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Persea americana attenuates inflammatory response associated with hyperlipidemia in ovariectomized and irradiated rats by regulating MMP-3/TIMP-1 levels

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ABSTRACT

Objective: To explore the effect of *Persea americana* supplementation on inflammation, oxidative stress, and lipid profiles in ovariectomized rats fed with a high-fat diet and exposed to radiation.

Methods: The control group was sham operated, while groups 2-5 were ovariectomized and fed a high-fat diet. Groups 4 and 5 were exposed to γ-radiation (1 Gy/week for 5 weeks) after ovariectomy. Groups 3 and 5 were treated with 1 mL/250 g/day of *Persea americana* for one month. Serum levels of estrogen, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides and lipoproteins were measured. Additionally, hepatic oxidative stress, inflammatory and fibrogenic markers were evaluated.

Results: *Persea americana* treatment reduced the oxidative stress markers as well as the levels of triglyceride, total cholesterol, and low-density lipoprotein cholesterol, which in turn lowered hepatic fat accumulation. Moreover, it suppressed hepatic inflammatory mediators (interleukin-6, tumor necrosis factor-α, and C-reactive protein) and downregulated pro-fibrogenic markers (transforming growth factor-β and tissue inhibitor of metalloproteinase-1).

Conclusions: *Persea americana* provides protection against ovariectomy, and gamma radiation-mediated hepatic inflammation not only through its antioxidant, anti-inflammatory, lipid-lowering effect but also by modulating the fibrogenic markers.

KEYWORDS: *Persea americana*; γ-Radiation; Postmenopausal; Oxidative stress; Lipid; Ovariectomy; Hepatic inflammation; Fibrogenic; Rat

1. Introduction

Postmenopausal women and ovariectomized (OVX) rodents develop metabolic dysfunction and obesity (including increased body weight)[1]. A postmenopausal state coupled with high fat intake triggers inflammation-based liver diseases[2]. The excessive accumulation of lipid in the hepatocytes promotes lipotoxicity and lipid toxic intermediates that, in turn, lead to mitochondrial defects and oxidative stress, triggering the inflammatory response that contributes to liver injury and the progression of nonalcoholic fatty liver disease (NAFLD)[3]. In addition to excessive natural background radiation from the sun (the principal source of ultraviolet radiation), terrestrial radiation (gamma-emitting radionuclides in the soil), inhalation of radon gas, and ingestion of radionuclides[4], postmenopausal women may be exposed to radiation through radiotherapy or widespread use of computed tomography, mammogram, and other radiologic diagnostic modalities, and some may be exposed to radiation occupationally.

Significance

The loss of estrogen is associated with liver inflammation and fibrosis. Our study showed that avocado oil (*Persea americana*) attentuated inflammatory response aasociated with hyperlipidemia in ovariectomized and irradiated rats by regulating MMP-3/TIMP-1 levels. Moreover, it can protect from the side effect of radiotherapy.

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Radiation exposure is associated with various complications like immunosuppression, endocrine dysfunction, and organ injury[5]. The deleterious effect of ionizing radiation (IR) is derived from its interaction with biological systems (proteins, nucleic acids, and lipids) and the production of free radicals or reactive oxygen species (ROS), leading to cell dysfunction and apoptotic cell death[6]. As a result of augmented oxidative stress and ROS production, not only direct negative side effects but also ROS-related diseases may develop. The early response of the immune system to IR is *via* inflammatory response and secretion of many inflammatory mediators[7].

Due to the high radiosensitivity of the liver, Kim *et al*.[8] showed that irradiation causes liver damage that exceeds the threshold ability of the liver to repair. Thus, it would lead to loss of regenerative capacity and increased susceptibility to hepatic disorders. Radiation-induced fibrosis (RIF) is a severe long-term complication in multiple normal tissues, including the skin, lung, and gastrointestinal tract following radiotherapy. The essential multi-step process of RIF includes the release of ROS, microvascular injury, recruitment of inflammatory cells, and activation of myofibroblasts[9]. Previous studies have shown that the initial inflammatory response after irradiation is not followed by a recovery phase, but triggers progressive liver fibrosis and cirrhosis[7].

Inflammation is the normal response of the immune system after exposure to harmful stimuli like radiation, diet, aging, and sex hormones, estrogens in particular[10]. It has a principal role in the development of liver fibrosis *via* the release of pro-inflammatory mediators that results in imbalance between extracellular matrix (ECM) production and fibrinolysis [matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs)] leading to accumulation of fibrosis instead of resolution and liver regeneration[11]. Consequently, besides the inflammatory markers related to liver injury, the disrupted expression of the MMP/TIMP promotes the development of NAFLD[12].

