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ORAL ADMINISTRATION OF A NUTRACEUTICAL COMBINATION IMPROVES ENDOTHELIAL DYSFUNCTION IN OVARIECTOMIZED DIABETIC RATS

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ABSTRACT

Sixty albino female rats were divided into 6 groups, 10 rats in each group, and were classified as follows: group I (sham group), group II (sham received myrtus and omega-3), group III (ovariectomized group), group IV (ovariectomized group treated with myrtus and omega-3) ,group V (ovariectomized diabetic group) and group VI (ovariectomized diabetic group treated with myrtus and omega-3). Plasma 15- lipoxygenase (15-LOX), 5-lipoxygenase (5-LOX), lipoxin-A4 (LXA4), von Willebrand factor (vWF), glucose, insulin and lipid profile were estimated. Superoxide dismutase (SOD), malondialdehyde (MDA) and catalase activity (CAT) enzymeswere measuredin pancreatic tissues. Alsohistopathology for pancreatic tissue was done.

Using a combination of *Myrtus communis* extract and omega-3 induced significant decrease in the plasma level of 15-LOX,5-LOX, vWF, MDA,CAT, glucose, LDL, triglyceride and cholesterol in all treated groups,whereas induced significant increase in lipoxin-A4,SOD and HDL.

We concluded that diabetes induced metabolic disturbances could get worse after ovariectomy due to oxidative stress and inflammatory condition . Whereas a combination of *Myrtus communis* serving as antioxidant agent due its highly content from flavonoids and phenolics compounds and omega-3 has potential effects in reducing endothelial dysfunction furthermore oxidative stress and inflammation associated with diabetes mellitus.

Keywords: Diabetes, Ovariecomized rats, *Myrtus communis,* Endothelial Dysfunction, Lipoxygenase, Von Willebrand factor

Introduction

After menopause estrogen deficiency is correlated with metabolic disorders like T2DM and cardiovascular disease; this indicates its significance in pathogensizing these conditions (Ko and Kim, 2020). Estrogen regulates several physiological and pathological processes in addition to its role in female physiology and reproduction. The association of estrogen levels with the risk of diabetes development is consistent with menopause. Margolis *et al*. (2004) revealed that the risk of diabetes in female taking hormone replacement therapy was lower. In the meantime, Ding *et al*. (2007) documented that insulin resistance and post-menopause diabetes in women with high endogenous estrogen levels.

Estrogen deficiency is combined with oxidative stress after menopause; a disorder arises when the development of reactive oxygen species (ROS) exceeds the body's anti-oxidant defenses. ROS are end products of physiological oxidative processes that bind protein, lipid, carbohydrates and DNA within cells, resulting in cellular damage to cell membranes and genetic materials. Since the elevated rates of ROS contribute to T2DM pathogenesis, this may indicate an increased risk of developing T2DM in postmenopausal women (Fahmy *et al*., 2018).

Recently trends in the use of the medicinal plant extracts such as *Myrtus communis* L. (common myrtus) containing phenolic compounds which have anti-hyperglycemic and anti-inflammatory agents for the modulation of adverse side effects occur as a result of the use of synthetic drugs for T2DM treatments. It has been confirmed that *M. communis* has antioxidant, antibacterial, anti-hemorrhagic, anti-hyperglycemic, antimutagenic antifungal, and hepatoprotective activities. This plant was considered in Iran as appreciable medicinal plant. This is due to its simple collection and its valuable biological activity (Rasheed *et al*., 2016).

Fish oil is obtained from oily fish tissues. It is suggested for a balanced diet because it is composed of omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), precursors to eicosanoids that reduce inflammation throughout the body (Innes and Calder 2020).

Arachidonic acid (AA) is metabolized by lipooxygenases (LOX), cytochrome P450 (CYP450) and cyclooxygenase (COX) enzymes involved in a number of diseases, such as type 1 & 2 diabetes, to form eicosanoids.

Eicosanoids are either derived from omega-6 (ω-6) or omega-3 (ω-3). The ω-6 eicosanoids are primarily pro-inflammatory; whereas ω-3 eicosanoids are primarily anti-inflammatory. In contrast to omega 3 fatty acids, AA metabolites from omega 6 fatty acids are essential factors in the degradation and dysfunction of beta-cells through COX, 12-lipoxygenase (12-LOX) and 5-lipoxygenase (5-LOX) pathways and ultimately, in diabetes pathogenesis and complications (Adkins and Kelley, 2010).

Many evidence show that 2 lipoxygenases (12-LOX and 15-LOX), and their products play important functions in different organs and tissues, like the kidney, vasculature, brain, adipose tissue, and the pancreatic islet. lipoxin A4 (LXA4),one member of the family of lipoxins, has antiestrogenic effect which greatly reduces the action of the estrogen released in the body-17-estradiol (E2) (Russell *et al.,* 2011).

5-LOX metabolizes free arachidonic acid which results in the production of proinflammatory leukotrienes participated in initiation and progression of atherosclerosis (Piper and Garelnabi, 2020).

Lipoxins-A4 (LXA4) are products of the metabolism of arachidonic acid formed by the action of lipoxygenase. LXA 4 acts as anti-inflammatory by inhibiting recruitment and activation of eosinophils and neutrophils, and by inhibiting the generation of pro-inflammatory cytokines and reactive oxygen species (Wenceslau *et al*., 2014).

