group was treated with normal saline for the experimental period. Group I: 1mL/kg of normal saline while Groups II-V were treated with TCA (250mg/kg, p.o.) by gavage for ten days, then fed with varying doses of virgin coconut oil diet for another 21 days ad libitum. Group III: TCA+5% of VCO/g of feed; Group IV: TCA+10% of VCO/g of feed; and Group V: TCA+15% of VCO/g of feed. The negative (Group I) and positive (Group II) control groups received normal saline and normal rat feed for a total of 31 days. Studies on animal experimentation were done following the Current Animal Care Regulations and Standards approved by (ILAR, 2011) and protocols approved by the Animal Ethics Committee of the College of Medicine, Federal University, Oye-Ekiti, Nigeria (ID: FUOYE/AECCM/PHS/2022/016).

The preparation of the 5%, 10% and 15% virgin coconut diet was achieved by mixing and mashing the 50g, 100g and 150g of virgin coconut oil with 950g, 900g and 850g of normal rat chow, respectively until there was homogeneity.

Biochemical Analysis

Blood was collected by cardiac puncture, then serum bilirubin levels (T.Bil), liver and renal function biomarkers, ALT, AST, ALP, urea, and creatinine were estimated using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK). The serum (0.1 mL) was mixed with 0.5 mL of phosphate buffer (L-alanine) and (L-aspartate) for ALT and AST, respectively. The mixture was incubated for 30 min at 37 °C, and then 0.5 mL of 2,4-dinitrophenylhydrazine was added and vortexed. The mixture was allowed to stand for 20 min at room temperature, then 5 mL of 0.4 mol/L sodium hydroxide was added, and the absorbance of the solution was read after 5 min at a wavelength of 546 nm. For the ALP activity estimation, serum was directly mixed with 2-amino, 2-methyl, 1-propanol (AMP) buffer at pH 10.5, followed by estimation of absorbance of the resultant yellow colour solution at 405 nm.

Oxidative stress and inflammatory cytokines assay

Following sacrifice by cervical dislocation, the kidneys and whole liver were immediately fetched and cut in between the right and left lobes. The right lobe and right kidney were sliced into small pieces, washed with PBS and prepared to spin for 10 minutes in a homogenizer. MDA concentration as an index of lipid peroxidation was quantified according to the method described by Varshney and Kale (1990). Briefly, 50 µL of the homogenate was dispensed into a clean test tube, 100 µL of Trichloroacetic acid (TCA)/ Thiobarbituric acid (TBA) working solution was added, followed by 1.85 mL of distilled water. The mixture was placed in a boiling water bath for 15 minutes and allowed to cool thereafter. The mixture was then centrifuged, and the absorbance was read at 535 nm using a microplate reader (BIOBASE, China BK-EL10A). Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972) but with slight modification. Superoxide dismutase (SOD) is an enzyme whose level corresponds to cellular bioprotection, and the activity is determined by the kinetic method. The ability of the enzyme to prevent epinephrine autoxidation in a basic medium was spectrophotometrically determined when 0.2 mL homogenate was added to 2.5 mL phosphate buffer (50 mM) at pH 10.4 and the reaction initiated by the addition of 0.3 mL Adrenaline (Sigma-Aldrich). The absorbance was later read at 420 nm. Calculation of the enzyme activity was in terms of nanomoles of unoxidized adrenaline per minute of protein using a molar extinction coefficient of 4.02×103 per M/cm. Catalase (CAT) activity using H2O2 as substrate was measured by the method of Claiborne (1995). H2O2 decomposition rate was measured in the mixture of 0.019 M H2O2, 50 µL of homogenate and 1.95 mL of 0.05 M phosphate buffer (pH 7.0), and absorbance was determined spectrophotometrically at 240 nm, and at 0 sec, 20 sec, 40 sec, 60 sec, and 80 sec for each sample as the nmol H2O2 consumed/min/mg protein at 240 nm. Tumor necrosis factorα (TNFα), interleukin1β (IL-1β), Nuclear factor-kappa B (NF-kB), Nuclear factor erythroid 2-related factor 2 (NrF2), Caspase-3 and BCl-2 levels were also analyzed with an ELISA kit (ElabScience, USA) following the manufacturer's protocol.

Histopathology and histomorphometry

The left lobe liver and left kidney were preserved in 10% formalin solution for 24 h and washed with 70% ethanol. Tissues were then prepared and embedded in paraffin blocks. The paraffin blocks were sectioned at 6- micrometres, distributed onto glass slides and then dried. Slides were observed under a light microscope after being stained with hematoxylin and eosin (H&E). Three independent histopathologists assessed the level of liver and kidney damage and graded it accordingly, as described by Gisder et al. (2022) (Table 1) and Toprak et al. (2020) (Table 2).