Appropriate lifestyles in the form of healthy and natural food may have a beneficial effect against metabolic diseases and exposure to radiation. *Persea americana* (Avocado) (PA) is a nutritious commercial fruit and is widely consumed around the world. Avocado is a well-known source of carotenoids, minerals, phenolics, vitamins, and fatty acids. Avocado possesses medicinal properties such as antioxidant, anti-inflammatory, anticarcinogenic, and hypocholesterolemic properties. Avocado oils are sources of phytosterols, tocols, and unsaturated fatty acids. These components can modulate the metabolic processes, thereby reducing the risk of certain chronic diseases such as arthritis, diabetes, obesity, hypercholesterolemia, cancer, and cardiovascular disease. These pharmacological and health benefits are attributable to the high content of unsaturated fatty acids (oleic and linolenic acids), tocopherols, and bioactive phytochemicals such as phytosterols, carotenoids, and polyphenols[13].

We hypothesized that radiation exposure would raise the risk of several metabolic syndromes and related co-morbidities in postmenopausal women, including obesity, dyslipidemia, chronic inflammatory diseases, and NAFLD in which the liver is the primary target organ of estrogen. Therefore, our study was designed to explore the medicinal effect of PA on these complications.

2. Materials and methods

2.1. Materials

Organic avocado oil (PA virgin oil) was obtained from Avogen® USA. All chemicals used in this study were purchased from Sigma-Aldrich® (St. Louis, Missouri, USA).

High-fat diet (HFD) was obtained from El-Nasr Co. (Cairo, Egypt), consisting of 50% carbohydrates/starch, 27% fat, 10% protein, 10% sucrose, 1.5% fiber, and 1.5% vitamins.

2.2. Radiation facility

The irradiation of whole-body gamma irradiation was performed at the National Center for Radiation Research and Technology (Cairo, Egypt) using Canadian gamma cell-40 (137Cesium) at a dose rate of 0.67 Gy/min.

2.3. Experimental animals

Fifty female Sprague-Dawley rats (12 weeks old) were obtained from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). Throughout the experimental period, rats were allowed *ad libitum* access to food and water and housed under the same laboratory conditions with a light/dark cycle of 12 h, humidity of (50±15)%, and temperature of (22±2)℃.

2.4. Methods

2.4.1. Ovariectomy procedure

After one week of adaptation, the rats were anesthetized by intraperitoneal injection of 25 mg/kg sodium thiopental. They were subjected to ovariectomy operation. The lower part of the back was shaved and a single 1.5 to 2 cm incision was made in the skin to expose the back muscles. An incision of 2 cm was made in the muscles overlying the ovaries on both sides, and the ovaries were isolated, tied off with a sterile suture, and removed. The muscles and the skin were sutured separately. To prevent wound damage from other animals, they were caged separately for post-surgical care. The surgical wound of the operated rats was cleaned with povidone-iodine twice a day for 5 d. Rats were also administered benzylpenicillin 40 000 U/kg intramuscularly for 7 d. In the sham group (Control), rats were subjected to all surgical procedures

of ovariectomy except the removal of ovaries. Two weeks after recovery from the ovariectomy, rats were fed a high fat diet (HFD) for 8 weeks.

2.4.2. Experimental groups

Group Ⅰ, Control group (sham): The surgical procedure for the sham-operated rats was the same except that the ovaries were not removed (n=10);

Group Ⅱ, OVX-HFD group: Ovariectomized rats were fed with an HFD (n=10);

Group Ⅲ, OVX-HFD+PA group: Ovariectomized rats were fed with HFD and were treated with 1 mL/250 g/day PA *via* oral gavage 6 weeks after surgery and continued for one month[14] (n=10);

Group Ⅳ, OVX-HFD+IR group: Ovariectomized rats were fed with HFD and were exposed to whole-body γ-radiation (1 Gy/week for 5 weeks) 5 weeks after surgery (n=10);

Group Ⅴ, OVX-HFD+IR+PA group: Ovariectomized rats were fed with HFD and were exposed to whole-body γ-radiation similar to group Ⅳ and treated with PA as in group Ⅲ (n=10).

