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Alleviation of oxidized lipid-induced oxidative stress and hypertension by estrogen and selected antihyperlipidemic drugs in post-menopausal Wistar rats

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Abstract

Lipid peroxidation is implicated in the development of hypertension and coronary artery disease, and its deleterious impact is exacerbated by estrogen (ETD) depletion in post-menopausal women. We hypothesize that treatment with ETD and antihyperlipidemic drugs, either alone or in combination, can alleviate the development of cardiovascular disease. In this study, female Wistar rats were divided into 10 groups (n = 6): Group 1 (control) underwent a Sham operation and was fed standard rat chow, whereas the other nine groups were ovariectomized (OVX) and received a diet containing either thermoxidized palm oil (TPO) or thermoxidized soya oil (TSO) for 12 weeks. ETD at 0.2 mg/kg/day, atorvastatin (ATV) at 10 mg/kg/day, and a combination of ezetimibe (EZE) and ATV (EZE at 3 mg/kg/day + ATV at 10 mg/kg/day) were administered for 12 weeks in both TSO and TPO diet groups. Blood pressure and electrocardiogram (ECG) parameters were assessed, along with serum lipid profile, atherogenic indices, and markers of oxidative stress. Both TPO and TSO diets significantly altered blood pressure and ECG parameters in OVX rats. Treatment with ATV, EZE+ATV, and ETD significantly reduced blood pressure parameters compared to the OVX+TPO group. Antihyperlipidemic drugs significantly decreased heart rate, QT interval, QRS duration, and QT corrected (QTc), whereas ETD similarly shortened the QRS and QTc duration. ATV and ETD also reduced total cholesterol, triglycerides, and very low-density lipoprotein levels, while boosting high-density lipoprotein concentrations compared to untreated OVX+TSO rats. This study demonstrates that thermoxidized oil has a deleterious effect on OVX rats by altering blood pressure, ECG parameters, and atherogenic indices. Treatment with antihyperlipidemic drugs and ETD normalized blood pressure and ECG parameters, reversed hyperlipidemia, and restored antioxidant system balance.

Keywords: Thermoxidized oil; Lipid peroxidation; Oxidized low-density lipid; Menopause; Cardiovascular diseases; Estrogen; Antihyperlipidemic drugs

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1. Introduction

Palm oil and soya oil are commonly used in food processing.[1] Palm oil is rich in saturated fatty acids, whereas soybean oil predominantly contains polyunsaturated fatty acids.[2] Saturated fats (SFs) are generally believed to raise low-density lipoprotein cholesterol (LDL-c) levels and contribute to atherosclerosis. However, a recent review found that plant-derived SFs, such as those in palm oil, increase both LDL-c and high-density lipoprotein cholesterol (HDL-c), potentially balancing pro-atherogenic and anti-atherogenic lipids.[2,3] While dietary advisories recommend reducing the intake of SFs, the health effects of these SFs remain controversial and vary across studies.[3]

Thermal oxidization of dietary oils, commonly practiced in both homes and industries to enhance palatability,[4] occurs when oils are heated during frying at temperatures of 180°C or higher. Oxidation occurs due to exposure to moisture and air, producing lipid oxidation products,[5] such as peroxides, hydroperoxides, and aldehydes, which compromise the biochemical and nutritional safety of the oil.[6] Lipid peroxidation is implicated in several diseases, including hypertension and coronary heart disease.[7]

The loss of estrogen (ETD) after menopause deprives blood vessels of significant protection, further compounding the harmful effects of the highly reactive products of thermoxidized oils on the vasculature. This can result in a loss of vascular integrity and accelerated development of atherosclerosis and cardiovascular disease (CVD).[8,9] CVD is an umbrella term for conditions that entail structural and functional abnormalities of the heart and blood vessels. These conditions include coronary artery disease, cerebrovascular disease, and acute coronary syndromes such as myocardial infarction, stroke, and peripheral artery disease. CVDs account for the highest rates of morbidity and mortality worldwide.[8,9]

Although the incidence of CVD is relatively low in young women, there is a noticeable increase in risk after menopause.[10] CVD is the primary cause of illness and death among women over 50, responsible for around 80% of fatalities in post-menopausal women.[10] Menopause is associated with a dramatic rise in blood pressure.[11] Physiologically, estradiol serves a modulatory role in the peripheral nervous system, particularly in the sympathetic arm, while also regulating the renin-angiotensin-aldosterone system and maintaining body mass. These effects essentially delay the onset of hypertension in premenopausal women.[12] However, in post-menopausal women, ETD depletion is associated with an increased risk of hypertension and other CVDs.[13] Furthermore, diets rich in SFs have been shown to exacerbate the deleterious effect of ETD depletion on blood pressure.[14,15]

In this study, we hypothesize that early control of circulating lipids through the administration of ETD and antihyperlipidemic drugs can prevent the development of oxidized lipid-induced oxidative stress and subsequently reduce the risk of hypertension and coronary artery disease.

2. Materials and methods

2.1. Preparation of thermoxidized oil diet

Palm oil (Ace, Nigeria) and soya oil (Sunola, Nigeria) were thermally oxidized following the method outlined previously.[16] Five liters of each oil were used to fry 2 kg of potatoes on a hot plate at 180°C for 20 min, and this process was repeated 5 times. After each frying session, the potatoes were removed, and the oil was allowed to cool for 5 h. No additional oil was added to maintain the volume, and the 5-h cooling period was consistent across all cycles. The resulting thermoxidized palm oil (TPO)/ thermoxidized soya oil (TSO) was then incorporated into diets (15% w/w) mixed with standard rat chow (Ladokun Feeds, Nigeria) and formed into pellets.

2.2. Experimental animals

For this study, 60 female Wistar rats, weighing between 200 and 250 g, were sourced from McTemmy Farms in Ogbomosho, Nigeria. The rats were individually housed in cages at the University of Ilorin Central Laboratory animal house, maintained at a temperature of 27±2°C, with a 12-h light/dark cycle and adequate ventilation. The rats had unrestricted access to food and water. Ethical approval for the study was granted by the University of Ilorin Ethical Review Committee under approval number UERC/ ASN/2021/2038.