Excess formation of free radicals like hydroxyl anions, peroxynitrite superoxide, and defective antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) have been associated with cardiovascular problems in diabetic patients by oxidizing low-density lipoprotein (LDL) to oxidized LDL and increasing the risk of developing atherosclerosis (Masoodi *et al*., 2015).

The vascular endothelium, under physiological conditions, develops several compounds that are closely associated with haemostasis, fibrinolysis, growth factors synthesis, and vessel tone control and permeability. Another such material that is synthesized by endothelial cells and retained in them is the von Willebrand factor (vWF). As release of vWF increases when endothelial cells are damaged, vWF levels have been suggested as a potential indicator of endothelial dysfunction (Lip and Blann, 1997).

In our previous works, we studied the beneficial effect of myrtus extract treatment against streptozotocin (STZ)-induced experimental diabetes (Rasheed *et al*., 2016); moreover, we studied how far combination between myrtus extract and fish oil against oxidative stress and inflammation induced by diabetes (Aly *et al*., 2017). So, in this study, we focusedon the detection of potential protecting effect andpossible benefit of a combination betweenphenolic compositionof myrtus extractand omega-3 against oxidative stress and inflammation induced by ovariectomized diabetes to confirm our postulation about the additive effect of myrtus extract and omega-3supplementationas an anti-inflammatory and anti-hyperglycemic agents to alleviate the side effects of synthetic medication widely used to treat harmful effects of diabetes or menopause.

Materials and Methods

Drugs and chemicals

Streptozotocin and all chemicals were bought from Sigma-Aldrich (USA). Refined omega-3 was purchased from local pharmacy.

Plant materials

Myrtus communis L. leaves have been collected from Faculty of Agriculture, Fayoum Univ., Egypt. A voucher specimen (p.g.n.1) is deposited at the Herbarium of the Biochemis. Dept., Fac. Agric., Fayoum Univ.The leaves of the plant were thoroughly washed with tap water, air dried in the shade and then powdered to 24 mesh by lab mill. Powdered material was kept in an airtight container at ambient temperature (28± 2º C) and protected from light until used (Messaoud *et al*., 2012).

Preparation of alcoholic extracts

The known weight of the dried air powdered leaves was extracted with successive chloroform and methanol at ambient temperature (28±2ºC). The process of extraction was repeated at least 5 times until each organic solvent was colorless. The extracts obtained were filtered using Whatman No.1 filtering paper and the combined extract (filtrate) was evaporated to dryness at 45ºC with vacuum rotary evaporator. Extracts of dried chloroform and methanol were stored in a desiccator at 4ºC until used (Messaoud *et al*., 2012).

Preliminary phytochemical screening

The preliminary screening of the extract for the following classes of phytoconstituents was preformed according to the methods described by Farnsworth, (1966).

High-performance liquid chromatography (HPLC)-Assay of Flavonoids and Phenolic compounds in Myrtus extract

HPLC analysis for flavonoids and phenolic compounds has been carried out using a liquid chromatography of the Agilent Technologies 1100 series equipped with an auto sampler and a diode-array detector. The analytical column has been an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2 percent of acetic acid in water (v/v) (solvent B). For a total runtime of 70 min, the flow rate has been maintained at 0.8 ml / min and the gradient system was as follows: 100 percent of B to 85 percent of B in 30 min, 85 percent of B to 50 percent of B in 20 min, 50 percent of B to 0 percent B in 5 min and 0 percent of B to 100 percent of B in 5 min.. The injection volume has been 50 µl, and the peaks of the benzoic acid and cinnamic acid derivatives were monitored at 280 and 320 nm, respectively. A 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) was used to filter all the samples prior to injection. The peaks have been determined by congruent retention times and UV spectra and compared to the standards ones (Kim *et al.,* 2006).

Animals

Sixty wistar strain albino female rats (weighting 180–200 g) have been collected from the author's institution animal house at the beginning of the experiment to be used for this research. These rats were kept in clean polypropylene cages individually and kept at controlled

temperature room (22 ±2ºC) in a12-hours light and a 12-hours dark cycle with free access to water and regular rat pellets. Fourteen days before the experiment, Animals could acclimatize to the laboratory conditions. All procedures were conducted and approved by National Research Centre's Ethical Committee in accordance with ethical standards.

Experimental design:

Sixty female rats have been classified into 6 groups, 10 rats in each group, and divided into the following groups: group I (sham group), group II (sham received myrtus and omega-3), group III (ovariectomized group), group IV (ovariectomized group treated with myrtus and omega-3),group V (ovariectomized diabetic group) and group VI (ovariectomized diabetic group treated with myrtus and omega-3) .Groups (II, IV,and VI) were received myrtus extract in a daily orally dose of 100 mg/kg body weight (Rasheed *et al*., 2016) and omega-3 in a daily orally dose of 1.2 ml /kg body weight /day orally (Aly *et al*., 2017) during the experimental period (8 weeks).