The samples were evaluated, and measurements were performed with an Olympus CX43 microscope with a colour digital camera connected to a computerized image analysis system (Image Pro, USA).

Statistical Analysis

Data were presented as Mean±standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA) using GraphPad prism (version 5.0). The Student's t-test was used to compare the differences between the groups, followed by the Bonferroni post hoc test. Statistical differences were reported significant at *p <0 .05

Table 1. Histological Scoring system for TCA-induced liver damage

Tabela 1. Ocenjevalni sistem za histologijo poškodb jeter pod vplivom TCA

Grade	Grade indication	Morphological criteria	Score
0	Normal	Hepatocytes with round euchromatic nuclei and prominent nucleoli, radiating sinusoids, and intact hepatic cords	0
I	Well-differentiated	Minimal atypia, Fatty change is frequent	1
II	Moderately differentiated	Abundant eosinophilic cytoplasm, round nuclei with distinct nucleoli, Bile or proteinaceous fluid within acini	2
III	Poor differentiated	Moderate to marked pleomorphism, Absence of sinusoid-like blood spaces, Larger, more hyperchromatic nuclei but less acidophilic cytoplasm	3
IV	Undifferentiated	Little / scanty cytoplasm with fewer granules, spindle, or round-shaped cells, Highly hyperchromatic nuclei	4

Table 2. EGTI histological scoring system for TCA-induced renal damage

Tabela 2. EGTI ocenjevalni sistem za histologijo poškodb ledvic pod vplivom TCA

Tissue type	Damage	Score
Tubular	No damage	0
	Loss of Brush Border (BB) in less than 25% of tubular cells. Integrity of basal membrane	1
	Loss of BB in more than 25% of tubular cells, Thickened basal membrane	2
	Inflammation, cast formation, necrosis up to 60% of tubular cells	3
	Necrosis in more than 60% of tubular cells	4
Endothelial	No damage	0
	Endothelial swelling	1
	Endothelial disruption	2
	Endothelial loss	3
Glomerular	No damage	0
	Thickening of Bowman Capsule	1
	Retraction of glomerular tuft	2
	Glomerular fibrosis	3
Tubulo/Interstitial	No damage	0
	Inflammation, haemorrhage in less than 25% of tissue	1
	Necrosis in less than 25% of tissue	2
	Necrosis up to 60%	3
	Necrosis of more than 60%	4

Results

Serum Biochemical Analysis

TCA caused an increase (P<0.05 Student t-test) in serum ALT, AST, ALP, T.Bil, urea and creatinine when compared with the negative control of the normal saline group (Table 3) by 50%, 197%, 263%, 118%, 70%, 137% respectively. However, treatment with 5% VCO, 10% VCO and 15% VCO diet reversed the increases significantly.

Lipid peroxidation and antioxidant enzymes

TCA caused oxidative damage in both liver and kidney tissues through significant elevation of MDA by 272% but a reduction in the activities of SOD, CAT, GPx and NrF2 by 56.6%, 61%, 68.9%, and 56.4%, respectively. Meanwhile, a VCO-rich diet not only quashed the TCA-induced lipid peroxidation but also enhanced the activities of the antioxidant system (Table 4).

Inflammation and Apoptosis

TCA caused a significant elevation in the level of the inflammatory cytokine in both liver and kidney tissues. We observed an increase in TNF-α and IL-1β by 141%, 135% and 133%, 120% for liver and kidney, respectively. Similarly, NF-kB, an important transcription factor in the inflammatory response, also increased by 160% in the liver but 100% in the kidney (Figure 1). Caspase-3 and BCl-2 serve as the pro-and anti-apoptotic markers employed in this study. While there was a significant increase in the Caspase-3 level in both liver (114%) and kidney (150%) homogenate, the BCl-2 level decreased (250%, 220%) in the TCA-treated rats without virgin coconut oil (Figure 2). Upon treatment with a VCO-rich diet, the inflammatory response was lessened, and the anti-apoptotic marker was favoured, thereby ameliorating the toxic effects of TCA.

Histopathology

Thorough histological evaluation of the slides revealed many cytoarchitectural alterations, including hepatocyte ballooning, pleomorphism and vascular congestion in the liver, and loss of tubular architecture, tubular congestion and leukocyte infiltration in the kidney, all occasioned by TCA-intoxication (Figure 4,5). Further, the histomorphological assessment using a standard scoring system (Table 1, 2) revealed the significant mitigation of all the TCA-induced cyto-architectuarl alteration by the VCO-rich diet (Figure 3).