2.3. Ovariectomy and study design

Rats were anesthetized with a ketamine/xylazine mixture (10:1). Incisions were made to access the abdominal cavity. The fat pad containing the ovary was externalized, and the anterior arm of the bifurcated uterus was traced up to the

uterine horn, where the horn was cut to excise the ovary. The uterus and fat pad were then returned to the abdominal cavity, and the muscle and skin incisions were sutured with 4/0 absorbable sutures (Chromic Catgut Suture, Nigeria). The same procedure was used to remove the contralateral ovary. After surgery, the suture sites were cleaned and treated with penicillin to prevent infection. The animals were administered ofloxacin (100 mg/kg orally) for 7 days to prevent infection.[17] Following surgery, the rats were allowed a 1-week recovery period with unrestricted access to food and water. A further week was allowed for the rats to attain a post-menopausal state before the start of treatment and diet administration.

The study consisted of 10 groups, each containing six rats. The first group was the Sham group, which underwent a simulated surgery for surgical stress and was fed normal rat chow. The other nine groups were ovariectomized (OVX) and fed the test diets containing TPO or TSO. Group 2 received the TSO diet, and Group 3 received the TPO diet. The remaining treatment groups were fed either the TSO or TPO diet and concurrently treated with one of the following: atorvastatin (ATV) at 10 mg/kg/day, a combination of ezetimibe (EZE) and ATV (EZE at 3 mg/kg/day + ATV at 10 mg/kg/day), or estradiol valerate (ETD) at 0.2 mg/kg/day. The diets and treatments were administered concurrently for 12 weeks, after which blood pressure parameters were measured.

2.4. Measurement of blood pressure and electrocardiogram (ECG) parameters

Systolic (SBP) and diastolic blood pressure, as well as mean arterial pressure (MAP), were measured using a non-invasive tail-cuff sphygmomanometer (CODA Tail Cuff Blood Pressure Monitor, Kent Scientific, United States of America). ECG parameters, including PR interval, QT interval, heart rate, QRS duration, P duration, R amplitude, and QT corrected (QTc), were measured using an electrocardiograph machine (VE 1010 Veterinary 6/7 Channel PC ECG Machine, EDAN, United States of America).

2.5. Serum lipid profile analyses

Serum total cholesterol, triglycerides, and HDL-c were measured using specialized kits from Randox (Randox Laboratories, United Kingdom). The concentrations of LDL and very LDL (VLDL), as well as the atherogenic index (AI), coronary risk index (CRI), and HDL-c/LDL-c ratio, were calculated using established formulas.[18-20]

2.6. Serum oxidative stress assessment

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances assay.[21] Markers of oxidative stress, including reduced glutathione (GSH),[22] catalase (CAT),[23] and superoxide dismutase (SOD).[24] activities were determined using previously reported methods.

2.7. Statistical analysis

All data were summarized as mean ± standard error of the mean. The normality of the data was confirmed, followed by a one-way analysis of variance (ANOVA). Bonferroni's multiple comparisons test was applied for *post-hoc* analysis. The confidence level was set at 95%.

3. Results

3.1. ETD and antihyperlipidemic drugs improve blood pressure parameters in OVX rats treated with thermoxidized oils

Blood pressure parameters, including SBP, DBP, and MAP, were not significantly affected in OVX rats compared to the Sham group. However, SBP and MAP were markedly elevated in OVX + TSO rats compared to Sham rats (Table 1). Treatment with EZE + ATV reduced SBP ($P < 0.05$) compared to the untreated OVX+TSO group.

In OVX+TPO rats, there were significant increases in SBP, DBP, and MAP compared to Sham rats (Table 2). However, treatment with ATV, EZE+ATV, and ETD normalized the SBP, DBP, and MAP parameters in these rats.

3.2. ETD and antihyperlipidemic drugs improve ECG parameters in OVX rats treated with thermoxidized oils

Heart rate (Figure 1A), QRS duration (Figure 1B), QT interval (Figure 1C), and QTc (Figure 1D) values were

Table 1. Effects of estradiol and antihyperlipidemic drugs on blood pressure parameters in ovariectomized Wistar rats fed thermoxidized soya oil

Treatment	Blood pressure parameters (mmHg)[a]		
	SBP	DBP	MAP
Sham	107.30±3.87	78.25±3.10	87.46±2.09
OVX	109.20±3.02	83.87±2.15	90.06±1.48
OVX+TSO	138.30±1.91#	98.58±2.99	111.50±2.41#
OVX+TSO+ATV	130.00±4.16	95. 88±4.61	106.9±4.25
OVX+TSO+EZE+ATV	111.80±7.10*	83.84±7.82	92.91±7.59
OVX+TSO+ETD	130.00±11.02	92.45±10.02	104.7±9.83

Notes: [a]Values are presented as mean±SEM. Statistical significance: #$P<0.05$ compared to Sham; *$P<0.05$ compared to OVX+TSO. Abbreviations: ATV: Atorvastatin; DBP: Diastolic blood pressure; ETD: Estradiol valerate; EZE: Ezetimibe; MAP: Mean arterial pressure; OVX: Ovariectomized; SBP: Systolic blood pressure; TSO: Thermoxidised soya oil.