At the end of the experiment, animals were weighed and sacrificed under diethyl ether anesthesia and whole blood was collected *via* cardiac puncture. The coagulated blood samples were centrifuged for 5 min at 2000 *g* at 4℃ and the serum was collected for further biochemical estimates. Liver tissues were excised, washed with 0.9% physiological saline, dried, and stored at –80℃ for further mRNA extraction.

2.4.3. Measurement of biochemical parameter

The serum levels of estrogen (E_2) were detected using rat enzyme-linked immunosorbent assay (ELISA) kits (Cat. No. 2011-11-0175; BioSource EuropeS.A., Nivelles, Belgium).

2.4.4. Estimation of liver function tests

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Reitman and Frankel[15] while gamma-glutamyl transferase (GGT) was determined according to Szasz's method[16].

2.4.5. Estimation of the lipid profile

Total cholesterol (TC) was measured in serum according to the method of Allain *et al*.[17], using a cholesterol kit from Biodiagnostic Company. Triglycerides (TG) levels were measured in serum according to the method described by Fossati and Prencipe[18], using a triglycerides kit purchased from Biodiagnostic Company. Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by the method of Lopes-Virella *et al*.[19].

2.4.6. Estimation of inflammatory markers

In the liver tissue homogenate, the levels of interleukin- 6 (IL-6) and tumor necrosis factor-(TNF)-α were evaluated using ELISA Kits (Biomed, Diepenbeek, Belgium; and RD system, Minneapolis, MN, United States, respectively) based on the manufacturer's instructions and the values were presented as pg/mL. Also, the levels of C-reactive protein (CRP) and TGF-β in liver tissue homogenate were determined using a rat ELISA kit (PTX1) (ab108827) and rat TGF-β ELISA kit (ab 119558).

2.4.7. Estimation of oxidative stress markers

In liver tissue, lipid peroxidation was measured according to the method of Yoshioka *et al*.[20]. The method is based on the determination of malondialdehyde (MDA), the end product of lipid peroxidation, which can react with thiobarbituric acid in an acidic medium to yield a pink-colored trimethine complex exhibiting an absorption maximum at 532 nm. The concentration of thiobarbituric acid in the sample is calculated as μmol/mg tissue. The activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were assayed *via* the method of Minami and Yoshikawa[21] and Sinha[22], respectively.

2.4.8. Determination of mRNA gene expression of MMP-3 and TIMP-1 by quantitative real-time PCR (qRT-PCR) in liver tissues

The change in mRNA expression of *MMP-3* and *TIMP-1* was examined in liver tissues. Using the manufacturer's instructions for TRIzol reagent (Life Technologies, USA), total RNA was isolated from 30 mg of liver tissues. Then 1% agarose gel electrophoresis stained with bromide of ethidium was used to confirm the integrity of RNA. The synthesis of the first strand of complementary DNA (cDNA) was done with reverse transcriptase (Invitrogen) using 1 μg of total RNA as the template according to the manufacturer's protocol. After that, qRT-PCRs were performed using the Sequence Detection Program (PE Biosystems, CA) in a thermal cycler stage one plus (Applied Biosystems, USA). Table 1 lists the primer pairs used in these experiments. A total of 25 μL reaction mixture consisted of 2×SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each prim, and 2 μL of cDNA. The conditions for PCR thermal cycling included an initial step at 95℃ for 5 min; 40 cycles at 95℃ for 20 s; 60℃ for 30 s; and 72℃ for 20 s. At the end of the reaction, the results were normalized using the *β-actin* gene. The relative expression of target mRNA was calculated using the method of comparative Ct[23].

Table 1. Sequence of the primers used for real-time PCR.

Genes	Primer's sequence
MMP-3	F: 5′- AAGATCCATGGAAGGCGTCG -3 R:5′- TCAGTGCGCCAAGTTTCAGA -3′
TIMP-1	F: 5′- CTGCAACTCGGACCTGGTTA -3 R: 5′- CAGCGTCGAATCCTTTGAGC- -3′
β-actin	F: 5′ CCAGGCTGGATTGCAGTT3′ R: 5′GATCACGAGGTCAGGAGATG3′

2.5. Statistical analysis

Data were analyzed using the statistical package SPSS (Statistical Program for Social Science) version 20 by applying a one-way ANOVA test followed by a *post hoc* test for multiple comparisons (Duncan test). All data are expressed as mean ± SE and differences between means are considered significant at $P<0.05$.