Table 3. Effects of Virgin coconut oil-rich diet on the hepatic and renal function markers in rats treated with trichloroacetic acid

Tabela 3. Vpliv diete bogate z deviškim kokosovim oljem na markerje jetrnih in ledvičnih funkcij pri podgana tretiranih s triklorocetno kislino

	ALT (U/L)	AST (U/L)	ALP (U/L)	Bil. mg/dL	Urea mg/dL	Creatinine mg/dL
NS (1mL/Kg)	12.2±2.0	3.85±0.5	0.8±0.05	7.6±2.5	30.55±5.1	0.40±0.07
TCA (250 mg/kg)	18.5±1.5*	9.9±0.9*	2.9±0.1*	16.5±3.0*	51.30±4.5*	0.95±0.1*
5%VCO Diet + TCA (250 mg/kg)	13±1.7[a]	6.2±0.4[a]	1.9±0.15[a]	14±1.7[a]	45.65±4.0[a]	0.80±0.05[a]
10%VCO Diet + TCA (250 mg/kg)	12.2±1.5[b]	6.1±1[b]	1.45±0.2[b]	13.5±1.5[b]	40.25±5.0[b]	0.70±0.09[b]
15%VCO Diet +TCA (250 mg/kg)	10.5±1.9[c]	4.5±0.5[c]	1.25±0.3[c]	11.5±1.0[c]	35.70±3.5[c]	0.55±0.1[c]

*p<0.05 Student t-test, TCA vs NS; [a,b,c] p<0.05 Student t-test, test groups vs TCA. NS=Normal saline, TCA=Trichloroacetic acid (250 mg/kg/day)

Table 4. Antioxidative effects of Virgin coconut oil-rich diet on the liver and kidney of rats treated with trichloroacetic acid

Tabela 4. Antioksidativni učinek diete bogate z deviškim kokosovim oljem na jetra in ledvice podgan tretiranih s triklorocetno kislino

	MDA (µM/g)	SOD (u/mg)	CAT (u/mg)	GPx (u/mg)	NrF2 (pg/mL)
			Liver		
NS (1mL/Kg)	1.18±0.1	15.5±1.3	10.5±1.5	2.25±0.5	19.5±2.5
TCA (250 mg/kg)	4.4±0.15*	7.5±0.55*	4.1±0.9*	0.7±0.1*	8.5±1.9*
5%VCO Diet + TCA (250 mg/kg)	4.29±0.2	8.5±0.9	5.6±0.5[a]	1.1±0.12[a]	9.0±1.5[a]
10%VCO Diet + TCA (250 mg/kg)	3.62±0.25[b]	10.8±1.85[b]	6.5±1.0[b]	1.5±0.2[b]	12.7±2.0[b]
15%VCO Diet +TCA (250 mg/kg)	3.24±0.15[c]	12.5±1.2[c]	7.5±1.2[c]	2.0±0.55[c]	13.5±2.5[c]
			Kidney		
NS (1mL/Kg)	0.6±0.1	12.2±2.8	14±2.0	1.1±0.2	29.4±5.5
TCA (250 mg/kg)	3.5±0.2*	4.9±1.2*	7.4±1.8*	0.5±0.1*	12.9±2.5*
5%VCO Diet + TCA (250 mg/kg)	3.0±0.2	5.8±1.1[a]	9.1±1.5[a]	0.6±0.2	17.7±2.0[a]
10%VCO Diet + TCA (250 mg/kg)	3.1±0.25	6.0±1.4[b]	10.5±1.8[b]	0.7±0.1b	18.3±2.5[b]
15%VCO Diet +TCA (250 mg/kg)	2.5±0.15[c]	8.5±2.0[c]	12.0±2.5[c]	0.9±0.2[c]	21.0±3.0[c]

*p<0.05 Student t-test, TCA vs NS; [a,b,c] p<0.05 Student t-test, test groups vs TCA. NS=Normal saline, TCA=Trichloroacetic acid (250 mg/kg/day)

