ORIGINAL ARTICLE

DOI: 10.4274/gulhane.galenos.2024.33603
Gulhane Med J

Virgin coconut oil reverses atrazine and diabetes-induced lipid profile derangements in male Wistar rats

Titilope Helen Olatunbosun[1], Elemi John Ani[2,3], Ezekiel Etim Ben[1], Idorenyin Udo Umoren[1], Daniel Ejike Eze[3], Jessica Utibe David[1], Stephanie Abiola Tijani[4], Jereton Ihoeghian Osaretin[1], Asuquo Etim Asuquo[1], Helen Peter Udo[1]

[1]University of Uyo Faculty of Basic Medical Sciences, Department of Physiology, Uyo, Nigeria
[2]University of Calabar Nigeria Faculty of Basic Medical Sciences, Department of Physiology, Calabar, Nigeria
[3]University of Rwanda, Department of Medical Physiology, Kigali, Rwanda
[4]University of Rwanda, Department of Biochemistry, Molecular Biology and Genetics, Kigali, Rwanda

Cite this article as: Olatunbosun TH, Ani EJ, Ben EE, et al. Virgin coconut oil reverses atrazine and diabetes-induced lipid profile derangements in male Wistar rats. Gulhane Med J. [Epub Ahead of Print]

Date submitted:
13.03.2024
Date accepted:
15.12.2024
Epub:
14.01.2025
Publication Date:

Corresponding Author:
Titilope Helen Olatunbosun, P.h.D., University of Uyo Faculty of Basic Medical Sciences, Department of Physiology, Uyo, Nigeria
helentolatunbosun@uniuyo.edu.ng
ORCID:
orcid.org/0000-0002-7528-7590

Keywords: Atrazine, lipids, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, virgin coconut oil

ABSTRACT

Aims: We assessed the ameliorative effect of virgin coconut oil (VCO) following atrazine (ATZ) and diabetes-induced lipid profile derangements in rats.

Methods: Male Wistar rats (n=35) in the test group were divided into five groups (n=7): Groups 1, 2, and 3 received 10 mL/kg distilled water, 10 mL/kg VCO, and 123 mg/kg ATZ, respectively. Group 4 consisted of the untreated diabetic animals and Group 5 consisted of diabetic animals treated with 10 mL/kg of VCO for 2 weeks. The recovery group rats (n=35) were divided into five groups (n=7): Groups 1 and 2 received 10 mL/kg of distilled water and VCO, respectively. Groups 3, 4, and 5 received 123 mg/kg ATZ for 2 weeks. After 2 weeks, Group 1, Group 2, and Group 3 continued the initial treatment, whereas Group 4 and Group 5 received 10 mL/kg of VCO and 10 mL/kg of distilled water, respectively. Blood samples were collected after 2 weeks.

Results: From Group 1 to Group 5 in the test group, differences were observed in high-density lipoprotein cholesterol (HDL-C) levels (56.20±0.86 mg/dL, 66.00±0.71 mg/dL, 42.60±0.93 mg/dL, 33.80±1.16 mg/dL, and 52.60±1.17 mg/dL, respectively, $p<0.05$) and low-density lipoprotein cholesterol (LDL-C) levels (39.20±1.16 mg/dL, 44.00±1.52 mg/dL, 69.80±0.37 mg/dL, 75.20±0.86 mg/dL, and 61.60±1.29 mg/dL, respectively, $p<0.05$). In the recovery group, HDL-C levels were higher in Group 4 than in Group 3 (40.40±0.51 mg/dL vs. 32.20±0.80 mg/dL, $p<0.001$), LDL-C levels were lower (45.40±0.87 mg/dL vs. 66.60±1.08 mg/dL, $p<0.001$).

Conclusions: ATZ and diabetes mellitus induction reduced HDL and increased LDL levels, which were reversed by VCO administration.

Introduction

Environmental pollutants are taken into the body by inhalation, swallowing, or skin contact (1). They have devastating effects on human health, causing diseases like diabetes mellitus (DM) (2). The high prevalence of DM is of great concern not only for human health but also for its social and economic implications and burden on national development. Genetic predisposition, overnutrition, and physical inactivity play key roles in the etiology of DM. Nevertheless, neither these well-recognized risk factors nor genetic predispositions can explain the rapid rise in the prevalence of DM. Increased manufacturing rate of pollutants coincides with the prevalence of diabetes (3),

constituting new risk factors for this multi-factorial disease. Thus, understanding how environmental risk factors influence the development and progression of DM can lead to further improvements in public health. Atrazine (ATZ) is one of the most widely used herbicides that was reported to be above the limits in water bodies (4). The Environment Protection Agency classifies ATZ as class 3 toxicity on a scale of 1-4 (scale 1 as the most toxic). ATZ is an endocrine-disrupting pesticide by the US Environmental Protection Agency (5). The primary target of ATZ in humans and animals is the endocrine (hormonal) system (6), which could ultimately disrupt the regulatory and metabolic activities of the body.