Surgical ovariectomy

Bilateral ovariectomy and sham surgeries were performed after the sedation and anesthetization of rats via the mid-abdominal route by intraperitoneal (i.p.) injection of xylazine and ketamine (Goseki *et al*., 1996). Total lack of limb retraction following painful stimulation was evaluated for anesthesia.

Induction of diabetes

Diabetes has been induced by intraperitoneal injections of freshly prepared streptozotocin (40 mg/kg of bodyweight) dissolved in 0.1 M chilled citrate buffer (pH 4.5) (Aly *et al*., 2018). The animals allowed 5% glucose solution to be drunk during the night to prevent hypoglycemic mortality initially caused by streptozotocin. 48 hours after the last dose of streptozotocin, fasting blood glucose levels have been tracked and glucose-levels of animals over 200 mg/dl have been considered diabetic and assigned to different treatment plans.

Collection of samples

After the research period, animals fasted for twelve hours before blood sample was collected by means of capillary tubes and blood was taken from the retro-orbital venous plexus of the eye and collected in:

a. Sodium fluoride tubes used for blood glucose estimation.
b. Heparinized tubes to additional biochemical markers.

The blood has been centrifuged for 10 minutes at 3000 rpm with cooling centrifuge. Instantly, plasma was split and frozen.

Sample of pancreatic tissue was taken for estimation of enzymes and for histopathology examination.

Homogenization of pancreatic tissue

Pancreatic tissues have been homogenized in 50 mmol/l phosphate buffer (pH 7.4) at 4°C for 30s (2 x 15s with a 15s cooling interval) by a Polytron homogenizer. The homogenate has been filtered via cheese cloth and the filtrate was centrifuged at 1000xg for 5 min with a cooling centrifuge (Beckman Model 52-21) (Rakesh *et al*., 1996).

Biochemical Analysis:

Evaluation of insulin resistance

Fasting blood glucose levels have been estimated by enzymatic colorimetric technique, using kits of Centronic Co. (Wartenberg, Germany), based on the method of Trinder, (1969). Plasma insulin levels have been assessed using an enzyme linked immunosorbent assay (ELISA) based on the method of Yallow and Bawman, (1983) using BioSource INSEASIA Co. (Nivelles, Belgium) Kit. Insulin resistance has been determined from this equation: Insulin resistance=fasting glucose (mg/dl) ×fasting insulin (µIU/ml)/405, according to Mathews *et al.* (1985).

Estimation of lipid profile

Cholesterol, HDL-C and triglyceride have been determined by the colorimetric enzymatic examines based on the method of Allain *et al*. (1974), Lopes-Virella *et al.* (1977) and Glick *et al.* (1986)] respectively. The kits have been supplied from Biocon Diagnostic (Germany).LDL-cholesterol has been determined utilizing this equation: LDL-C (mg/dl) = Total cholesterol - (HDL-C+ TG/5) according to Friedewald *et al.* (1972).

Estimation of vWF, 5-LOX, 15-LOX and LA-4 levels

Plasma levels of von Willebrand factor (vWF) and LA-4 have been determined by the method of (1998) and Dobrian *et al*. (2011) respectively. Whereas, according to the method of Hardya *et al*. (2005), the levels of 5-LOX and 15-LOX were determined using manufacturers protocols (R&D systems).The kits have been purchased from Biosource, Belgium.

Estimation of oxidant and antioxidant enzyme activitiesin pancreatic tissue homogenate

The lipid peroxidation is a good way for evaluation the tissue damage caused by oxidant stress. Hence levels of malondialdehyde (MDA) in pancreatic tissue homogenateas, thiobarbituric acid-reactive substances measured using the method of Esterbauer and Cheeseman (1990). The activity of superoxide dismutase (SOD) and catalase activity (CAT) enzyme in pancreatichomogenatewere measured using standard spectrophotometric assay according to Stephen *et al.* (2006).

Histological analysis

The animals have been scarified, rapidly dissected after blood sampling for the biochemical analysis, and small slices of pancreas have been taken in 10 percent formalin and fixed. The specimens have been dehydrated in ascending grades of ethanol, clarified in xylene, and embedded in paraffin wax. Sections with a pancreatic thickness of 6 µm have been prepared and stained with Haematoxylin and Eosin and then examined under light microscope (Drury and Wallington, 1980).

Statistical Analysis

All data was stated as mean ± SE. The normal distribution of data has been confirmed with the standard state test (SPSS package) (version 18). Statistical significance was examined by one-way variance analysis (ANOVA) trailed by post hoc Tukey's tests (experiments with more than two groups and one variable). Pearson's correlation coefficient was obtained. (P) Value<0.05was considered as statistically significant.

Results

Table 1: Phytochemical screening tests for constituents of *Myrtus communis* leaves extract.