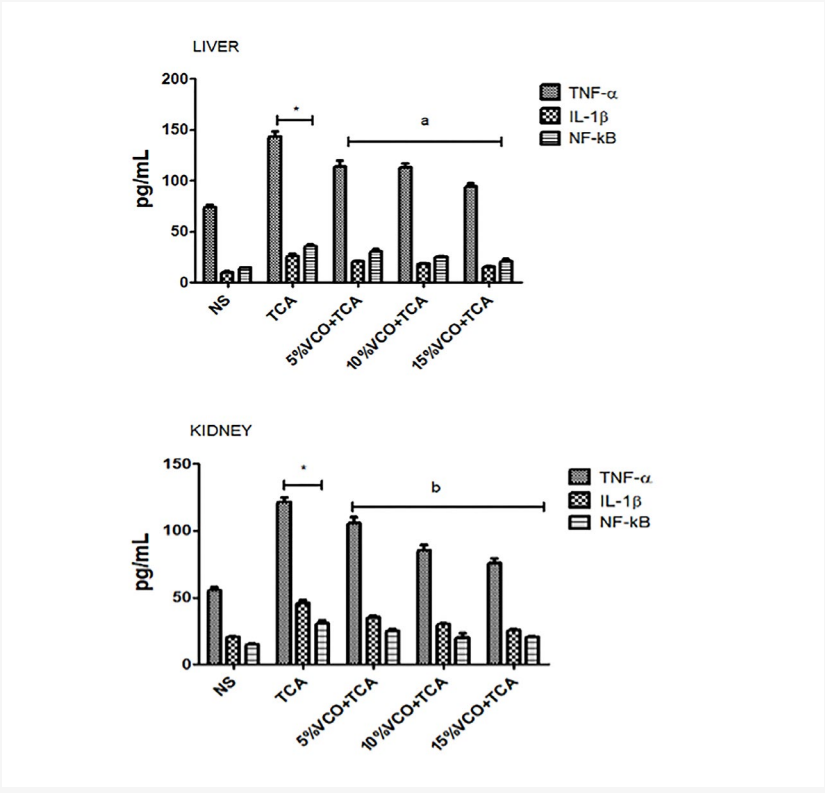

Figure 1. Effect of Virgin coconut oil-rich diet on liver and kidney inflammatory markers in trichloroacetic treated rats. *p<0.05 Student t test, TCA vs NS; a,bp<0.05 Student t test, test groups vs TCA. NS=Normal saline, TCA=Trichloroacetic acid (250 mg/kg/day)

Slika 1. Učinek prehrane, bogate z deviškim kokosovim oljem, na označevalce vnetja jeter in ledvic pri podganah, zdravljenih s triklorocetno kislino. *p<0,05 Student t test, TCA proti NS; a,bp<0,05 Student t test, testne skupine proti TCA. NS=običajna fiziološka raztopina, TCA=trikloroocetna kislina (250 mg/kg/dan)

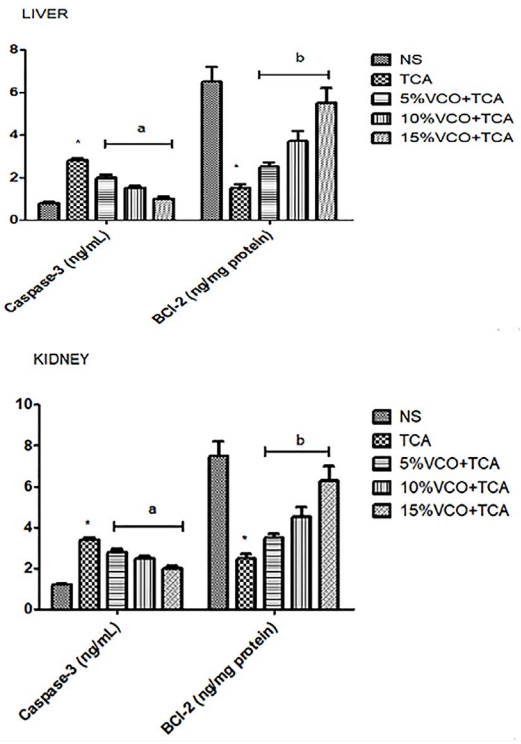

Figure 2. Effect of Virgin coconut oil-rich diet on liver and kidney apoptotic markers in trichloroacetic treated rats. *p<0.05 Student t test, TCA vs NS; a,bp<0.05 Student t test, test groups vs TCA. NS=Normal saline, TCA=Trichloroacetic acid (250 mg/kg/day)

Slika 2. Vpliv prehrane, bogate z deviškim kokosovim oljem, na apoptotične označevalce jeter in ledvic pri podganah, zdravljenih s trikloroocetom. *p<0,05 Student t test, TCA proti NS; a,bp<0,05 Student t test, testne skupine proti TCA. NS=običajna fiziološka raztopina, TCA=trikloroocetna kislina (250 mg/kg/dan)