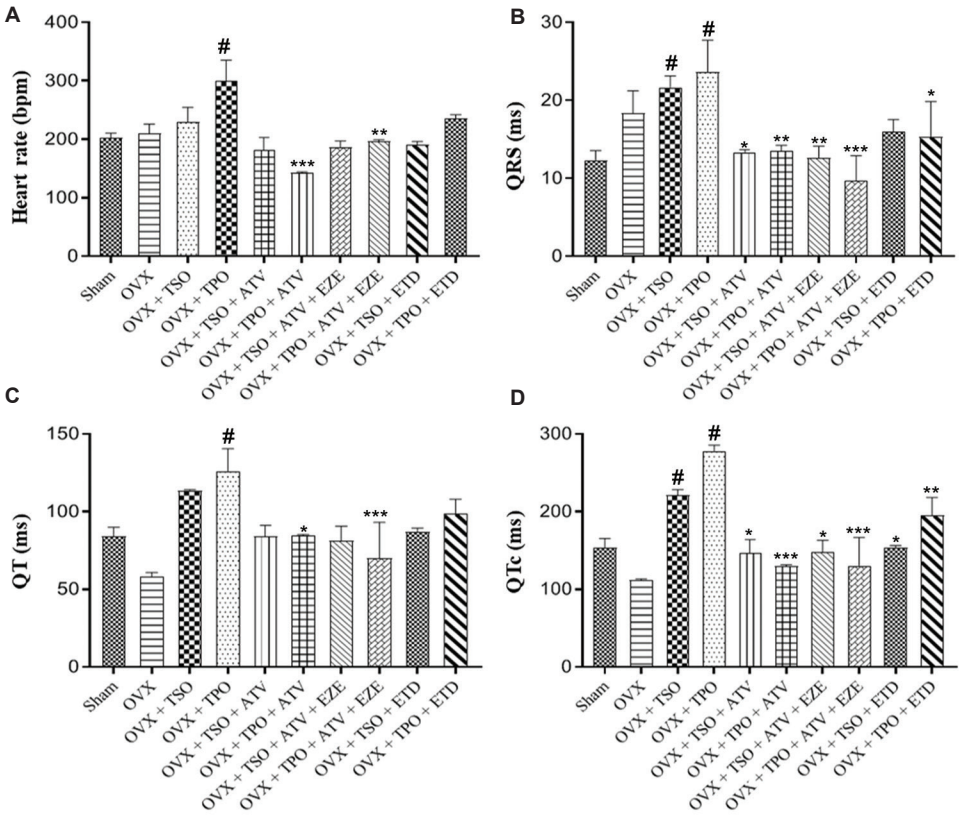


Figure 1. Effects of estradiol and antihyperlipidemic drugs on electrocardiogram parameters in OVX Wistar rats fed thermoxidized soya and palm oil diets. (A) Heart rate; (B) QRS interval; (C) QT interval; (D) QT corrected. Notes: Values are presented as mean±SEM. Statistical significance: #*P*<0.05 compared to Sham; **P*<0.05 compared to OVX+TSO/TPO; ***P*<0.01 compared to OVX+TSO/TPO; ****P*<0.001 compared to OVX+TPO
Abbreviations: ATV: Atorvastatin; ETD: Estradiol valerate; EZE: Ezetimibe; OVX: Ovariectomized; TPO: Thermoxidized palm oil; TSO: Thermoxidized soya oil.

Table 2. Effects of estradiol and antihyperlipidemic drugs on blood pressure parameters in ovariectomized Wistar rats fed thermoxidized palm oil

Treatment	Blood pressure parameters (mmHg)[a]		
	SBP	DBP	MAP
Sham	107.30±3.87	78.25±3.10	87.46±2.09
OVX	109.20±3.02	83.87±2.15	90.06±1.48
OVX+TPO	145.10±4.48#	105.90±3.43#	118.20±3.84#
OVX+TPO+ATV	108.90±8.69**	76.58±4.75*	86.98±6.01*
OVX+TPO+EZE+ATV	110.00±4.52*	76.70±3.59*	87.55±3.31*
OVX+TPO+ETD	110.60±7.60*	77.54±8.01*	88.18±7.74*

Notes: [a]Values are presented as mean±SEM. Statistical significance: #*P*<0.05 compared to Sham; **P*<0.05 compared to OVX+TPO; **P<0.01.
Abbreviations: ATV: Atorvastatin; DBP: Diastolic blood pressure; ETD: Estradiol valerate; EZE: Ezetimibe; MAP: Mean arterial pressure; OVX: Ovariectomized; SBP: Systolic blood pressure; TPO: Thermoxidised palm oil.

largely unchanged in the OVX group relative to the Sham group. Conversely, QRS and QTc ($P < 0.05$) were significantly prolonged in the OVX + TSO and OVX + TPO groups compared to the Sham group. Treatment with ETD, ATV, and EZE + ATV reversed these effects, significantly reducing QRS duration and QTc relative to the diet-only TSO and TPO groups.

3.3. ETD and antihyperlipidemic drugs improve lipid metabolism in OVX rats treated with thermoxidized oils

Lipid metabolism was disrupted in OVX rats, as indicated by significantly elevated total levels of cholesterol, triglycerides, HDL-c, LDL-c, and VLDL compared to Sham rats. OVX + TSO (Table 3) and OVX+TPO (Table 4) rats exhibited similar effects, though more pronounced. CVD indices, such as AI and CRI, were elevated, whereas high-density lipoprotein (HDL)/LDL ratios were significantly decreased ($P < 0.05$) in the OVX and diet-only groups (Tables 5 and 6).

Our results demonstrate that treatment with ETD and antihyperlipidemic drugs restored normal lipid metabolism in the treated rats, although each intervention exhibited a distinct mode of action. ETD increased HDL-c

Table 3. Effects of estrogen and antihyperlipidemic drugs on serum lipid profiles in ovariectomized Wistar rats fed thermoxidized soya oil

Treatment	Serum lipid profile (mg/dL)				
	T-CHOL	TRIG	HDL	LDL	VLDL
Sham	60.79±7.78	73.25±7.49	26.09±3.40	20.05±7.47	14.65±1.49
OVX	79.24±4.13#	150.51±7.57#	19.65±0.83#	23.97±1.06#	31.96±2.23#
OVX+TSO	80.41±3.55#	185.83±39.94#	19.76±1.01#	23.59±1.32#	37.16±7.98#
OVX+TSO+ATV	57.49±6.14***	108.52±31.89***	24.18±2.54***	14.01±1.60***	21.71±6.38***
OVX+TSO+EZE+ATV	69.58±6.08	148.54±45.85	28.03±1.04***	11.84±0.87***	29.71±9.17
OVX+TSO+ETD	74.29±6.24	113.81±23.79***	33.18±1.51***	18.36±0.95***	22.76±4.76***

Notes: Values are expressed as mean±*SEM*. Statistical significance: #*P*<0.05 compared to Sham. **P*<0.05 compared to OVX+TSO; ***P*<0.01 compared to OVX+TSO; ****P*<0.001 compared to OVX+TSO.
Abbreviations: ATV: Atorvastatin; ETD: Estradiol valerate; EZE: Ezetimibe; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; OVX: Ovariectomized; T-Chol: Total cholesterol; TRIG: Triglycerides; TSO: Thermoxidized soya oil; VLDL: Very low-density lipoprotein.