Constituent	Detection test	Result
Saponins	Froth	+
Sterols and/or triterpenoids	Libermann-Burchard and Salkowski	+
Tannins	Ferric chloride	+
Flavonoids	Sodium hydroxide	+
Alkaloids	Mayer Wagner's and modified Dragendorff's	-
Glycosides and/or carbohydrates	Molisch	+

Table 2 : High-performance liquid chromatography assay of flavonoids and phenolic compounds in myrtus extract

Compound	Retention Time (min)	Myrtus extract (µg/g)
Gallic acid	5.90	794.54
p-hydroxybenzoic acid	15.22	2789.45
Catechin	18.37	6780.06
Ferulic acid	32.17	144.99
Sinapic acid	33.56	96.54
Rutin	36.18	1621.08
p-coumaric acid	36.95	1026.99

In agreement with previously studies Rasheed *et al.* (2016) & Dellaoui and Berroukche (2019), the HPLC analysis (Table-2 and Fig. 1) of the alcoholic extract of the myrtle showed its highly content from effective secondary metabolites, flavonids as Catechin (6780.06 µg/g) & Rutin (1621.08 µg/g) and phenolic compounds as p-hydroxybenzoic acid (2789.45µg/g), p-coumaric acid (1026.99µg/g), Gallic acid (794.54 µg/g), Ferulic acid (144.99 µg/g) and Sinapic acid (96.54µg/g).

The oral administration of *Myrtus communis* extract combined with omega-3 play an important role to reduce the levels of the blood sugars significantly due to the presence of the biologically active constitutes specially the flavonoids, by adjusting the various signaling pathways in *β*-cells of pancreas, adipocytes, hepatocytes, and skeletal myofibers, furthermore the highly content of phenolics and flavonoids of the extract affect positively each of the following oxidative stress, HDL and LDL levels in the blood serum which can be attributed to the ability of these secondary metabolites to serve as free radical scavenging.

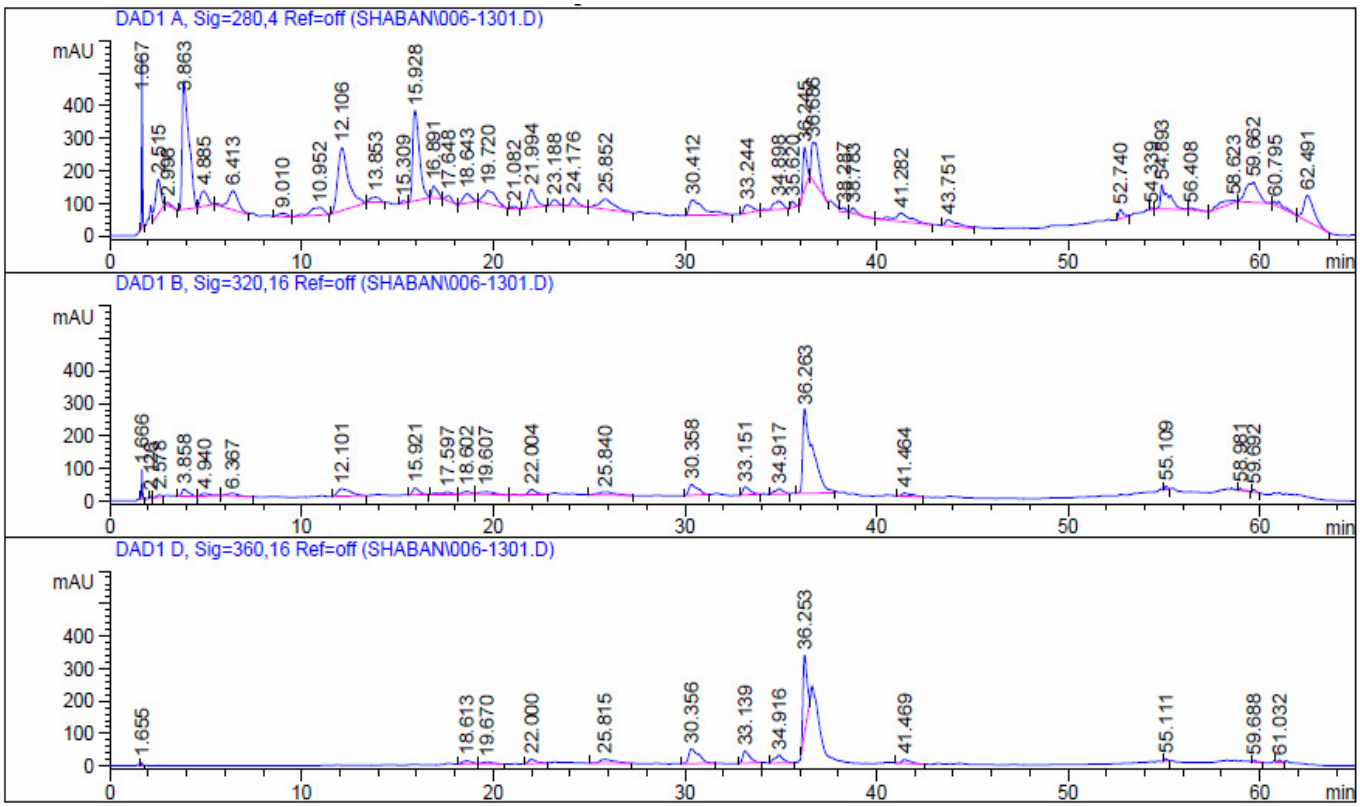

Fig. 1: High- performance liquid chromatography chromatograms of myrtle extract, at wavelength 280 nm, 330 nm and 360nm.

Table 3: Mean levels of blood glucose, plasma insulin and insulin resistance in the different studied groups.