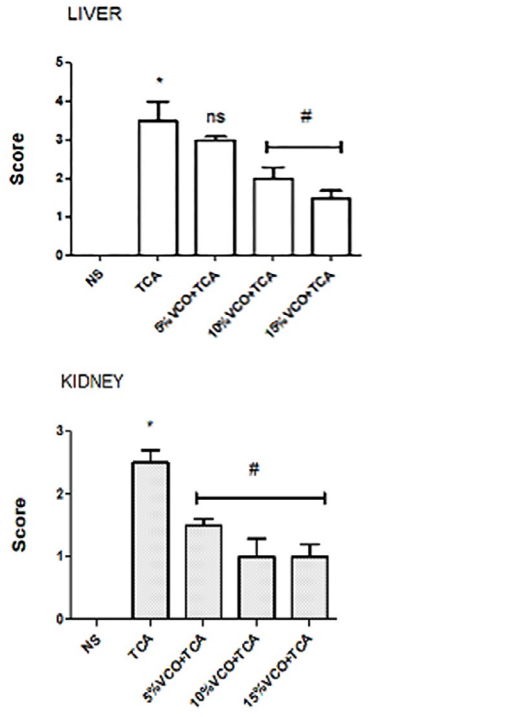

Figure 3. Liver and Kidney Histomorphomological Assessment of TCA-induced toxicity upon treatment with Virgin Coconut Oil-rich diet: Grading of hepatic and renal cytoarchitectural changes. *p<0.05 Student t test, TCA vs NS; #p<0.05 Student t test, test groups vs TCA. NS=Normal saline, TCA=Trichloroacetic acid (250 mg/kg/day)

Slika 3. Histomorfomološka ocena jeter in ledvic toksičnosti, ki jo povzroči TCA, po zdravljenju z dieto, bogato z deviškim kokosovim oljem: Razvrščanje jetrnih in ledvičnih citoarhitekturnih sprememb. *p<0,05 Student t test, TCA proti NS; #p<0,05 Student t test, testne skupine proti TCA. NS=običajna fiziološka raztopina, TCA=trikloroocetna kislina (250 mg/kg/dan)

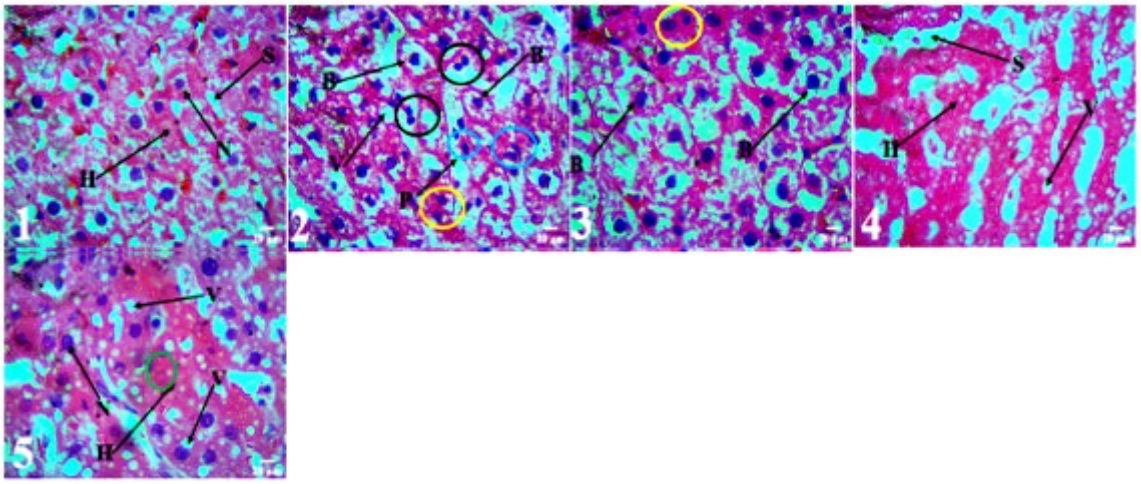

Figure 4. Representative fields of TCA-intoxicated liver sections were treated with a virgin coconut oil-rich diet. (1): Cords of hepatocytes (H) with prominent basophilic round nuclei (N) and radiating sinusoid (S). (2): Hydropic changes (ballooning of hepatocytes) with darkly-stained nuclei (B) and small-droplet steatosis (V). Phagocytic Kupffer cells (black circle) are numerous, and varying degrees of cellular and nuclear pleomorphism were also seen (blue circle). Also, note the binucleated cell (yellow circle). (3): Ballooned hepatocytes with Mallory-Denk body (B), with darkly-stained nuclei and small-droplet steatosis (V). Also note the binucleated cell (yellow circle) (4): Anucleated hepatocytes (H), small-droplet steatosis (V), near-normal cords of hepatocytes (H) (5): Small-droplet steatosis (V), Karyolysis - faded basophilia of the chromatin (green circle). Scale bars - 20µm, light microscopy, captured field – midzone, H and E – Hematoxylin and Eosin.