2.6. Ethical statement

All experimental procedures were performed in compliance with the standards and guidelines of the National Research Centre Ethics Committee, issued by the U.S. National Institutes of Health, "Guide for the treatment and use of laboratory animals" for the use and protection of experimental animals (NIH publication No. 85-23, 1996). It was approved by the Institutional Animal Care and Use Committee (Vet CU 3/2021/139).

3. Results

3.1. Effect of PA on body weight, body mass index, and estrogen levels in the OVX and irradiated rat

Compared with the sham group, both OVX and OVX+IR groups exhibited markedly increased body weight and body mass index which may be due to HFD-induced obesity and metabolic dysregulation associated with ovarian hormone deficiency (Figures 1A and B). Conversely, PA administration decreased the body weight and body mass index.

As shown in Figure 1C, exposure to gamma radiation potentiated the effect of OVX and resulted in an extensive reduction in the estrogen level. In contrast, treatment with PA showed a marked increase in the levels of estrogen.

3.2. Effect of PA on the lipid profile in serum of the OVX and irradiated rat

As shown in Table 2, both the OVX and OVX+IR groups showed a significant increase in serum levels of TG, TC, LDL-C and a significant decrease in the HDL-C level as compared to the sham group. In contrast, PA supplementation reduced the levels of TG, TC, and LDL-C levels that were slightly near to those of the sham group. Moreover, PA increased the HDL-C level.

3.3. Effect of PA on hepatic oxidative stress status in the OVX and irradiated rat

As shown in Table 3, the level of hepatic oxidative stress marker (MDA) was significantly increased accompanied by a significant reduction in the activities of antioxidant enzymes (SOD and CAT) in OVX and OVX+IR groups compared to the sham group. On the other hand, treatment with PA induced a significant reduction in MDA and an increase in antioxidant defense enzymes (SOD and CAT), respectively.

Table 2. Effect of *Persea americana* on serum lipid parameters (mg/dL).

Groups	TC	TG	LDL-C	HDL-C
Control	139.0±0.6^{d}	96.0±2.0^{e}	70.7±1.4^{e}	48.0±1.7^{b}
OVX	230.0±1.6^{b}	142.0±2.2^{b}	158.0±2.7^{b}	29.2±1.6^{a}
OVX+PA	149.0±2.9^{c}	106.0±2.1^{d}	80.0±3.5^{d}	46.0±2.1^{b}
OVX+IR	233.0±2.7^{a}	173.0±1.9^{a}	166.0±2.6^{a}	28.6±2.5^{a}
OVX+IR+PA	150.0±1.8^{c}	118.0±1.3^{c}	90.9±2.6^{c}	43.9±1.9^{b}
F	2 751.7	8 288.5	1 253.8	167.6
P	<0.05	<0.05	<0.05	<0.05

TG: triglycerides; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol. Values were expressed as mean ± SE, $n=6$. Values sharing different letters are significantly different ($P<0.05$) while those with similar letters are non-significant. OVX: ovariectomized rats. OVX+PA: ovariectomized rats treated with *Persea americana*. OVX+IR: ovariectomized rats exposed to gamma radiation. OVX+PA+IR: ovariectomized rats exposed to gamma radiation and treated with *Persea americana*.