Virgin coconut oil (VCO) is an unprocessed oil obtained from the mature and fresh kernel of the coconut fruit by mechanical or natural means, with or without mild heat (7). VCO is rich in lauric acid, an essential fatty acid that transforms into monolaurin acid which is an anti-viral and anti-parasite (8). VCO shows hypoglycemic actions by enhancing glucose, insulin, and estrogen metabolism (9) and by ameliorating oxidative stress induced in type 1 DM-induced rats (10). VCO also ameliorates low-density lipoprotein (LDL) in diabetes-induced male rats (11) and restores ATZ-deranged glucose transporter-4 receptors in male Wistar rats (12).

Because plasma lipid level follow-up and proper diet are paramount in preventing the prevalence of certain risk factors (e.g., obesity) associated with DM (11), VCO's effects on high-density lipoprotein may be used in the management and prevention of diabetes and possibly ATZ toxicity. Thus, this study aimed to test the effects of VCO administration following ATZ and diabetes-induced lipid profile derangements in rats.

Methods

Experimental design and groups

Adult male Wistar rats (180-200 g) maintained at the animal house unit of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar were kept in a well-ventilated space to acclimatize, fed with rat chow, and allowed water "ad libitum" for two weeks. After the acclimatization period, the animals were weighed, and fasting blood glucose levels were measured before commencing the experimental treatment.

The rats were randomly divided into two main experimental groups (the test and recovery groups), with 35 rats in each group. The experiments lasted two weeks in the test group and four weeks in the recovery group.

The rats in the test group were randomly divided into five subgroups of 7 animals (n=7) and fed oral gavage. Group 1 served as the healthy controls and received 10 mL/kg distilled water. Group 2 received 10 mL/kg VCO, and Group 3 received 123 mg/kg (20% of lethal dose) ATZ. Group 4 consisted of untreated diabetic animals and Group 5 consisted of diabetic animals treated with 10 mL/kg of VCO. The animals were examined after 2 weeks, and blood samples were collected (Table 1).

During the two weeks, 35 rats in the recovery group were also divided into five subgroups of 7 rats (n=7) and received the following treatments: Group 1 served as healthy controls and received 10 mL/kg body weight of distilled water, Group 2 received 10 mL/kg of VCO, and Group 3, Group 4, and Group 5 received 123 mg/kg of ATZ. After 2 weeks, the animals were re-treated for recovery and were treated as follows: Group 1 continued the initial treatment, receiving 10 mL/kg body weight of distilled water; Group 2 received 10 mL/kg of VCO; Group 3 received 123 mg/kg of ATZ; Group 4 received 10 mL/kg of VCO; and Group 5 received 10 mL/kg of distilled water. After two weeks of the recovery treatment period, the animals were sacrificed and blood samples were collected (Table 1).

Experimental procedures

Induction of diabetes mellitus

Diabetes was induced intraperitoneally using 150 mg/kg body weight of alloxan monohydrate (13). Polyuria and glucosuria were observed for approximately 48 hours. After 72

Table 1. Experimental groups and treatments

Groups	Treatments		
	Test group (n=35)	Recovery group (n=35) (4 weeks)	
	2 weeks	1st 2 weeks	2nd 2 weeks
Group 1* (n=7)	10 mL/kg of distilled water	10 mL/kg of distilled water	10 mL/kg of distilled water
Group 2* (n=7)	10 mL/kg of virgin coconut oil	10 mL/kg of virgin coconut oil	10 mL/kg of virgin coconut oil
Group 3* (n=7)	123 mg/kg (20% of lethal dose) of atrazine	123 mg/kg (20% of lethal dose) of atrazine	123 mg/kg (20% of lethal dose) of Atrazine
Group 4** (n=7)	10 mL/kg of distilled water	123 mg/kg (20% of lethal dose) of atrazine	10 mL/kg of virgin coconut oil
Group 5** (n=7)	10 mL/kg of virgin coconut oil	123 mg/kg (20% of lethal dose) of atrazine	10 mL/kg of distilled water

*Normal rat, ** Diabetic rat

hours, diabetes was confirmed with a blood glucose level of 180-200 mg/dL and above using a glucometer (ACCU-CHECK Active) and ACCU-CHECK compatible glucose test strips.