Slika 4. Reprezentativna polja delov jeter, zastrupljenih s TCA, zdravljenih z dieto, bogato z deviškim kokosovim oljem. (1): Niti hepatocitov (H) z izrazitimi bazofilnimi okroglimi jedri (N) in sevajočim sinusoidom (S). (2): Hidropične spremembe (baloniranje hepatocitov) s temno obarvanimi jedri (B) in drobnokapljično steatozo (V). Fagocitne Kupfferjeve celice (črni krog) so številne, opažene pa so bile tudi različne stopnje celičnega in jedrnega pleomorfizma (modri krog). Upoštevajte tudi dvojedrno celico (rumeni krog). (3): Balonirani hepatociti z Mallory-Denkovim telesom (B), s temno obarvanimi jedri in drobnokapljično steatozo (V). Upoštevajte tudi dvojedrno celico (rumeni krog) (4): jedrni hepatociti (H), drobnokapljična steatoza (V), skoraj normalne vrvice hepatocitov (H) (5): drobnokapljična steatoza (V), karioliza - zbledela bazofilija kromatina (zelen krog). Merilne lestvice - 20 µm, svetlobna mikroskopija, zajeto polje – srednji del, H in E – hematoksilin in eozin.

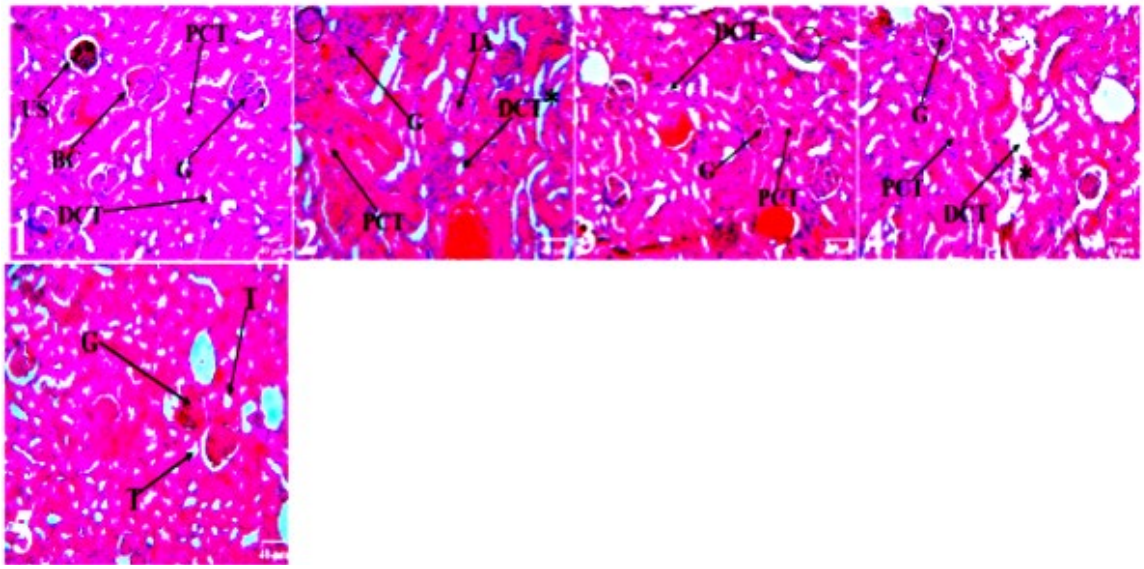

Figure 5. Representative fields of TCA-intoxicated kidney sections treated with a virgin coconut oil-rich diet.

(1): the intact architectural appearance of the renal cortex with a normal outline of the glomerular tuft (G), urinary space (US), and simple cuboidal epithelium of the tubular cells (PCT, DCT). (2, 3): some adhesion of the glomerular basement membrane to Bowman's capsule (upper left and centre glomeruli in 2, centre and lower right glomeruli in 3), thickening of the interlobular artery and obliteration of vascular lumen in 2 (IA), leukocyte infiltration (black circle), loss of tubular architecture as evidenced by interruptions in the otherwise continuous epithelial wall in 2 (right tubules - asterisk). Tubular congestion in 3. (4): Wrinkled glomerular capillary loops (upper and lower right glomeruli), loss of tubular architecture as evidenced by interruptions in the otherwise continuous epithelial wall (asterisks). (5): Adhesion of the GBM to Bowman's capsule (centre of the micrograph), glomerular atrophy and tubular degeneration evidenced by loss of cuboidal epithelial cells.Scale bars - 40µm, light microscopy, captured field: renal cortex, H and E – Hematoxylin and Eosin.