Parameters / Groups	Glucose (mg/dl)	Insulin (µIU/ml)	Insulin Resistance (mg/dl µIU/ml)
Sham Group	90.4± 2.8	6.9 ± 2.6	1.5±0.1
TreatedSham	79 ± 3.7[a]	7.0 ± 1.2	1.4±0.1
OVX Group	108 ± 3.8[a]	14.0 ± 3. 2 [a]	3.7±0.8 [a]
Treated OVX Group	98 ± 4.7[a,b]	9.0 ± 1.4[a,b]	2.2±0.2[a,b]
OVX Diabetic Group	222±8.7 [a]	6.1 ± 1.1	3.3±0.4 [a]
Treated OVX Diabetic Group	116.9±3.7[a,b]	12.4±0.5[a,b]	3.6±0.6 [a]

Values *are expressed as mean ± standard error (SE).*
a: significant difference at p<0.05 compared to sham (SH) group;
b*: significant difference at p<0.05 compared to the blank of each group*

Table 3 showed increase in glucose, insulin and insulin resistance in both OVX and OVX diabetic groups as compared with sham group; these results improved after treatment with a combination of myrtus and omega-3.

Table 4: Mean levels of lipid profile in the different studied groups.

Parameters / Groups	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Sham Group	60.8 ± 9.5	29.22±2.5	99.9 ± 1.4	110± 3.1
Treated Sham	70.1 ± 5.1[a]	13.7±1.9 [a]	80.3 ± 2.7 [a]	99.8 ± 1.7 [a]
OVX Group	40.9 ± 6.7 [a]	105.1±5.6 [a]	120 ± 10.6 [a]	170 ± 4.6 [a]
Treated OVX Group	48.9 ± 4.8 [a]	60.96±4.5[a,b]	100.7 ± 7.2[a,b]	130.2 ± 5.4[a,b]
OVX Diabetic Group	30.8± 2.7 [a]	172.2±5.8 [a]	135±6.7 [a]	230±5.5 [a]
Treated OVX Diabetic Group	48.9±4.6[a,b]	119.1±4.8[a,b]	110±5.2[a,b]	190±5.0[a,b]

Values *are expressed as mean ± standard error (SE).*
a: significant difference at p<0.05 compared to sham (SH) group;
b*: significant difference at p<0.05 compared to the blank of each group*

Table 4 showed significant increase in lipid profile (cholesterol, triglyceridesand LDL) with a significant reduction in HDL in both OVX and OVX diabetic groups as compared to sham group; these results showed significant improvement in lipid profile aftertreatment with myrtus and omega-3.

Table 5: Mean levels of oxidative stress in the pancreatic tissue of different studied groups.

Parameters / Groups	SOD (U/mg protein)	MDA (nmol/mg protein	Catalase activity (U/ mg protein)
Sham Group	11.2 ± 0.73	0.39± 0.03	0.61 ± 0.06
Treated Sham	15.1 ± 0.11[a]	0.31 ± 0.07	0.58 ± 0.08
OVX Group	6.5 ± 0.6[a]	0.83 ± 0.05[a]	0.87 ± 0.01[a]
Treated OVX Group	9.5 ± 0.88[a,b]	0.46 ± 0.06[b]	0.48 ± 0.04[a,b]
OVX Diabetic Group	5.2 ± 0.13[a]	1.07 ± 0.18[a]	0.98± 0.03[a]
Treated OVX Diabetic Group	8.1 ± 0.11[a,b]	0.7 ± 0.48[a,b]	0.42 ± 0.01[a,b]

Values *are expressed as mean ± standard error (SE).*
a: significant difference at p<0.05 compared to sham (SH) group;
b*: significant difference at p<0.05 compared to the blank of each group*

Table 5 showed significant increase in MDA& CAT levels and significant decreasein SOD in both OVX and OVX diabetic groups as compared with sham group; these results improved after treatment with myrtus and omega-3supplementation.

Table 6: Mean levels of 5, 15-lipoxygenase, lipoxin-A4 and vWF in plasma of different studied groups.

Parameters / Groups	15-LOX (U/L)	5-LOX (U/L)	LXA4 (Pg/L)	vWF (ng/L)
Sham Group	8.4 ± 0.76	7.2 ± 0.6	78.6 ± 1.5	534.0 ± 62.0
Treated Sham	6.6 ± 0.89[a]	5.4 ± 0.2[a]	149 ± 5.7[a]	513.0 ± 36.0[a]
OVX Group	16.9 ± 0.77[a]	15.3 ± 1.15[a]	94.0± 7.4[a]	753.0 ± 30.0[a]
Treated OVX Group	10.2 ± 1.31[b]	11.5 ± 1.5[a,b]	156.0 ± 9.0[a,b]	637.0 ± 82.0[a,b]
OVX Diabetic Group	19.1 ± 0.61[a]	20.0 ± 1.3[a]	153.0 ±7.1[a]	808.0 ± 24.0[a]
Treated OVX Diabetic Group	15.4 ± 1.45[a,b]	15.9 ± 0.1[a,b]	172.0 ± 2.8[a,b]	720.0 ± 98.0[a,b]

Values *are expressed as mean ± standard error (SE).*
a: significant difference at p<0.05 compared to sham (SH) group;
b*: significant difference at p<0.05 compared to the blank of each group*

Table 6 showed significant increase in 15-LOX,5-LOX,LXA4and vWF in both OVX and OVX diabetic groups as compared with sham group; these results improved after treatment with a combination of myrtus and omega-3supplementation. And the data showed significant decrease in 15-LOX, 5-LOXand vWF with significant increase ofLXA4 in sham group received combination of myrtus and omega-3 as compared to sham group.