Virgin coconut oil preparation

VCO was extracted from mature dried coconut using a modified wet extraction method (14). The solid endosperm of mature coconut was crushed into a thick slurry. Approximately 500 mL of water was added to the slurry and squeezed through a fine sieve to obtain coconut milk. The resultant coconut milk was left for approximately 18 hours to facilitate the gravitational separation of the emulsion. Demulsification produced layers of an aqueous phase (water) at the bottom, an oil phase in the middle layer, and an emulsion phase (cream) on top. The cream on the top was removed, and the oil was scooped and warmed for about 5 minutes to remove moisture. The obtained oil was then filtered and stored at room temperature.

Median lethal dose

The toxicity of ATZ was assessed using Lorke's method (15), involving the administration of the chemical to the animals and observation for mortality within 24 hours, which was achieved as follows:

a. Twelve Swiss albino mice were fasted and weighed.
b. The animals were subgrouped into four groups for graded intraperitoneal doses of 1250 mg/kg (1.25 g/kg), 1000 mg/kg (1.00 g/kg), 750 mg/kg (0.75 g/kg), and 500 mg/kg (0.50 g/kg).
c. The administration was based on body weight, and the experimental mice were examined 24 hours after dosage administration.
d. There were physical signs of toxicity in the groups administered with doses of 1,250, 1,000, and 750 mg/kg, and mortality was recorded.
e. No physical signs of toxicity or mortality were recorded in animals administered a 500 mg/kg dose (Table 2).

Evaluation of the lipid profile

Measurement of high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and very low-density lipoprotein-cholesterol concentrations

We used a Cell Biolabs HDL-C and LDL-C/VLDL-C assay kit [United States (USA)] to measure the HDL-C, LDL-C, and VLDL-C levels (16).

Preparation of high-density lipoprotein cholesterol, low-density lipoprotein-cholesterol, and very low-density lipoprotein-cholesterol fractions

First, 200 µL of serum and 200 µL of precipitation reagent were added to a microcentrifuge tube and mixed well with cortexin. The mixture was incubated for 5-10 minutes at room temperature (20-25°C) for precipitation and centrifuged at 2000 x g (5000 rpm) for 20 min (the pellet should be visible). The supernatant (HDL-C fraction) was carefully transferred into a new tube, leaving the pellet (LDL-C/VLDL-C fraction). The pellet was resuspended and dissolved in 400 µL of phosphate-buffered saline and mixed well to ensure that the pellet (LDL-C/VLDL-C fraction) was completely dissolved before testing.

Assays for high-density lipoprotein cholesterol and low-density lipoprotein-cholesterol levels

First, 50 µL of the diluted cholesterol standard or sample was added to a 96-well microtiter plate. Then, 50 µL of the prepared cholesterol reaction reagent was added and mixed thoroughly. The wells were covered to protect them from light and incubated for 45 minutes at 37 ºC. The plate was immediately read with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and emission in the 590-600 nm range.

Estimation of total cholesterol concentration

We used a Cell Biolabs total cholesterol (TC) assay kit (USA) to measure TC levels as described by Ghezzi et al. (16).

Assay procedure

First, 50 µL of the diluted cholesterol standard or sample was added to a 96-well microtiter plate. Then, 50 µL of the pre-pared cholesterol reaction reagent was added and mixed thoroughly. The plate was covered to protect it from light and incubated for 45 minutes at 37 ºC. The plate was immediately

Table 2. Dosages of lethal concentrations

Dose-1250 mg/kg		
Mice	**Body weight**	**Dosage**
T_1	25.60 g	0.64 mL
T_2	25.23 g	0.63 mL
T_3	26.04 g	0.66 mL
Dose-1000 mg/kg		
Mice	**Body weight**	**Dosage**
T_4	28.18 g	0.56 mL
T_5	27.63 g	0.55 mL
T_6	24.45 g	0.49 mL
Dose-750 mg/kg		
Mice	**Body weight**	**Dosage**
T_7	26.37 g	0.40 mL
T_8	27.53 g	0.41 mL
T_9	22.90 g	0.34 mL
Dose-500 mg/kg		
Mice	**Body weight**	**Dosage**
T_{10}	19.70 g	0.20 mL
T_{11}	21.40 g	0.21 mL
T_{12}	27.60 g	0.28 mL

read with a spectrophotometric microplate reader in the 540-570 nm range. The cholesterol concentration was calculated by comparing the sample absorbance values with the cholesterol standard curve. The study was approved by the Local Ethics Committee for Animal Experiments (decision no: 022PY30417, date: 27.06.2017).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY: USA, released 2011). The data were analyzed using the one-way analysis of variance followed by Tukey's post-hoc test and expressed as mean±standard error of mean. A p-value <0.05 was considered significant.