Table 4. Effects of estrogen and antihyperlipidemic drugs on serum lipid profiles in ovariectomized Wistar rats fed thermoxidized palm oil

Treatment	Serum lipid profile parameters (mg/dL)				
	T-CHOL	TRIG	HDL	LDL	VLDL
Sham	60.79±7.78	73.25±7.49	26.09±3.40	20.05±7.47	14.65±1.49
OVX	79.24±4.13#	150.51±7.57#	19.65±0.83#	23.97±1.06#	31.96±2.23#
OVX+TPO	80.27±4.21#	192.40±9.05#	19.69±1.96#	22.10±1.20	38.48±1.81#
OVX+TPO+ATV	77.09±1.91	121.09±15.12***	24.45±2.18***	27.75±1.37	24.22±3.02***
OVX+TPO+EZE+ATV	69.99±8.20	74.24±3.52***	28.24±4.21***	26.90±1.54	14.85±0.70***
OVX+TPO+ETD	61.61±2.26***	103.77±11.39***	24.84±2.01***	16.02±1.02***	20.75±2.28***

Notes: Values are expressed as mean±SEM. Statistical significance: #*P*<0.05 compared to Sham; **P*<0.05 compared to OVX+TPO; ***P*<0.01 compared to OVX+TPO; ****P*<0.001 compared to OVX+TPO.
Abbreviations: ATV: Atorvastatin; ETD: Estradiol valerate; EZE: Ezetimibe; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; OVX: Ovariectomized; T-Chol: Total cholesterol; TPO: Thermoxidized palm oil; TRIG: Triglycerides; VLDL: Very low-density lipoprotein.

Table 5. Effects of estrogen and antihyperlipidemic drugs on serum atherogenic indices in ovariectomized Wistar rats fed thermoxidized soya oil

Treatment	Atherogenic indices		
	AI	CRI	HDL/LDL
Sham	2.03±0.49	2.51±0.46	4.42±2.14
OVX	7.67±0.58#	3.94±0.2#	0.69±0.11#
OVX+TSO	8.87±2.38#	4.19±0.29#	−0.12±1.01#
OVX+TSO+ATV	3.72±1.37***	2.58±0.28***	6.47±5.89***
OVX+TSO+EZE+ATV	4.91±1.91***	3.09±0.76**	0.44±0.71
OVX+TSO+ETD	2.62±0.65***	2.63±0.57***	0.30±2.24

Notes: Values are expressed as mean±SEM. Statistical significance: #*P*<0.05 compared to Sham; **P*<0.05 compared to OVX+TSO; ***P*<0.01 compared to OVX+TSO; ****P*<0.001 compared to OVX+TSO.
Abbreviations: AI: Atherogenic index; ATV: Atorvastatin; CRI: Coronary risk index; ETD: Estradiol valerate; EZE: Ezetimibe; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; OVX: Ovariectomized; TSO: Thermoxidized soya oil.

Table 6. Effects of estrogen and antihyperlipidemic drugs on serum atherogenic indices in ovariectomized Wistar rats fed thermoxidized palm oil

Treatment	Atherogenic indices		
	AI	CRI	HDL/LDL
Sham	2.03±0.49	2.51±0.46	4.42±2.14
OVX	7.67±0.58#	3.94±0.2#	0.69±0.11#
OVX+TPO	9.429±1.39#	4.26±0.43#	1.19±0.34#
OVX+TPO+ATV	4.22±0.89***	3.27±0.27*	0.94±0.16
OVX+TPO+EZE+ATV	1.99±0.57***	2.91±0.76***	3.03±1.67
OVX+TPO+ETD	3.41±0.69***	2.55±0.21***	1.78±0.35

Notes: Values are expressed as mean±*SEM*. Statistical significance: #*P*<0.05 compared to Sham; **P*<0.05 compared to OVX+TPO; ***P*<0.01 compared to OVX+TPO; ****P*<0.001 compared to OVX+TPO.
Abbreviations: ATV: Atorvastatin; AI: Atherogenic index; CRI: Coronary risk index; ETD: Estradiol valerate; EZE: Ezetimibe; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; OVX: Ovariectomized; TPO: Thermoxidized palm oil.

levels, whereas ATV and EZE + ATV reduced triglyceride levels. All treatments significantly decreased AI and CRI indices.

3.4. ETD and antihyperlipidemic drugs alleviate oxidative stress in OVX rats treated with thermoxidized oils

The concentrations of oxidative stress markers – GSH, MDA, and CAT – in OVX rats were comparable to those of Sham rats, except for SOD, which was significantly depleted in OVX rats compared to Sham. Diets containing TSO (Table 7) and TPO (Table 8) markedly ($P < 0.05$) reduced serum GSH, CAT, and SOD levels while significantly increasing MDA levels in comparison to Sham. Treatment with ATV + NCN significantly increased serum GSH levels. In addition, treatment with ATV, EZE + ATV, and ETD reversed the oxidative stress induced by thermoxidized oils, as revealed by the significantly decreased ($P < 0.05$) MDA levels and increased CAT and SOD levels, in contrast to the untreated TSO and TPO groups.

4. Discussion

Post-menopausal women experience a marked increase in CVD risk, including hypertension, hyperlipidemia, and atherosclerosis.[25] Studies have also shown that long-term feeding of TPO or TSO to OVX rats increases the risk of CVD by accelerating the development of hyperlipidemia and atherosclerosis.[26-27] This effect is a result of the generation of oxidized products, such as oxidized LDL, and their interaction with blood vessel components.[25,28,29] Our study explored the potential ameliorative effects of ETD, ATV, and EZE + ATV in this context.