Slika 5. Reprezentativna polja delov ledvic, zastrupljenih s TCA, zdravljenih z dieto, bogato z deviškim kokosovim oljem.

(1) : nedotaknjen arhitekturni videz ledvične skorje z normalnim obrisom glomerularnega snopa (G), urinskega prostora (US) in enostavnega kuboidnega epitelija tubularnih celic (PCT, DCT). (2, 3): določena adhezija glomerularne bazalne membrane na Bowmanovo kapsulo (zgornji levi in srednji glomeruli pri 2, srednji in spodnji desni glomeruli pri 3), zadebelitev interlobularne arterije in obliteracija vaskularnega lumna pri 2 (IA), levkocitna infiltracija (črni krog), izguba tubularne arhitekture, kar je razvidno iz prekinitev v sicer neprekinjeni epitelijski steni v 2 (desni tubuli - zvezdica). Tubularna kongestija v 3. (4): Nagubane glomerularne kapilarne zanke (zgornji in spodnji desni glomeruli), izguba tubularne arhitekture, kar je razvidno iz prekinitev v sicer neprekinjeni epitelijski steni (zvezdice). (5): Adhezija GBM na Bowmanovo kapsulo (sredina mikrofotografije), glomerularna atrofija in tubularna degeneracija, ki jo dokazuje izguba kuboidnih epitelijskih celic. Merilna lestvica - 40 µm, svetlobna mikroskopija, zajeto polje: ledvična skorja, H in E – hematoksilin in eozin.

Discussion

The results of the present study are in harmony with previous findings on the deleterious effects that trichloroacetic acid has on the kidneys and liver of experimental animals (El Arem et al., 2013; Mokhamer et al., 2022).

The Aminotransferases— Alanine aminotransferase and Aspartate aminotransferase are indicators of hepatocellular injury; the level of ALT in normal liver is higher than AST (Lala et al., 2023). An increase in the circulating aminotransferases signifies that the membrane permeability of the liver cells is altered (Saad et al., 2014), as observed in the study. Moreover, the high level of circulating bilirubin in the TCA-assaulted rats also suggests that the liver functions were distorted, as established by Abdel-Hamid et al. (2011) and Mokhamer et al. (2022). TCA is a known chemical used to induce experimental hepatocellular injury by increasing the oxidative stress level and inflammation in the cells (Fouad *et al.*, 2013), though a significant reduction in values associated with the above-named conditions was seen in groups treated with a VCO-rich diet.

Hepatocytes make an important layer that isolates sinusoidal blood from the bile in the canaliculi (Gissen and Arias, 2015). The histomorphological assessment of the liver queries the cellular arrangement and their integrity with or without exposure to TCA. The liver cells of the animals exposed to TCA had a deformed arrangement that can be seen in some cases of liver diseases. There was ballooning of the hepatocytes and pleomorphism, which indicates the presence of a tumour which could likely be cancerous. The noticeable cellular (leucocytes) infiltration and phagocytic kupffer cells are considered to be part of the cellular responses to injury (Alzergy et al., 2018) caused by the toxicity of TCA. Nonetheless, the intervention of a VCO-rich diet showed it has cytoprotective effects against the injury initiated by TCA, as demonstrated in the histomorphometric grading of the liver slides. It was observed that the suspected and/or possible tumour progression was altered in the animals treated with the VCO-rich diet.

Plasma urea and creatinine are useful markers in assessing renal functions (El Arem et al., 2013). An abnormal rise in plasma urea indicates a problem with the glomerular filtration rate (Kang et al., 2002; Michael and Sircar, 2010). TCA induction in the experimental animals caused reduced glomerular filtration rate and renal dysfunction, as evidenced by the elevated urea and creatinine levels in the blood. These findings are in alignment with various works done using TCA (Pereira et al. 2001). Moreover, renal dysfunction in TCA-administered animals might be secondary because TCA distorted liver functions and might be unable to clear urea (which is the end product of protein catabolism) from the blood (El Arem et al., 2013). Leucocyte infiltration, adhesion of glomerular basement membrane to the Bowman's capsule, thickened interlobular artery, and loss of tubular architecture are clear indicators of the toxic effects that TCA had on renal integrity. Infiltration of leucocytes observed in the kidneys suggests that the glomeruli were inflamed. However, this renal dysfunction was evidently reversed in animals treated with VCO dose-dependently, which is also evidenced by the findings of the histomorphometric grading of the kidney slides.

The increased production and accumulation of reactive oxygen species and free radicals tend to disrupt the body's homeostasis (Granger and Kvietys, 2015). One of the mechanisms by which TCA induces its toxicity is via oxidative stress, which has been implicated in renal and hepatic injury (El Arem et al., 2013; Liu et al., 2016; Pinegin et al., 2018).