Results

High-density lipoprotein cholesterol levels in the test groups

There was a significant increase in the HDL-C levels in Group 2 compared with Group 1 (66.00±0.71 mg/dL vs. 56.20±0.86 mg/dL, p<0.001), and in Group 3 compared with Group 1 and Group 2 (42.60±0.93 mg/dL vs. 56.20±0.86 mg/dL, p<0.001 and 66.00±0.71 mg/dL, p<0.001, respectively). There was a reduction in HDL-C levels In Group 4, compared with Groups 1, 2, and 3 (33.80±1.16 mg/dL vs. 56.20±0.86 mg/dL, 66.00±0.71 mg/dL, and 42.60±0.93 mg/dL, p<0.001, respectively). HDL-C levels significantly increased in Group 5, compared with Group 3 and Group 4 (52.60±1.17 mg/dL vs. 42.60±0.93 mg/dL, and 33.80±1.16 mg/dL, p<0.001, respectively) (Table 3).

High-density lipoprotein cholesterol levels in the recovery group

HDL-C levels decreased significantly in Group 3 compared with Group 1 and Group 2 (32.20±0.80 mg/dL vs. 57.52±0.35 mg/dL, and 66.25±0.82 mg/dL, p<0.001, respectively). HDL-C levels increased in Group 4 compared with Group 3 (40.40±0.51 mg/dL vs. 32.20±0.80 mg/dL, p<0.001) but decreased when compared with Group 1 and Group 2 (40.40±0.51 mg/dL vs. 57.52±0.35 mg/dL and 66.25±0.82 mg/dL, p<0.001, respectively). There was no significant difference in the HDL-C levels between Group 5 and Group 3 (p=0.204), while it was significantly lower than in Group 1, Group 2, and Group 4 (p<0.001 for all) (Table 4).

Low-density lipoprotein-cholesterol levels in the test groups

The mean values for LDL-C levels were 39.20±1.16 mg/dL, 44.00±1.52 mg/dL, 69.80±0.37 mg/dL, 75.20±0.86 mg/dL and 61.60±1.29 mg/dL in Group 1, Group 2, Group 3, Group 4, and Group 5, respectively. There was a significant increase in LDL-C levels in Group 2 compared with Group 1 (p=0.044). There was an increase in LDL-C levels in Group 3 compared with Group 1 and Group 2 (p<0.001, for both). LDL-C level was also higher in Group 4 than in Group 1 (p<0.001), Group 2 (p<0.001), and Group 3 (p=0.02). LDL-C level decreased significantly in Group 5 compared with Group 3 and Group 4 but remained higher than in Group 1 and Group 2 (Table 3).

Table 3. Comparison of HDL-C, LDL-C, and TC levels among the test groups (n=35)

Groups	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	Group 4 (n=7)	Group 5 (n=7)
HDL-C (mean±SEM)	56.20±0.86	66.00±0.71[a]	42.60±0.93[a,b]	33.80±1.16[a,b,c]	52.60±1.17[b,c,d]
LDL-C (mean±SEM)	39.20±1.16	44.00±1.52	69.80±0.37[a,b]	75.20±0.86[a,b,c]	61.60±1.29[a,b,c,d]
TC (mean±SEM)	39.80±1.16	44.40±1.08[a]	65.60±1.03[a,b]	72.20±0.86[a,b,c]	58.60±1.29[a,b,c,d]

a= p<0.05 vs. Group 1, b= p<0.05 vs. Group 2, c= p<0.05 vs. Group 3, d= p<0.05 vs. Group 4.
HDL-C: High-density-lipoprotein cholesterol, LDL-C: Low-density-lipoprotein cholesterol, TC: Total cholesterol, SEM: Standard error of the mean

Table 4. Comparison of HDL-C, LDL-C, and TC levels among the recovery groups (n=35)

Groups	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	Group 4 (n=7)	Group 5 (n=7)
HDL-C (mean±SEM)	57.52±0.35	66.25±0.82[a]	32.20±0.80[a,b]	40.40±0.51[a,b,c]	34.40±0.51[a,b,d]
LDL-C (mean±SEM)	39.82±1.78	44.32±1.65	66.60±1.08[a,b]	45.40±0.87[a,c]	56.80±1.46[a,b,c,d]
TC (mean±SEM)	40.00±1.20	44.55±0.84[a2]	73.00±0.71[a,b]	57.40±0.51[a,b,c]	64.20±0.80[a,b,c,d]

a= p<0.05 vs. Group 1, b= p<0.05 vs. Group 2, c= p<0.05 vs. Group 3, d= p<0.05 vs. Group 4.
HDL-C: High-density-lipoprotein cholesterol, LDL-C: Low-density-lipoprotein cholesterol, TC: Total cholesterol, SEM: Standard error of the mean