OVX rats not exposed to thermoxidized oil maintained normal levels of SBP, DBP, and MAP. In contrast, OVX rats fed thermoxidized oil diets showed elevated blood pressure compared to the Sham group. These results indicate that prolonged exposure to thermoxidized oil induces hypertension, which aligns with findings from previous studies.[17,30,31] However, treatment with antihyperlipidemic drugs resulted in significant reductions in SBP, DBP, and

Table 7. Effects of estrogen and antihyperlipidemic drugs on serum oxidative stress markers in ovariectomized Wistar rats fed thermoxidized soya oil

Treatment	Serum oxidative stress parameters			
	GSH (µM/mg protein)	MDA (nM/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Sham	0.38±0.03	2.20±0.06	6.02±0.34	1.22±0.05
OVX	0.34±0.05	2.61±0.16	5.24±0.31	0.42±0.03$^{\#}$
OVX+TSO	0.20±0.03$^{\#}$	3.09±0.08$^{\#}$	4.26±0.16$^{\#}$	0.38±0.04$^{\#}$
OVX+TSO+ATV	0.41±0.03***	2.39±0.13***	6.26±0.31**	1.48±0.09***
OVX+TSO+EZE+ATV	0.31±0.02	2.28±0.11***	5.12±0.30	0.91±0.06***
OVX+TSO+ETD	0.24±0.04	2.45±0.14**	5.41±0.28	0.66±0.10

Notes: Values are expressed as mean±*SEM*. Statistical significance: $^{\#}P<0.05$ compared to Sham; *$P<0.05$ compared to OVX+TSO; **$P<0.01$ compared to OVX+TSO; ***$P<0.001$ compared to OVX+TSO/OVX+TPO.
Abbreviations: ATV: Atorvastatin; CAT: Catalase; ETD: Estradiol valerate; EZE: Ezetimibe; GSH: Glutathione; MDA: Malondialdehyde; OVX: Ovariectomized; SOD: Superoxide dismutase; TSO: Thermoxidised soya oil.

Table 8. Effects of estrogen and antihyperlipidemic drugs on serum oxidative stress markers in ovariectomized Wistar rats fed thermoxidized palm oil

Treatment	Serum oxidative stress parameters			
	GSH (µM/mg protein)	MDA (nM/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Sham	0.38±0.03	2.20±0.06	6.02±0.34	1.22±0.05
OVX	0.34±0.05	2.61±0.16	5.24±0.31	0.42±0.03$^{\#}$
OVX+TPO	0.18±0.02$^{\#}$	3.35±0.14$^{\#}$	4.25±0.20$^{\#}$	0.47±0.06$^{\#}$
OVX+TPO+ATV	0.26±0.04	2.14±0.04***	6.11±0.49**	1.42±0.15***
OVX+TPO+EZE+ATV	0.28±0.05	2.38±0.16***	5.21±0.18	0.47±0.03
OVX+TPO+ETD	0.24±0.03	2.30±0.09***	4.80±0.14	0.69±0.03

Notes: Values are expressed as mean±SEM. Statistical significance: $^{\#}P<0.05$ compared to Sham; *$P<0.05$ compared to OVX+TPO; **$P<0.01$ compared to OVX+TPO; ***$P<0.001$ compared to OVX+TPO.
Abbreviations: ATV: Atorvastatin; CAT: Catalase; ETD: Estradiol valerate; EZE: Ezetimibe; GSH: Glutathione; MDA: Malondialdehyde; OVX: Ovariectomized; SOD: Superoxide dismutase; TPO: Thermoxidized palm oil.

MAP. These reductions are consistent with previous studies demonstrating that lipid control can play a crucial role in managing hypertension and other metabolic syndrome-related diseases.[32,33]

In addition, evaluation of ECG parameters revealed significantly prolonged QTc and QRS intervals in OVX + TSO rats, and prolonged QRS, QT, and QTc in OVX+TPO rats. This finding is consistent with an earlier report where ovariectomy in rats affected all recorded ECG parameters.[34] Prolonged QTc indicates cardiotoxicity and an elevated risk of fatal cardiac arrhythmias.[35,36] A decreasing trend in QRS, QT, and QTc intervals was observed across all treatment groups, suggesting a cardioprotective effect. TPO increases CVD risk, as evidenced by the significant increase in heart rate, QRS, QT, and QTc. Treatment with antihyperlipidemic drugs offers cardioprotection, as demonstrated by the significant decrease in heart rate and reductions in QRS, QT, and QTc upon completion of treatment with ATV and EZE + ATV.

Evaluation of serum lipid profile and atherogenic indices revealed the development of hyperlipidemia, characterized by hypercholesterolemia, hypertriglyceridemia, and overproduction of VLDL, following ovariectomy and thermoxidized oil treatment. The significant increases in total cholesterol, triglycerides, VLDL, and LDL-c in levels in the diet-only groups support this finding. These results also suggest the presence of metabolic syndrome in these groups. Treatments with ATV, EZE + ATV, and ETD effectively reversed hyperlipidemia in OVX + TSO rats. ATV and ETD significantly decreased VLDL levels, whereas EZE + ATV and ETD treatments significantly increased HDL-c levels. HDL-c plays a crucial role in reverse cholesterol transport by scavenging excess cholesterol from tissues and transporting it to the liver for metabolism and excretion. This action of HDL-c can attenuate oxidized LDL-mediated atherogenic progression in the artery walls.[37] Similarly, ETD and antihyperlipidemic drug treatments reversed hyperlipidemia in OVX + TPO rats. This was evident from the significant reductions in triglyceride and VLDL levels across all treatment groups. In addition, EZE + ATV significantly increased HDL-c levels, while ETD treatment significantly decreased triglyceride levels. Overproduction of VLDL is a hallmark of dyslipidemia in metabolic syndrome.[38] Increased VLDL levels negatively impact the composition of HDL, facilitating its degradation through the actions of hepatic lipase and cholesterol ester transfer protein.[38] Thus, reducing VLDL levels helps preserve HDL-c concentration.