The antioxidant system of both enzyme and non-enzymatic defends the body against ROS effects (Paiva et al., 2018). Lipid peroxidation increases after the administration of TCA supports previous findings (Celik and Tuluce, 2007; Celik et al., 2009). Malondialdehyde (MDA) is a significant indicator of this activity, which is a result of released free radicals by TCA (Austin et al., 1996). However, a reduced level of MDA was observed in the groups treated with a VCO-rich diet, thus validating its anti-inflammatory potential.

Superoxide Dismutase (SOD) and Catalase (CAT) are primary antioxidant enzymes that defend cells against injury occasioned by the presence of increased free radicals (Srinivasan et al., 2007). One of the many functions of cells is the maintenance of an equilibrium between antioxidants and reactive oxygen species (ROS). Thus, oxidative stress in the cell must be at a minimal level to achieve and maintain redox homeostasis (Doulias et al., 2013; Aparicio-Trejo et al., 2019). To maintain this, various signalling pathways are employed by activating concerned transcription factors (Irazabal and Torres, 2020; Aranda-Rivera et al., 2022). Glutathione peroxidase (GPx) is one of the detoxifying enzymes that are modulated by upregulated Nuclear factor erythroid 2-related factor (Nrf2) (Kaspar et al., 2009; Cuadrado et al., 2014). There was an increase in the GPx quantified in the animals treated with a VCO-rich diet alongside up-regulated Nrf2, which indicates that VCO also employed this pathway (Nrf2/GPx) to establish its effects.

Anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) is one of the proteins that regulate cell death (Cory and Adams, 2002; Danial and Korsmeyer, 2004). The low expression of Nrf2 observed in the TCA-induced liver and kidney results in altered Bcl-2 induction and apoptosis. The rich expression of Bcl-2 can be stimulated by the binding of Nrf2 to the Antioxidant Response Element (ARE) (Kaspar et al., 2009; Niture and Jaiswal, 2012). This pathway helps in preventing chemical-induced cell death (and other types of cell death) (Niture and Jaiswal, 2011; Dodson et al., 2019). Consequently, there would be a reduced expression of proapoptotic proteins like Caspase 3 and 7. As seen in the results, a VCO-rich diet enhanced the expression of Bcl-2 while it reduced the activity of Caspase 3.

Mohan et al. (2020) and Liebman and Le (2021) reported that activation of Nrf2 reduces accumulated ROS levels and motivates hepatoprotection and nephroprotection. Both Nrf2 and NF-κB have a close relationship in regulating inflammation in the liver and kidney (Alshehri et al., 2022). Li et al. (2021) and Deng et al. (2020) highlighted the NF-κB/ Nrf2 signalling pathway in the liver and kidney of experimental animals; Nrf2 quashes inflammation through cytokine release regulation (Keleku-Lukwete et al., 2018).

Tumour Necrotic Factor-α (TNF-α) and Interleukin-1β (IL-1β) are examples of pro-inflammatory mediators (Colombo et al., 2018; Courtois and Fauvarque, 2018) which are activated by NF-κB signalling pathway. These factors were up-regulated by TCA-induced toxicity in the liver and kidney. Interestingly, the administration of varying percentages of a VCO-rich diet reduced TNF-α, and IL-1β levels, thereby explaining its capacity to reverse the liver and kidney damages induced by TCA.

NF-κB is a main regulator of inflammation (Shih et al., 2015; Colomer et al., 2017) and one of the mediators of apoptosis by influencing the expression of apoptosis-related genes (Canbay et al., 2003). Reactive Oxygen Species can exacerbate inflammatory response, causing tissue damage and activation of the NF-κB signalling pathway (Zhao and Wen, 2018). The production of apoptotic cells, which are later consumed by Kupffer cells, stimulates the expression of TNF-α, resulting in hepatic inflammation and hepatocyte apoptosis (Serhan et al., 2012; Wang and Lin, 2013). In this study, VCO probably de-activated the apoptosis cascade and inflammation by down-regulating the expression of NF-κB, TNF-α, Caspase-3 and IL-1β while stimulating Nrf2, Bcl-2 and GPx.

In conclusion, Virgin coconut oil enacted its anti-apoptotic, anti-inflammatory, and antioxidative effects, as demonstrated by the findings in this study. Although the 15% VCO/gram of feed appears to be most effective in the reversal of TCA-induced hepatic and renal damages, the suggestion of dose-dependent effects may still suffice.

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Data Availability

Data generated from this study can easily be made available on request.

Conflicts of Interest

The authors declare no conflict of interest.

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