Low-density lipoprotein-cholesterol levels in the recovery group

LDL-C levels increased significantly with continuous use of ATZ in Group 3 compared with Group 1 (66.60±1.08 mg/dL vs. 39.82±1.78 mg/dL, p<0.001), and Group 2 (66.60±1.08 mg/dL vs. 44.32±1.65 mg/dL, p<0.001). LDL-C level was higher in Group 4 than in Group 1 (45.40±0.87 mg/dL vs. 39.82±1.78 mg/dL, p=0.016), but lower than in Group 3 (45.40±0.87 mg/dL vs. 66.60±1.08 mg/dL, p<0.001). LDL-C levels were higher in Group 5 than Group 1 (p<0.001), Group 2 (p<0.001), and Group 4 (p<0.001) but lower than Group 3 (p<0.001) (Table 4).

Total cholesterol levels in the test groups

There was a significant increase in TC levels in Group 2 compared with Group 1 (44.40±1.08 mg/dL vs. 39.80±1.16 mg/dL, p=0.044). TC levels were significantly higher in Group 3 than in Group 1 and Group 2 (65.60±1.03 mg/dL vs. 39.80±1.16 mg/dL, p<0.001, and 44.40±1.08 mg/dL, p<0.001, respectively). A significant increase in TC levels was observed in Group 4 compared with Group 1 (p<0.001), Group 2 (p<0.001), and Group 3 (p=0.020). There was a decrease in TC levels in Group 5 compared with Group 3 and Group 4 (58.60±1.29 mg/dL vs. 65.60±1.03 mg/dL, p<0.001, and 72.20±0.86 mg/dL, p=0.001, respectively) but an increase in TC levels compared with Group 1 and Group 2 (58.60±1.29 mg/dL vs. 39.80±1.16 mg/dL, p<0.001, and 44.40±1.08 mg/dL, p<0.001, respectively) (Table 3).

Total cholesterol levels in the recovery group

There was an increase in the TC levels in Group 3 compared with Group 1 and Group 2 (73.00±0.71 mg/dL vs. 40.00±1.20 mg/dL, p<0.001, and 44.55±0.84 mg/dL, p=0.026, respectively). Following VCO administration, TC levels in Group 4 decreased when compared with Group 3 (57.40±0.51 mg/dL vs. 73.00±0.71 mg/dL, p<0.001) and increased when compared with Group 1 and Group 2 (57.40±0.51 mg/dL vs. 40.00±1.20 mg/dL, p<0.001, and 44.55±0.84 mg/dL, p<0.001, respectively). TC levels in Group 5 were lower than Group 3 but higher than Group 1 (p<0.001), Group 2 (p<0.001), and Group 4 (p=0.001) (Table 4).

Discussion

This study determined the effects of VCO treatment in diabetes and ATZ-induced lipid profile derangements in rats. ATZ decreased HDL-C levels and increased LDL-C and TC levels, which reversed after VCO treatment. VCO administration for 14 days did not alter LDL-C and TC levels in control rats, suggesting that VCO did not negatively impact the lipid profile in healthy animals. Dyslipidemia in diabetes is characterized by reduced HDL-C and elevated LDL-C or triglyceride (TG) levels (17,18). The current findings are in agreement with a study by Eleazu et al. (19) that also reported no alterations in lipid profiles in healthy rats fed with 5 mL/kg VCO for 21 days. Margata et al. (20) reported that rats fed VCO and hydrolyzed VCO had higher TC levels than rats administered atorvastatin. Famurewa et al. (21) also reported that 10% and 15% VCO supplementation for 35 days considerably reduced TC, TG, LDL-C, and VLDL-C levels, accompanied by a significant increase in HDL-C levels, compared with the control rats. Nevin and Rajamohan (22) observed that VCO supplementation improved TG and LDL-C levels but not serum and tissue HDL-C levels. In another study, VCO reduced serum TC and prevented LDL-C oxidation, a vital process in atherosclerotic plaque formation (14). It was suggested that approximately 60% of the medium-chain fatty acids, pre-dominantly lauric acid and myristic acid (52% and 15%, respectively (23), present in VCO and may mediate the beneficial effect of VCO on lipid metabolism. The discrepancy in these results could be due to the length of the experiments and the different doses administered, as also suggested by Famurewa et al. (21). The results of this study indicated alterations in the lipid profiles of ATZ-treated and diabetic rats compared with healthy rats fed distilled water and VCO.