Ovariectomy and thermoxidized oil treatment appear to increase the risk of developing atherosclerosis and coronary artery disease in OVX+TSO and OVX+TPO rats. This is evidenced by the significant elevation of AI and CRI, as well as the significant reduction in the HDL/LDL cholesterol ratio. While these indices were mildly elevated in OVX animals, consumption of thermoxidized oil exacerbated them. ATV treatment reversed these effects by significantly increasing the HDL/LDL cholesterol ratio in OVX+TSO rats, and AI and CRI were significantly decreased across all treatment groups. ETD administration effectively attenuated atherogenesis in OVX + TSO rats, with a notable 75% decrease in AI. AI is a predictor of CVD risk, with a higher value indicating a heightened risk of atherosclerosis.[39] Treatments with ETH, ATV, and EZE significantly decreased AI and CRI, though their effects on the HDL-c/LDL-c cholesterol ratio were less pronounced.

These results suggest that early administration of low-dose ETD and antihyperlipidemic drugs in individuals exposed to CVD risk factors, such as menopause and thermoxidized oil consumption, can reduce the risk of CVDs.

Previous studies have proposed that hypertension is an inflammatory disease and have explored the possible links between oxidative stress, hypertension, and CVDs.[40,41] Oxidative stress occurs when there is an imbalance between free radicals and the cellular antioxidant system, with pro-oxidant molecules overwhelming the system. Vascular oxidative stress is one of the leading factors for CVDs.[42,43] It is implicated in endothelial dysfunction, which is an initial step in the development of atherosclerosis and other CVDs. The generation of reactive oxygen species (ROS) and the degradation of nitric oxide are major outcomes of oxidative stress. This process promotes endothelial dysfunction by reducing endothelium-mediated vasorelaxation and disrupting the non-thrombogenic surface of the endothelium, exposing the vessel wall to thrombogenic components in the blood.[44,45] Impairment of endothelial vasodilation due to endothelial dysfunction disrupts the regulation of vascular tone, potentially resulting in paradoxical vasoconstriction, cardiac ischemia, heart failure, increased renal vascular constriction, sodium reabsorption, and hypertension.[40,44] In this study, serum levels of GSH, MDA, CAT, and SOD were examined as markers of oxidative stress in OVX rats fed TSO and TPO.

The findings of this study suggest that ovariectomy alone does not significantly impact oxidative stress. However, treatment with thermoxidized oils increases ROS production and disrupts redox balance, as revealed by the results of this study.

Treatment with ETD appeared to normalize lipid metabolism and reduce ROS production in both the TSO and TPO diet groups. These results support previous

findings where ETD was shown to offer protection against oxidative stress in OVX rats.[46,47] ETD reduces ROS production by downregulating mitogen-activated protein kinase activity and also decreases inflammatory markers and lipid peroxidation.[48,49]

In rats fed with TSO, treatment with EZE+ATV similarly restored SOD levels, while ATV treatment increased GSH, SOD, and CAT levels. Elevated expression of CAT inhibits the stimulation of ROS. As a crucial enzyme in the antioxidant defense system, CAT interacts with two molecules of hydrogen peroxide, breaking them down into water and oxygen[50] in a free radical scavenging process.

The results of this study suggest that ovariectomy alone contributes to dyslipidemia; however, ovariectomy does not independently lead to significant elevation in blood pressure or oxidative stress. However, dyslipidemia, which is a direct consequence of ovariectomy, is a well-established risk factor for CVDs. Furthermore, the consumption of thermoxidized oil exacerbates the effects of ETD withdrawal, resulting in elevated blood pressure, AI, lipid profiles, and oxidative stress markers.

The health effects of chronic consumption of TSO or TPO have been previously described.[51,52] Both have been shown to negatively impact lipid levels and general cardiovascular health. Based on their major fatty acid components, palm oil is classified as SFs, while soya oil is classified as a polyunsaturated fatty acid.[2] A recent systematic review showed the health impact of plant-derived SFs, such as palm oil, remains inconclusive, as both LDL-c and HDL-c levels increase following its consumption.[2]

TPO significantly elevated DBP, MAP, and SBP, while TSO raised SBP alone. Regarding their effects on ECG parameters, TPO increased heart rate and prolonged the QT interval, an effect not observed in the TSO group. Although TSO also had adverse health impacts, these results suggest that its impact is less severe than that of TPO. These results align with dietary guidelines advocating for the modification of dietary fats, specifically recommending reducing the intake of SFs and replacing them with unsaturated fatty acids to lower CVD risk.[2]

5. Conclusion

Our study demonstrates that the combination of menopause and thermoxidized oil consumption has a synergistically deleterious effect on cardiovascular health and the antioxidant system, with TPO exerting the most adverse impact. We propose that ETD supplementation and treatment with antihyperlipidemic drugs offer cardioprotective, antihyperlipidemic, and antioxidant benefits in OVX rats exposed to thermoxidized oils.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethical Review Committee of the University of Ilorin, Nigeria, under approval number UERC/ASN/2021/2038.

Consent for publication

Not applicable.

Availability of data

All data generated or analyzed during this study are included in this published article.

References

1. United States Department of Agriculture. *Washington: Global Oilseed Demand Growth Forecast to Outpace Production (Again). Oilseeds: World Markets and Trade*; 2018. Available from: https://www.fas.usda.gov/data/oilseedsworldmarkets-and-trade [Last accessed on 2021 Apr 23].
2. Unhapipatpong C, Shantavasinkul PC, Kasemsup V, *et al*. Tropical oil consumption and cardiovascular disease: An umbrella review of systematic reviews and meta analyses. *Nutrients*. 2021;13(5):1549.

   doi: 10.3390/nu13051549
3. Sacks FM, Lichtenstein AH, Wu J, *et al*. Dietary fats and cardiovascular disease: A presidential advisory from the

American heart association. *Circulation*. 2017;136(3):e1-e23.

doi: 10.1161/CIR.0000000000000510

4. Falade AO, Oboh G. Thermal oxidation induces lipid peroxidation and changes in the physicochemical properties and β-carotene content of *Arachis* oil. *Int J Food Sci*. 2015;2015:806524.

doi: 10.1155/2015/806524

5. Awney HA. The effects of Bifidobacteria on the lipid profile and oxidative stress biomarkers of male rats fed thermally oxidized soybean oil. *Biomarkers*. 2011;16(5):445-452.

doi: 10.3109/1354750X.2011.590228.