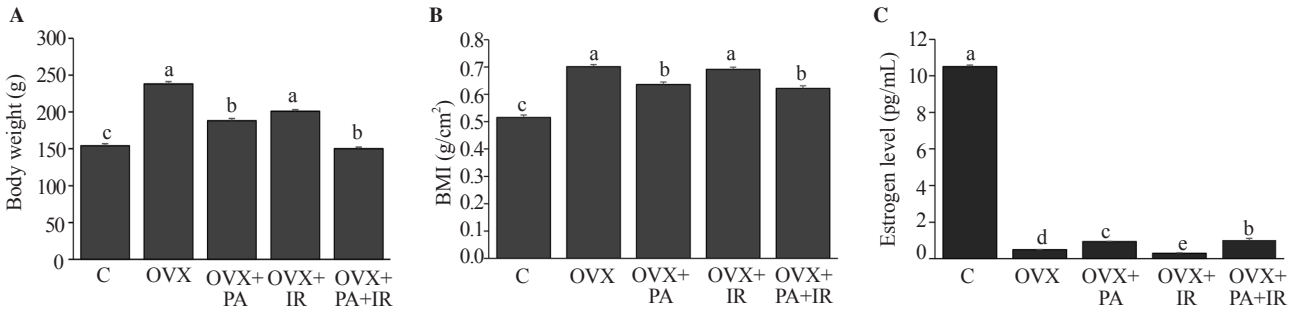

Figure 1. Effect of *Persea americana* on body weight (A), body mass index (BMI) (B), and estrogen levels (C), respectively. The lines represent the SE, and the bar graphs represent the mean value of the group. Values sharing different letters are significantly different ($P<0.05$) while those with similar letters are non-significant. The *F* values were 99.8, 64.9, and 35 257.7, respectively. C: control, OVX: ovariectomized rats. OVX+PA: ovariectomized rats treated with *Persea americana*. OVX+IR: ovariectomized rats exposed to gamma radiation. OVX+PA+IR: ovariectomized rats exposed to gamma radiation and treated with *Persea americana*.

Table 3. Effect of *Persea americana* on hepatic oxidative stress status in ovariectomized and irradiated rat.

Groups	MDA (μmol/mg tissue)	SOD (U/mg protein)	CAT (U/mg protein)
Control	40.6±1.3^{e}	45.4±2.3^{a}	71.8±2.8^{a}
OVX	73.5±3.7^{b}	29.6±2.1^{c}	51.0±1.8^{c}
OVX+PA	44.2±2.1^{d}	44.6±2.0^{a}	62.6±2.0^{b}
OVX+IR	109.0±2.0^{a}	22.7±1.6^{d}	41.0±3.0^{d}
OVX+IR+PA	57.1±3.9^{c}	35.8±2.3^{b}	60.9±3.6^{b}
F	1 310.2	338.5	215.1
P	<0.05	<0.05	<0.05

MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase. Values were expressed as mean ± SE, n=6. Values sharing different letters are significantly different (P<0.05) while those with similar letters are non-significant.

3.4. Effect of PA on the liver function in the OVX and irradiated rat

The results showed significant liver injury and impaired liver function accompanied by extensive leakage of the hepatic enzymes (GGT, ALT, and AST) into the blood, thus increasing their levels in the blood in response to ovariectomy and gamma irradiation. However, treatment with PA markedly inhibited the increment in these liver function parameters as illustrated in Figure 2.

3.5. Effect of PA on the hepatic inflammatory markers in the OVX and irradiated rat

The results showed increased levels of the hepatic pro-inflammatory cytokines IL-6, TNF-α, and CRP in both the OVX and OVX+IR groups. Supplementation with PA alleviated the inflammatory response induced either by OVX alone or combined with gamma irradiation (Figure 3).

3.6. Effect of PA on hepatic fibrosis and fibrogenic markers in OVX and irradiated rat

TGF-β is a well-characterized, pro-fibrotic cytokine that activates HSCs, induces the expression of matrix-producing genes, inhibits degradation of ECM leading to the excessive deposition of collagen fibers, and promotes liver fibrosis. Figure 4 reveals a significant increase in the level of TGF-β in OVX and OVX+IR groups compared with the sham group. Meanwhile, treatment with PA reduced the level of TGF-β.

Additionally, both ovariectomy and irradiation downregulated the mRNA expressions of collagenase (*MMP-3*), whereas the mRNA expressions of *TIMP-1* (the main MMP inhibitor) were upregulated as compared to the sham group. In contrast, administration of PA upregulated *MMP-3* and downregulated *TIMP-1* mRNA expression (Figure 5).