We observed a significant reduction in the HDL-C levels in Group 4. HDL-C is “The good cholesterol” because it is a free radical scavenger that can prevent lipoprotein peroxidation. High levels of HDL-C have been considered a good indicator of a healthy heart (20). Hypertriglyceridemia and low levels of HDL-C are the most common lipid abnormalities related to DM (24). This finding is in line with the studies by Sheweita et al. (25) and Sadri et al. (26) that also reported a significant decrease in HDL-C levels in streptozotocin-induced diabetic rats compared with healthy rats. Upon administration of VCO to the treated diabetic rats in Group 5, we observed a significant increase in the HDL-C levels compared with Group 4, which was the untreated diabetic group, indicating that the administration of VCO to diabetic rats helped increase their HDL levels. VCO has anti-oxidant, hypolipidemic, and anti-thrombotic properties (27), supporting the study by Margata et al. (20) that reported an improvement in HDL-C levels in dyslipidemic rats following VCO administration. Feoli et al. (28) also reported an increase in HDL levels in rats fed coconut oil (CO). When VCO was administered for longer, HDL-C levels may be restored to normal in diabetic rats because a slight enhancement was noticed in Group 2 compared with Group 1.

LDL-C and TC levels increased in Group 4; however, LDL-C and TC levels decreased after VCO treatment in Group 5. Thus, although diabetes is associated with an increased lipid profile, VCO administration can mitigate lipid derangement over time (29). This finding is consistent with a previous study that showed the anti-atherogenic and hypocholesterolemic activity of VCO (22). Another research group reported the hypolipidemic and anti-peroxidative effects of CO proteins in hypercholesterolemic rats fed a high-fat diet (30). The beneficial effects of CO on reducing

circulating lipoprotein levels and lipoprotein disposition may be associated with its biologically active polyphenol compounds (30,31). VCO has numerous beneficial effects on health, including reducing total cholesterol, TG, and phospholipid levels (32). The decreased TC and LDL-C levels in the VCO-treated group suggested reduced lipolysis by hormone-sensitive lipase due to increased insulin secretion or sensitivity (19).

ATZ administration in Group 3 caused a significant decrease in HDL-C levels, whereas there was a concomitant elevation in TC and LDL-C levels. This finding is consistent with a report by Mohammad et al. (33) that also observed a similar disruption in lipid profile after ATZ supplementation. This observation could result from the down-regulation of the steroidogenic activity of cholesterol, a precursor for steroidogenesis that decreases the end products and cholesterol production (34). The increase in serum LDL-C levels may be associated with the inhibition of scavenger receptor β1 by ATZ, as suggested by Pogrmic et al. (34). The inhibition of the scavenger receptor β1 increases LDL-C levels (35), whereas its overexpression decreases LDL-C levels (36). Such lipid profile changes may be related to the toxic effects of ATZ on lipid metabolism.

In the recovery groups, we observed that VCO supplementation did not significantly increase HDL-C levels in Group 4 compared with Group 5, although there was a marginal increase, which may be associated with the length of the recovery period of the experiment. However, LDL-C levels decreased in Group 4, which possibly contributed to recovery after VCO administration (22). Other studies reported that VCO supplementation considerably reduced TC, TG, LDL-C, and VLDL-C levels while increasing the HDL-C levels, which were higher than those of the controls (21,22). VCO also restored lipid parameters in hyperlipidemic animal models (20,21). Mohammad et al. (33) reported that clomiphene citrate treatment resulted in a dose-dependent improvement in serum lipid levels, a decrease in serum cholesterol, TG, LDL-C, and VLDL-C levels, and an increase in serum HDL-C levels in male and female rats (33). Ngala et al. (37) observed increases in TC levels with groundnut oil (GO) and CO administration. In addition, LDL-C levels increased in the CO group, HDL-C levels increased in the GO and CO groups, and TG levels increased in the GO group. Although TC levels increased in the CO group, cardiovascular risk decreased.

The polyphenol fraction from VCO has an advantage over other oils in reducing LDL-C oxidation (22). Several studies have revealed the anti-oxidant activity of polyphenolic substances, especially in red wine and olive oil (38). These polyphenolic compounds might trap reactive oxygen species in aqueous components such as plasma and interstitial fluid of the arterial wall, thereby inhibiting LDL oxidation and showing anti-atherogenic activity. In addition, these compounds can reverse cholesterol transport and reduce intestinal cholesterol absorption (39). The cholesterol-lowering activity of VCO may be partly attributed to this process. Previous research has demonstrated the mitigation of ATZ and diabetes-induced oxidative stress by VCO administration by enhancing deranged oxidative stress markers such as superoxide dismutase, catalase, and glutathione peroxidase (40).