6. Hur SJ, Min B, Nam KC, Lee EJ, Ahn DU. Effect of dietary cholesterol and cholesterol oxides on blood cholesterol, lipids, and the development of atherosclerosis in rabbits. *Int J Mol Sci*. 2013;14(6):12593-12606.

doi: 10.3390/ijms140612593

7. Ng CY, Leong XF, Masbah N, Adam SK, Kamisah Y, Jaarin K. Reprint of "heated vegetable oils and cardiovascular disease risk factors". *Vasc Pharmacol*. 2014;62(1):38-46.

doi: 10.1016/j.vph.2014.05.003

8. Anand SS, Islam S, Rosengren A, *et al*. Risk factors for myocardial infarction in women and men: Insights from the interheart study. *Eur Heart J*. 2008;29(7):932-940.

doi: 10.1093/eurheartj/ehn018

9. Rosano GM, Vitale C, Marazzi G, Volterrani M. Menopause and cardiovascular disease: The evidence. *Climacteric*. 2007;10(Suppl 1):19-24.

doi: 10.1080/13697130601114917

10. El Khoudary SR, Aggarwal B, Beckie TM, *et al*. Menopause transition and cardiovascular disease risk: Implications for timing of early prevention: A scientific statement from the American heart association. *Circulation*. 2020;142(25):e506-e532.

doi: 10.1161/CIR.0000000000000912

11. Ashraf MS, Vongpatanasin W. Estrogen and hypertension. *Curr Hypertens Rep.* 2006;8(5):368-376.

doi: 10.1007/s11906-006-0080-1

12. Maas AHEM, Franke HR. Women's health in menopause with a focus on hypertension. *Neth Heart J*. 2009;17(2):68-72.

doi: 10.1007/BF03086220

13. Sabbatini AR, Kararigas G. Estrogen-related mechanisms in sex differences of hypertension and target organ damage. *Biol Sex Differ*. 2020;11:1-17.

doi: 10.1186/s13293-020-00306-7

14. Patki G, Allam FH, Atrooz F, *et al*. Grape powder intake prevents ovariectomy-induced anxiety-like behavior, memory Impairment and high blood pressure in female Wistar rats. *PLoS One*. 2013;8(9):e74522.

doi: 10.1371/journal.pone.0074522

15. Ko SH, Kim HS. Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women. *Nutrients*. 2020;12(1):202.

doi: 10.3390/nu12010202

16. Owu DU, Osim EE, Ebong PE. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta Trop.* 1998;69(1):65-73.

doi: 10.1016/s0001-706x(97)00115-0

17. Chong C, Othman F, Hussan F. Vascular protective effects of *Morinda citrifolia* leaf extract on postmenopausal rats fed with thermoxidized palm oil diet: Evidence at microscopic level. *Int J Vasc Med*. 2018;2018:6317434.

doi: 10.1155/2018/6317434

18. Singh SV, Shrivastava A, Chaturvedi U, *et al*. A mechanism-based pharmacological evaluation of efficacy of *Flacourtia indica* in management of dyslipidemia and oxidative stress in hyperlipidemic rats. *J Basic Clin Physiol Pharmacol*. 2016;27(2):121-129.

doi: 10.1515/jbcpp-2015-0017

19. Harnafi H, Caid HS, El Houda Bouanani N, Aziz M, Amrani S. Hypolipidaemic activity of aqueous Ocimum basilicum extract in acute hyperlipidaemia induced by triton WR-1339 in rats and its antioxidant property. *Food Chem*. 2008;108(1):205-212.

doi: 10.1002/ptr.1961

20. Joerin L, Kauschka M, Bonnländer B, Pischel I, Benedek B, Butterweck V. *Ficus carica* leaf extract modulates the lipid profile of rats fed with a high-fat diet through an increase of HDL-C. *Phytother Res*. 2014;28(2):261-267.

doi: 10.1002/ptr.4994

21. Nagababu E, Rifkind JM, Boindala S, Nakka L. Assessment of antioxidant activity of eugenol *in vitro* and *in vivo*. In: *Free Radicals and Antioxidant Protocols*. United States: Humana Press; 2010. p. 165-180.

doi: 10.1007/978-1-60327-029-8_10

22. Jollow DJ, Mitchell JR, Zampaglione NA, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*. 1974;11(3):151-169.

doi: 10.1159/000136485

23. Goth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta*. 1991;196(2-3):143-151.

doi: 10.1016/0009-8981(91)90067-m

24. Misra, HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for

superoxide dismutase. *J Biol Chem*. 1972;247:3170-3175.