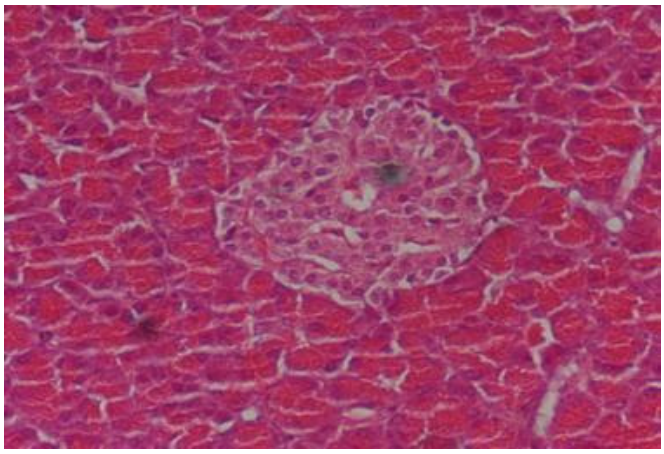

Fig. 2: A section of sham pancreas shows the normal architecture of the exocrine (acini) and endocrine (islets) compartment (H & E stain-X 400).

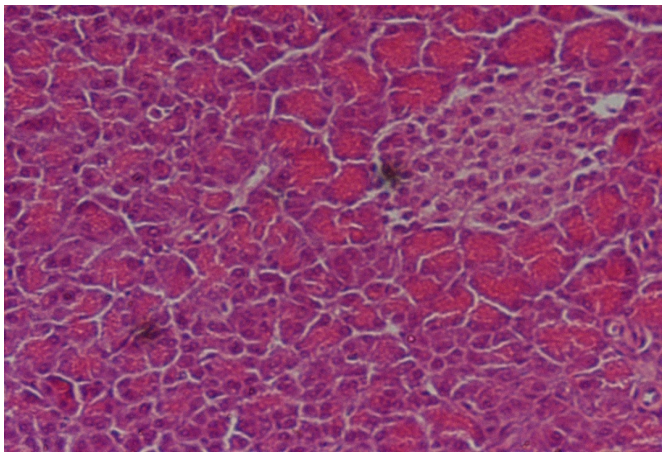

Fig. 3: A section of pancreas of ovariectomizedrat shows the disturbance of thearchitecture of the endocrine part associated with islet degeneration (H & E stain-X 400).

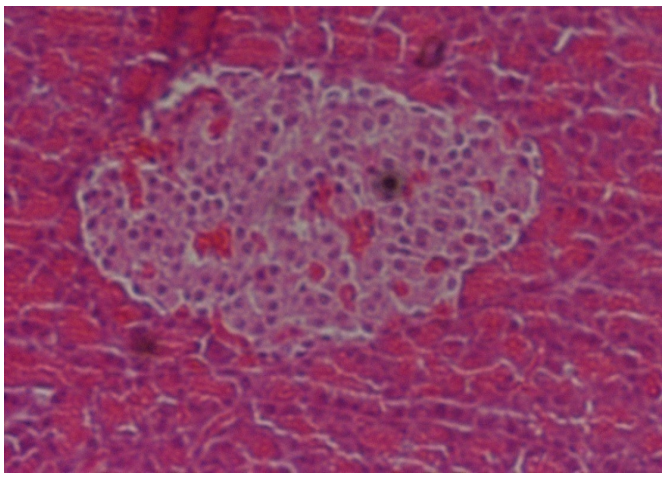

Fig. 4: A section of pancreas of ovariectomized rat given the combination of myrtle and omega-3 shows the exocrine part appears more or less like normal. Notice areas of eosinophlic stain (H & E stain-X 150).

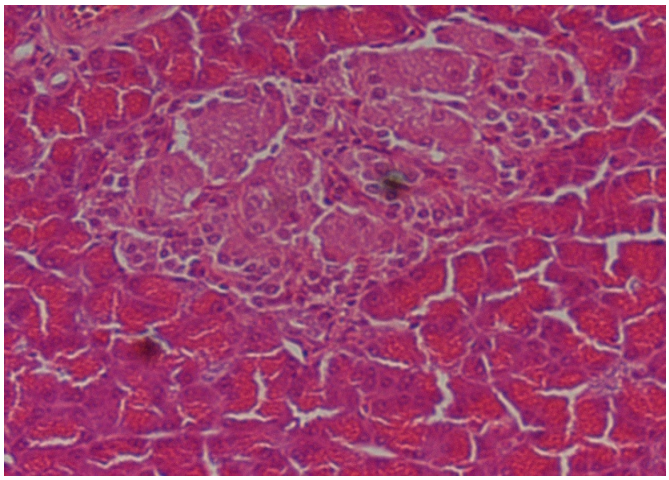

Fig. 5 : A section of pancreas of ovariectomized diabetic rat shows the normal structure of the exocrine parts. The islet exhibit degeneration associated with a reduction in the number of beta cells as compared with the control (H & E stain-X 150).