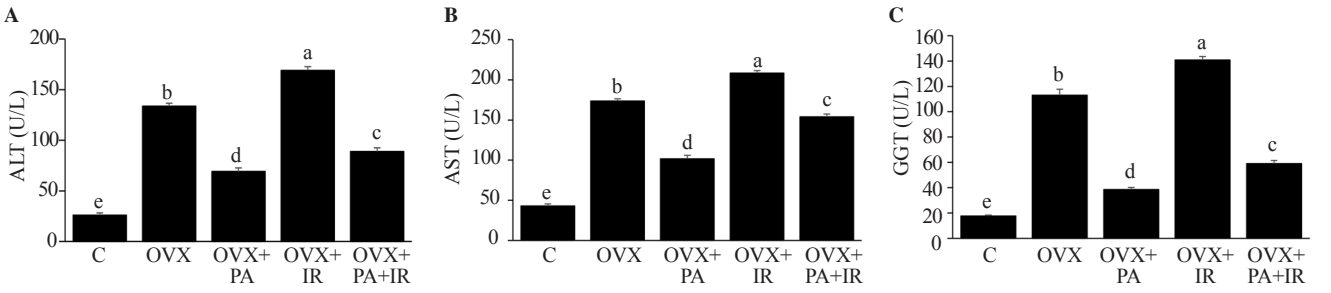

Figure 2. Effect of *Persea americana* on alanine aminotransferase (ALT) (A), aspartate aminotransferase (AST) (B), and gamma-glutamyl transferase (GGT) (C). The lines represent the SE, and the bar graphs represent the mean value of the group. Values sharing different letters are significantly different (P<0.05) while those with similar letters are non-significant. The F values were 2 876.7, 3 296.5, and 4 064.1, respectively.

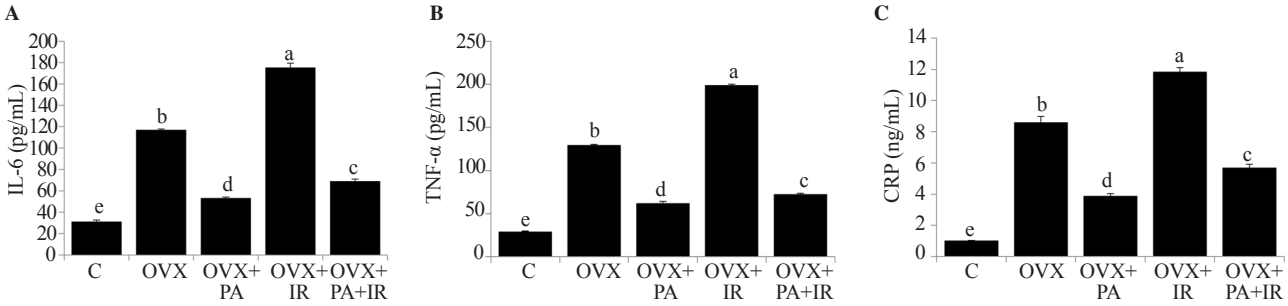

Figure 3. Effect of *Persea americana* on interleukin- 6 (IL-6) (A), tumor necrosis factor-α (TNF-α) (B), and C-reactive protein (CRP) (C). The lines represent the SE, and the bar graphs represent the mean value of the group. Values sharing different letters are significantly different (P<0.05) while those with similar letters are non-significant. The F values were 6 540.7, 10 269.8, and 1 832.8, respectively.

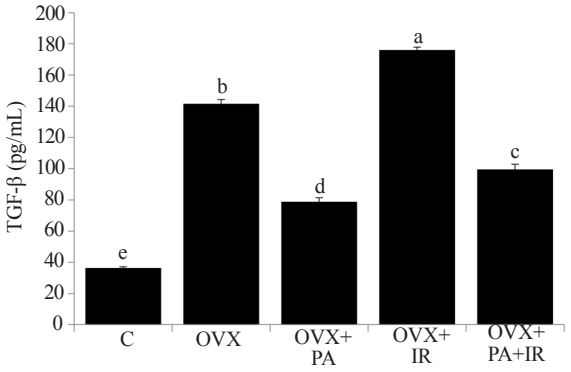

Figure 4. Effect of *Persea americana* on hepatic transforming growth factor-β (TGF-β). The lines represent the SE, and the bar graphs represent the mean value of the group. Values sharing different letters are significantly different (P<0.05) while those with similar letters are non-significant. The F value was 5 338.

4. Discussion

Intake of a high-fat diet increases obesity, moreover, it promotes inflammation-mediated liver injury in combination with ovariectomy[24]. Ovariectomy resulted in estrogen deficiency which in turn disrupts energy metabolism and increases the accumulation of lipid and related metabolic diseases, including dyslipidemia and obesity[25], consequently, enhancing the hepatic inflammation. Additionally, exposure to high-dose radiation increases the incidence of fatty liver and fibrosis[26].

In contrast, the administration of PA increases estrogen levels. This may be due to the presence of phytosterol specially β-sitosterol (1.91-2.47 g/kg) in avocado oil[13]. Phytoestrogens (phytosterol) are a class of compounds having a similar structure to estrogen and can bind the estrogen receptor. β-Sitosterol exhibits a phytoestrogenic effect and can be used as an alternative to hormone replacement therapy[27].