This study has several limitations. First, we did not determine the fatty acid content of the samples. Second, we did not perform a phytochemical analysis of the active components of VCO.

Conclusion

In conclusion, this study elucidated diabetes- and ATZ-induced lipid profile derangement and confirmed the role of VCO in restoring the deranged lipid profile.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee for Animal Experiments (decision no: 022PY30417, date: 27.06.2017)

Informed Consent: Not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: T.H.O., E.J.A., D.E.E., Concept: T.H.O., E.J.A., E.E.B., I.U.U., J.U.D., A.E.A., H.P.U., Design: T.H.O., E.J.A., E.E.B., I.U.U., D.E.E., J.U.D., S.A.T., J.I.O., A.E.A., H.P.U., Data Collection or Processing: T.H.O., E.E.B., I.U.U., J.U.D., J.I.O., Analysis or Interpretation: E.J.A., I.U.U., D.E.E., J.I.O., A.E.A., Literature Search: T.H.O., E.J.A., E.E.B., I.U.U., D.E.E., J.U.D., J.I.O., S.A.T., A.E.A., H.P.U., Writing: T.H.O., E.E.B., I.U.U., J.U.D., S.A.T., H.P.U.

Conflict of Interest: No conflict of interest was declared bythe authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Agency for Toxic Substances and Disease Registry. Public Health Statement for Atrazine Buford Hwy NE, Atlanta 2003. Retrieved from: https://www.atsdr.cdc.gov/phs/phs.
2. Rylander L, Rignell-Hydbom A, Hagmar L. A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. Environ Health. 2005;4:28.
3. Neel BA, Sargis RM. The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. Diabetes. 2011;60:1838-1848.
4. Graymore M, Stagnitti F, Allinson G. Impacts of atrazine in aquatic ecosystems. Environ Int. 2001:26:483-495.
5. Morales-Pérez AA, Arias C, Ramírez-Zamora RM. Removal of atrazine from water using an iron photo catalyst supported on activated carbon. Adsorption. 2016;22:49-58.

6. Available from: https://www.epa.gov/ingredients-used-pesticide-products/atrazine

7. Villarino B, Dy L, Lizada C. Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. LWT-Food Science and Technology. 2007;40:193-199.

8. Bergsson G, Arnfinnsson J, Karlsson S, Steingrimsson O, Thormar H. *In vitro* inactivation of Chlamydia tracomatis by fatty acids and monoglycerides. Antimicrob Agents Chemother. 1998;42:2290-2294.

9. Olatunbosun TH, Ani EJ, Francis UE, Asuquo EA. Protective role of virgin coconut oil against adverse effects of atrazine toxicity on glucose and oestradiol metabolism in male Wistar rats. J Bio sci. 2021;10:57-66.

10. Iranloye B, Oludare G, Olubiyi M. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. Journal of Diabetes Mellitus. 2013;3:221-226.

11. Akinnuga AM, Jeje SO, Bamidele O, Amaku EE, Otogo FO, Sunday VE. Virgin coconut oil: remedial effects on renal dysfunction in diabetic rats. Physiology Journal. 2014:495926.

12. Olatunbosun TH, Osim EE, Asuquo EA, David JU. Atrazine derangement of GLUT-4 and insulin metabolism and the ameliorative effect of virgin coconut oil in male Wistar rats. Int. J. Curr. Res. Physiol. Pharmacol. 2021;5:1-9

13. Katsumata K, Katsumata Y, Ozawa T, Katsumata K Jr. Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan diabetes in rats. Horm Metab Res. 1993;25:125-126.

14. Nevin and Rajamohan. Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague-Dawley rats. E Spen Eur E J Clin Nutr Metab. 2008;1: e1-e8

15. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-287.

16. Ghezzi AC, Cambri LT, Botezelli JD, Ribeiro C, Dalia RA, de Mello MA. Metabolic syndrome markers in wistar rats of different ages. Diabetol Metab Syndr. 2012;4:16.

17. Smith S, Lall AM. A Study on lipid profile levels of diabetics and non-diabetics among Naini region of Allahabad, India. Turk J Biochem. 2008;33:138-141.

18. Mahato RV, Gyawali P, Raut PP, et al. Association between glycaemic control and serum lipid profile in type 2 diabetic patients: glycated haemoglobin as a dual biomarker. Biomed Res. 2011;22:375-380.