25. El Khoudary, SR, Thurston RC. Cardiovascular implications of the menopause transition: endogenous sex hormones and vasomotor symptoms. *Obstetr Gynecol Clin North Am*. 2018;45(4):641-661.

    doi: 10.1016/j.ogc.2018.07.006

26. Xian TK, Omar, NA, Ying LW, *et al*. Reheated palm oil consumption and risk of atherosclerosis: Evidence at ultrastructural level. *Evid Based Complement Alternat Med*. 2012;2012:828170.

    doi: 10.1155/2012/828170

27. Che Idris CA, Sundram K, Abdull Razis AF. Correction: Che Idris *et al*. Effect of consumption heated oils with or without dietary cholesterol on the development of atherosclerosis. Nutrients 2018;10:1527. *Nutrients*. 2018;10(12):1839.

    doi: 10.3390/nu10121839

28. Brandsch C, Eder K. Effects of peroxidation products in thermoxidised dietary oil in female rats during rearing, pregnancy and lactation on their reproductive performance and the antioxidative status of their offspring. *Br J Nutr*. 2004;92(2):267-275.

    doi: 10.1079/BJN20041195

29. Choe E, Min DB. Chemistry of deep-fat frying oils. *J Food Sci*. 2007;72(5):R77-R86.

    doi: 10.1111/j.1750-3841.2007.00352.x

30. Leong XF, Mustafa MR, Das S, Jaarin K. Association of elevated blood pressure and impaired vasorelaxation in experimental Sprague-Dawley rats fed with heated vegetable oil. *Lipids Health Dis*. 2010;9:66.

    doi: 10.1186/1476-511X-9-66

31. Jaarin K, Mustafa MR, Leong XF. The effects of heated vegetable oils on blood pressure in rats. *Clinics* (*Sao Paulo*). 2011;66(12):2125-2132.

    doi: 10.1590/s1807-59322011001200020

32. Ruixing Y, Jinzhen W, Weixiong L, Yuming C, Dezhai Y, Shangling, P. The environmental and genetic evidence for the association of hyperlipidemia and hypertension. *J Hypertens*. 2009;27(2):251-258.

    doi: 10.1097/hjh.0b013e32831bc74d

33. Cífková R. Antiplatelet and lipid-lowering drugs in hypertension. *Eur Cardiol*. 2009;9(1):16-20.

    doi: 10.15420/ecr.2014.9.1.16

34. Kesmati M, Torabi M, Pourreza N, Tayebkhah M, Asadi F. Effects of anxiolytic doses of ZnO nanoparticle on ECG parameters in restraint and non-restraint ovariectomized female rats. *Nanomed Res J*. 2019;4(4):253-260.

    doi: 10.22034/nmrj.2019.04.008

35. Cubeddu LX. QT prolongation and fatal arrhythmias: A review of clinical implications and effects of drugs. *Am J Ther*. 2003;10(6):452-457.

    doi: 10.1097/00045391-200311000-00013

36. White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infect Dis*. 2007;7(8):549-558.

    doi: 10.1016/s1473-3099(07)70187-1

37. Brites F, Martin M, Guillas I, Kontush A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. *BBA Clin*. 2017;8:66-77.

    doi: 10.1016/j.bbacli.2017.07.002

38. Adiels M, Olofsson SO, Taskinen MR, Borén J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2008;28(7):1225-1236.

    doi: 10.1161/atvbaha.107.160192

39. Nayak IN, Chinta R, Jetti R. Anti-atherosclerotic potential of aqueous extract of *Cinnamomum zeylanicum* bark against glucocorticoid induced atherosclerosis in Wistar rats. *J Clin Diagn Res*. 2017;11(5):FC19.

    doi: 10.7860/jcdr/2017/23910.9864

40. Marvar PJ, Lob H, Vinh A, Zarreen F, Harrison DG. The central nervous system and inflammation in hypertension. *Curr Opinion Pharmacol*. 2011;11(2):156.

    doi: 10.1016/j.coph.2010.12.001

41. Drummond GR, Vinh A, Guzik TJ, Sobey CG. Immune mechanisms of hypertension. *Nat Rev Immunol*. 2019;19(8):517-532.

    doi: 10.1038/s41577-019-0160-5

42. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circ Res*. 2017;120(4):713-735.

    doi: 10.1161/circresaha.116.309326

43. Vinh A, Drummond GR, Sobey CG. Immunity and hypertension: New targets to lighten the pressure. *Br J Pharmacol*. 2019;176(12):1813-1817.

    doi: 10.1111/bph.14659

44. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. *Oxid Med Cell Longev*. 2009;2(5):259-269.

    doi: 10.4161/oxim.2.5.9441

45. Agita A, Alsagaff MT. Inflammation, immunity, and hypertension. *Acta Med Indones*. 2017;49(2):158-165.

46. Florian M, Freiman A, Magder S. Treatment with 17-β-estradiol reduces superoxide production in aorta of ovariectomized rats. *Steroids*. 2004;69(13-14):779-787.

    doi: 10.1016/j.steroids.2004.09.008

47. Zhang L, Fujii S, Kosaka H. Effect of oestrogen on reactive

oxygen species production in the aortas of ovariectomized Dahl salt-sensitive rats. *J Hypertension*. 2007;25(2):407-414.

doi: 10.1097/hjh.0b013e328010beee

48. Jeanes HL, Tabor C, Black D, Ederveen A, Gray GA. Oestrogen-mediated cardioprotection following ischaemia and reperfusion is mimicked by an oestrogen receptor (ER) alpha agonist and unaffected by an ER beta antagonist. *J Endocrinol*. 2008;197(3):493-501.

doi: 10.1677/joe-08-0071

49. Xiang D, Liu Y, Zhou S, Zhou E, Wang Y. Protective effects of estrogen on cardiovascular disease mediated by oxidative stress. *Oxid Med Cell Longev*. 2021;2021:5523516.

doi: 10.1155/2021/5523516

50. Dhaunsi GS, Yousif MH, Akhtar S, Chappell MC, Diz DI, Benter IF. Angiotensin-(1-7) prevents diabetes-induced attenuation in PPAR-gamma and catalase activities. *Eur J Pharmacol*. 2010;638(1-3):108-114.

doi: 10.1016/j.ejphar.2010.04.030

51. Adam SK, Das S, Soelaiman IN, Umar NA, Jaarin K. Consumption of repeatedly heated soy oil increases the serum parameters related to atherosclerosis in ovariectomized rats. *Tohoku J Exp Med*. 2008;215(3):219-226.

doi: 10.1620/tjem.215.219

52. Chong CL, Hussan F, Othman F. Hepatoprotective effects of morinda citrifolia leaf extract on ovariectomized rats fed with thermoxidized palm oil diet: Evidence at histological and ultrastructural level. *Oxid Med Cell Longev*. 2019;2019:9714302.

doi: 10.1155/2019/9714302