The obtained results revealed that estrogen insufficiency coupled with nutrition overload caused hypertriglyceridemia, hypercholesterolemia as well as increased LDL-C and decreased HDL-C levels to a greater extent, which ultimately lead to intrahepatic lipid accumulation. These findings are in agreement with that of Jiang *et al.*[28] in OVX animals. Moreover, Kilim and Chandala[29] reported that the remarkable impairment of lipid and lipoprotein metabolism in postmenopausal women, through impaired fatty acid beta-oxidation, increased free fatty acid uptake, and *de novo* lipogenesis for increased TG synthesis increase the incidence of obesity, hepatic steatosis, and NAFLD[30]. Moreover, the irradiation-induced hyperlipidemia may be attributed to changes in the liver lipid metabolism and serum lipoproteins and may be due to the indirect effect of radiation through the release of different inflammatory mediators[31].

However, avocado oil supplementation markedly reversed these changes. These results are in line with Shehata and Soltan[32] who depicted that avocado oil significantly decreased the serum levels of TC, TG, and LDL-C. The hypolipidemic effect of avocado oil was attributed mainly to its high content of monounsaturated fatty acids particularly oleic acid and other phytochemicals[13]. Accordingly, this confirms the lowering effect of PA on lipid parameters.

The accumulation of fatty acids in hepatocytes results in lipotoxicity which in turn induces the formation of reactive oxygen species (ROS), associated with mitochondrial dysfunction, release of cytokines from hepatic stellate and Kupffer cells which augmented the inflammatory response, cellular death, thereby, fibrotic changes[3]. Our results showed a marked increase in lipid peroxidation combined with lower activities of antioxidant enzymes. These results agree with that of Ohashi *et al.*[33], which confirm that estrogen deficiency is associated with increased oxidative stress in the liver, thus hepatic degeneration. Furthermore, Vares *et al.*[34] indicated that an HFD combined with exposure to radiation increases radio-sensitivity of hepatic cells to damage *via* mitochondrial dysfunction and ROS production, which consequently increases the hepatic steatosis and serum liver enzyme activities.

In contrast, *Persea americana* attenuated the impaired oxidative

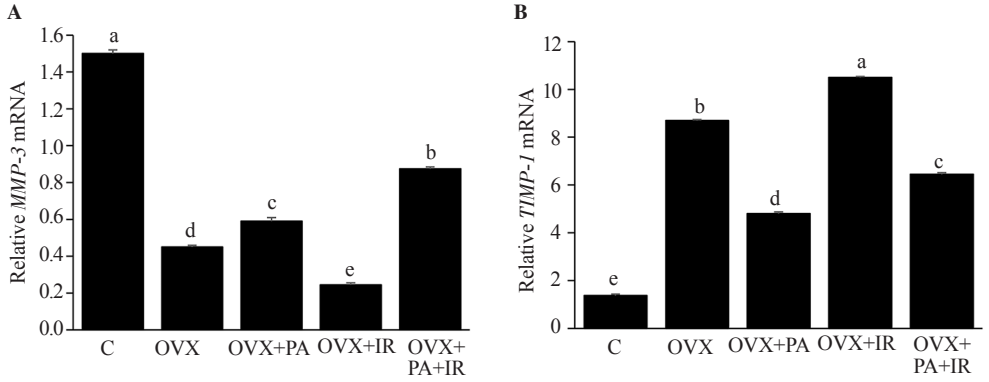

Figure 5. Effect of *Persea americana* on matrix metalloproteinase-3 (*MMP-3*) (A) and tissue inhibitors of MMP-1 (*TIMP-1*) (B) gene expression. The lines represent the SE, and the bar graphs represent the mean value of the group. Values sharing different letters are significantly different (P<0.05) while those with similar letters are non-significant. The F values were 284.4 and 4 267.0, respectively.

stress due to the presence of many compounds with antioxidant activity, such as phenolics, ascorbic acid, vitamin E, and carotenoids that quench free radicals and thus, neutralize oxidative stress and cellular damage. The antioxidant potential of avocado oil was attributed to attenuation of mitochondrial ROS generation along with scavenging of ROS, chelation of transition metal besides, and enhancement of the activity of the antioxidant enzymes (SOD and CAT)[35].