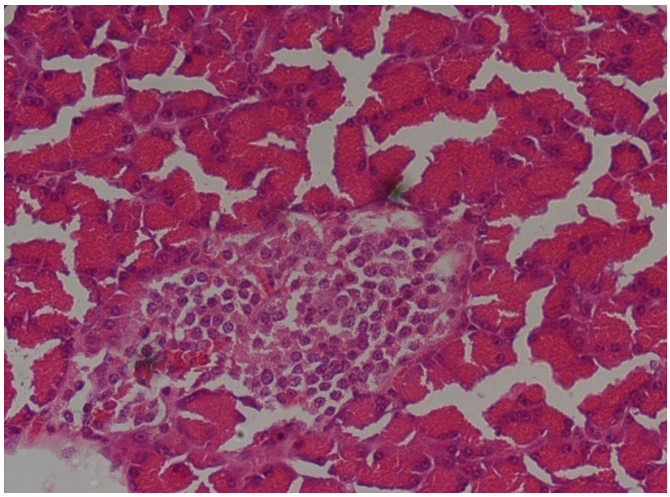

Fig. 6 : A section of pancreas of ovariectomized diabetic rat given the combination of myrtle and omega-3shows an improvement in the islet associated with an increase in the number of beta cells that appear more than with the control (H & E stain-X 150).

Histopathological examination of the pancreas of sham operated control group exhibited that normal appearance of the islet of Langerhans that seen scattered in-between the acini, and appeared as a paler structure formed of clusters of different cells. The acinar cells are formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm (Figure 2). In ovaroctomized rats, microscopic investigation showed a few of islets, appeared with fewer number in cells, and most of them were attenuated with very few or lacking cells in the center (Figure 3).

In ovariectomized-group that given both myrtus and omega-3, examination of pancreas showed that the exocrine and endocrine parts appeared more or less like normal (Figure 4).

Sections of pancreas of ovariectomized diabetic rats showed the hypertrophic feature of the islets that exhibited degeneration associated with a reduction in the number of beta cells as compared with the control (Figure 5).

Slight hypertrophy of Langerhans islets was shown in the pancreas of treated rats with the combination of myrtus and omega-3. The islet associated with an increase in the

number of beta cells that appear more than with the control (Figure 6).

Discussion

Endothelial cells (EC) are the lining of the interior surface of all blood vessels which are in direct contact with the blood stream. Dysfunction of the endothelium and atherosclerosis are the commonest problems with diabetes and menopause (Taddei *et al.,* 1996).

As compared to men of similar age, premenopausal women are found to be safe against the danger of developing metabolic syndrome. That protection is lost after menopause (Sankar *et al.,* 2015). Menopause is a significant stage in the life of women, marked by the absence of reproductive hormones and changed internal state (Fahmy *et al.,* 2018).

Estrogen, a female sex hormone, plays a crucial role in controlling homeostasis of glucose, and postmenopause estrogen deficiency contributes to disruption of the metabolism of glucose (Gravholt, 2005).

In this research, we evaluated the influence of ovariectomy (estrogen depletion) on oxidative damage and its complications especially increase glucose and insulin resistance in female rats. We found that ovariectomy led to the development of oxidative stress and insulin resistance and these complications were further aggravated by the induction of diabetes. Drug therapy will significantly improve these complications, but the combination of myrtus and omega-3 may be an efficient supplement for the treatment of postmenopausal complications.

In this study the level of blood glucose was significantly reduced in treated groups with combination of myrtus and omega-3 compared with sham, ovariectomized and ovariectomized-diabetics groups as in table (3). This combination can exert its effect by several mechanisms such as inhibition of glycogenesis, stimulation of glycolysis, stimulation of insulin release, inhibition of glucose absorption from intestinal tract and having insulin like action. Another possibility is that this combination induces blockage of ATP-dependent potassium channels in pancreatic beta cells and increases intracellular calcium by decreasing the voltage of the cell membrane as shown in Figure (4, 6). Increasing intracellular calcium in turn triggers insulin release, leading to reduced glucose levels. Therefore a significant decrease in glucose concentration in the treated groups can be observed (Johari *et al.,* 2014).

In agreement with our findings Sepici *et al.* (2004) reported that the myrtus extract can inhibit α-glucosidases of the small intestine, thereby decreasing intestinal glucose absorption and delaying the release of glucose from complex carbohydrates.

Our results revealed significant improvement in lipid profile (cholesterol, triglyceride and LDL-cholesterol) levels in all treated groups when compared with counter blanks (Table 4).

Hunkar *et al.* (2002) showed that omega-3 treatment lowers plasma cholesterol, triacylglycerol and lipid peroxidation products, and provides better control of glucose in diabetic animals. This is due to the antioxidant activities of unsaturated fatty acids in omega-3 which alone can play an important role in lipid oxidation prevention. Also myrtus extract contains active compounds that cause decrease in lipid profile level might be due to the inactivation of β-hydroxy β-methyl glutaryl CoA reductase enzyme concerned with cholesterol synthesis (Ahmet, 2004).