19. Eleazu C, Egedigwe-Ekeleme C, Famurewa A, et al. Modulation of the lipid profile, hepatic and renal antioxidant activities, and markers of hepatic and renal dysfunctions in alloxan-induced diabetic rats by virgin coconut oil. Endocr Metab Immune Disord Drug Targets. 2019;19:1032-1040.

20. Margata L, Silalahi J, Harahap U, Satria D. The effect of hydrolyzed virgin coconut oil on lipid profile and liver enzymes in dyslipidemic rats. Asian Journal of Pharmaceutical and Clinical Research. 2018;11:406.

21. Famurewa AC, Folawiyo AM, Elizabeth B, et al. Beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats. Integr Med Res. 2018;7:257-263.

22. Nevin and Rajamohan. Beneficial effects of virgin coconut oil on lipid parameters and *in vitro* LDL oxidation. Clin Biochem. 2004;37:830-835.

23. 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. 2018;98:1757-1764.

24. O'Brien T, Nguyen T, Zimmerman BR. Hyperlipidemia and diabetes mellitus. Mayo Clinic Proc. 1998;73:969-976.

25. Sheweita SA, Mashaly S, Newairy AA, Abdou HM, Eweda SM. Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced diabetes mellitus in rats: role of alhagi maurorum extracts. Oxid Med Cell Longev. 2016;2016:5264064.

26. Sadri H, Mohammad T, Zahra S, Morteza S. Antioxidant effects of biochanin a in streptozotocin induced diabetic rats. Brazilian archives of biology and technology. 2017;60:1716-1741.

27. Zakaria ZA, Somchit MN, Mat Jais AM, Teh LK, Salleh MZ, Long K. *In vivo* antinociceptive and anti-inflammatory activities of dried and fermented processed virgin coconut oil. Med Princ Pract. 2011;20:231-236.

28. Feoli A. M, Roehrig C, Rotta LN, et al. Serum and liver lipids in rats and chicks fed with diets containing different oils. Nutrition. 2003;19:789-793.

29. Pehwick DJ, Gomes AV, Barnes JA. Fatty acid composition and possible health effects of coconut constituents. West Indian Med J. 2000;49:128-133.

30. Salil G, Rajamohan T. Hypolipidemic and antiperoxidative effect of coconut protein in hypercholesterolemic rats. Indian J Exp Biol. 2001;39:1028-1034.

31. Muller H, Lindman AS, Blomfeldt A, Seljefolt I, Pedersen JI. A diet rich in coconut oil reduces diurnal postprandial variations in circulating tissue plasminogen activator antigen and fasting lipoprotein (a) compared with a diet rich in unsaturated fat in women. J Nutr. 2003;133:3422-3427.

32. Famurewa AC, Ekeleme-Egedigwe CA, Nwali SC, et al. 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. 2018;15:330-342.

33. Mohammad RS, Attabi A, Diwan AL. Protective role of clomiphene citrate from the biochemical effects of atrazine exposure in adult male rats. Basrah Journal of Veterinary Research. 2012;11:82-92.

34. Pogrmic K, Fa S, Dakic V, Kaisarevic S, Kovacevic R. Atrazine oral exposure of peripubertal male rats downregulates steroidogenesis gene expression in Leydig cells. Toxicol Sci. 2009;111:189-197.

35. Huszar D, Varban M, Rinninger F, et al. Increased LDL cholesterol and atherosclerosis in LDL receptor-deficient mice with attenuated expression of scavenger receptor B1. Arterioscler Thromb Vasc Biol 2000. 2000;20:1068-1073.

36. Ueda Y, Royer L, Gong E, et al. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL) and

non-HDL cholesterol in scavenger receptor class B type I transgenic mice. J Biol Chem. 1999;274:7165-7171.

37. Ngala R, Isaac A, Samuel A, Enoch O. Effect of dietary vegetable oil consumption on blood glucose levels, lipid profile and weight in diabetic mice: an experimental case-control study. BMC Nutrition. 2016;2:28.

38. Andrikopoulos NK, Kaliora AC, Assimopoulou AN, Papgeorgion VP. Inhibitory activity of minor polyphenolic and non-polyphenolic constituents of olive oil against *in vitro* low density lipoprotein oxidation. J Med Food. 2002;5:1-7.

39. Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. J Nutr. 1994;124:2451-2457.

40. Olatunbosun Titilope, Uka Emmanuel. Atrazine-induced/ streptozotocin-induced oxidative stress and mitigating effect of virgin coconut oil in male Wistar rats. International Journal of Research Publications. 2021;75:145-160.