Ovariectomy with the loss of hepatoprotective capacity of estrogen markedly enhanced liver cell destruction. Herein, our results showed notably increased levels of ALT, AST, and GGT in the OVX and/or irradiated groups, which confirm hepatocellular damage and hepatic dysfunction. Panchal *et al*.[36] showed mainly the same results where liver dysfunction displayed through the leakage of cellular enzymes: ALT, AST, and GGT were increased in postmenopausal women. Furthermore, Chen *et al*.[37] revealed this elevation in liver enzymes to radiation-derived free radicals (ROS), which attacks the hepatic polyunsaturated fatty acids cellular membranes leading to loss of membrane fluidity and permeability and leakage of the enzymes from the liver cytosol into the blood.

Meanwhile, the increase in the activities of these enzymes was alleviated by PA treatment, demonstrating an attenuation effect on liver dysfunction, due to maintenance of hepatocytes membrane integrity and enhanced liver regeneration[38]. Likewise, the restored levels of estrogen promote liver cell regeneration, which in turn alleviates liver functions[39].

Estrogen controls immune cell activity through regulation of cellular metabolism *via* its receptors by activating or repressing several immunomodulatory cytokines, which contribute to disease pathogenesis[40]. Interestingly, the loss of the immunomodulatory activity of estrogen mainly increased liver destruction *via* activation of inflammatory response.

The results of the current study revealed higher levels of the pro-inflammatory mediators (IL-6, TNF-α, and CRP) in both OVX and OVX+IR groups. Our results are in harmony with that of Monteiro *et al*.[41] who showed that estrogen deficiency provokes hepatic inflammation thus release of pro-inflammatory cytokines (IL-6 and TNF-α) along with increased levels of CRP leading to increased liver damage.

However, PA supplementation inhibited the pro-inflammatory cytokines released from the liver of the OVX and irradiated rats. This anti-inflammatory effect was due to the presence of oleic acid, tocopherols, and polyphenols in avocado oil[35]. Estrogens have been found to directly suppress inflammatory processes in the liver by inhibiting pro-inflammatory cytokine production and oxidative stress[42]. Moreover, the improved level of estrogen after PA supplementation enhances the resolution of inflammation and tissue repair in the liver.

The deficiency of estrogen is associated with collagen deposition and inflammation in the liver. Chronic inflammation and repair cause liver fibrosis *via* excessive accumulation of fibronectin and collagens. The MMP/TIMP imbalance during liver injury, with increased TIMP expression by activated HSCs, inhibited MMPs, thereby reducing matrix degradation with increased ECM production by activated HSCs[11].

Radiation-induced fibrosis is associated with increased collagen synthesis, altered remodeling, and sequential activation of key fibrogenic growth factors and cytokines, including TGF-β1, which is activated *via* ROS leading to an imbalance between TIMPs and MMPs[43]. The current results showed a marked increase in the levels of TGF-β concomitant with downregulation of *MMP–3* and upregulation of *TIMP–1*. Parallel to the obtained results, Martínez-Uña *et al*.[44] reported that the increased expression of TGF-β activates the HSCs, consequently, upregulating the collagen type Ⅰ, and *TIMP-1* gene expression, therefore, inhibiting collagen degradation or suppressing *MMP-3* gene expression leading to excessive collagen fiber accumulation and ultimately hepatic fibrosis.

However, PA supplementation reduced the levels of the pro-fibrotic cytokine TGF-β thus, upregulating the *MMP–3* expression, and enhancing fibrinolysis and liver regeneration. This may be linked to the anti-inflammatory effect of PA. Moreover, Brady[45] reported that estrogen attenuation prevents the proliferation of stellate cells and fibrogenesis *via* impeding the deposition of collagen, scavenging free radicals, as well as suppressing the pro-inflammatory mediators. Unfortunately, one of the limitations of this work is the unavailability to perform histopathological analysis for the liver tissue, which may further confirm the effect of avocado oil on the hepatic fibrosis and hepatic damage. Additionally, we would complete this study to evaluate if avocado oil protects from NAFLD.

It could be concluded that PA supplementation has a therapeutic effect against inflammatory responses associated with OVX and gamma irradiation. PA not only improves liver functions and serum lipid biomarkers (TC, TG, LDL-C, and HDL-C) but also reduced liver lipogenesis and oxidative stress. Moreover, PA ameliorates hepatic inflammatory response and alleviates hepatic regeneration *via* modulation of MMP-3/TIMP-1 expressions.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

DFE designed the experimental study, performed the methodology parts, and revised the manuscript. FAMS prepared materials, collected the data, and wrote the part of the manuscript. ESAA corrected the manuscript and contributed to the materials and data analysis. All authors read and approved the final manuscript.

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