Our results revealed that pancreatic SOD was significantly decreased in OVX and OVX- diabetic rats compared with sham group as shown in table (5).The decreasing trend of SOD activity observed in the tissue with progression of OVX, OVX diabetes might be because of glycation of the enzyme which occurs in this condition. Glycation of SOD leads to its inactivation and contributes to oxidative damage through formation of superoxide radical and hydrogen peroxide (Kakkar *et al.,* 1998). After treatment with myrtus and omega-3 there is an increase in the antioxidant enzyme SOD. It has been found that omega-3 has a free radicals scavenging activity that may have a beneficial impact on pathological alterations caused by the presences of O^{-2} and OH^{-} (Harding *et al.,* 2004).

More importance has been focused to the importance of oxidative stress, which can be the essential and common events in the pathogenesis of diverse diabetic complications (Ceriello, 2000). In the present research, the levels of MDA and CAT in pancreatic tissue extract of OVX and OVX-diabetic rats are significantly higher than in sham rats as shown in tables (5). Our results are in agreement with Tourandokht *et al.* (2012). The pancreatic β-cells are highly vulnerable to oxidative stress and damage because they have low antioxidant enzymes expression and activity which are the first line of defense against oxidative insults (Lenzen, 2008).

Habiballah *et al.* (2014) reported that myrtus leaf flavonoids may increase glucose-6-phosphate dehydrogenase enzyme, the main regulatory enzyme of pentose phosphate or phosphogluconate pathway. This pathway provides NADPH required for the hydrogen peroxide to decompose. With increased pentose phosphate pathway activity, hydrogen peroxide removal increases where this reaction is followed by the increase of superoxide dismutase, catalase and glutathione peroxidase.

Because endothelial cell injury is important in atherosclerosis development, elevation of vWF activity may be associated with well-known risk factors for these diseases: hypertension, smoking, obesity, diabetes and dyslipidemia (Horvath *et al.*, 2004). The finding of this study revealed a significant rise in the plasma level in the vWFin the OVX and OVX-diabetics relative to sham group and this result improved after myrtus and omega-3 supplementation as shown in table (6).

A non heme iron containing dioxygenases known Lipoxygenases (LOXs) catalyzes the process of dioxygenation of polyunsaturated fatty acids, involving at least two isolated cis-double bonds. The principal products of the LOX pathway are transformed into a great amount of biologically active lipid mediators including pro-inflammatory mediators, such as leukotrienes (LTs) which is synthesized with arachidonic liopxygenase-5 (ALOX5) (Savari *et al.,* 2014) and also with anti-inflammatory mediators like lipoxins (LA4, LB4) (Romano, 2010).

15-LOX activities have been considered proatherogenic, promoting more atherogenic oxidized LDL cholesterol esters in LDL particle oxidation (Bender *et al.* 2016). Also its pathway induces inflammation in various

tissues by rising the development of inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (Suzuk *et al.* 2015).

In accordance with Suzuk *et al.* (2015), significant increase in the levels of 15LOX, 5LOX were observed in OVX and OVX-diabetic groups as opposed to sham group. Yet after treatment, these levels were increased as shown in table (6).

Furthermore, it has been reported that myrtus extract potently suppresses 5-lipoxygenase (5LOX) biosynthesis. Its ability to suppress typical pro-inflammatory cellular responses has suggested its therapeutic use in the treatment of inflammatory and allergic diseases (Feisst *et al.,* 2005).

Innes and Calder (2020) demonstrated that EPA and DHA (omega 3) have beneficially modulated a variety of recognized risk factors such as blood lipids, endothelial function and inflammation. In addition (chen, 2016) documented that the protective effect of myrtus extract may be related to flavonids compounds as catechin, rutin and phenolic compounds like p-hydroxybenzoic acid, p-coumaric acid, gallic acid, ferulic acid, sinapic acid, which act as antioxidants, anti-inflammatory, anticancer, antimutagenic, antiglycemic & have neuroprotective activities and scavenging radiation-induced free radicals.

Conclusion

The present study demonstrated that both myrtus extract and omega-3 have the ability to restore endothelial dysfunction in menopause or even in diabetes associated with menopause owing to the additive effect of the two therapeutic agents which give this combination a powerful effect, including antioxidant, anti-inflammatory, and antihyperglycemic properties.

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Abbreviations

AA	Arachidonic Acid
ALT	Alanine Aminotransferase
AST	Aspartate Aminitransferase
CAT	Catalase Activity
COX	Cyclooxygenase
CYP450	Cytochrome P450
DHA	Docosahexaenoic Acid
E2	Estradiol
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Eicosapentaenoic Acid
GPX	Glutathione Peroxidase
HDL	High- Density Lipoprotein
HPLC	High-Performance Liquid Chromatography
LC	Liquid Chromatography
LDL	Low-Density Lipoprotein
LOX	Lipooxygenases
LXA4	Lipoxin-A4
MDA	Malondialdehyde
PBS	Phosphate Buffered Saline
ROS	Reactive Oxygen Species
SE	Standard Error
SOD	Superoxide Dismutase
SPSS	Statistical Package For The Social Science
STZ	Streptozotocin
T2DM	Type 2 Diabetes Mellitus
vWF	Von Willebrand Factor
ω-3	Omega-3
ω-6	Omega-6

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