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Characterization of antihypertensive and cardioprotective effects of extra virgin olive oil against doxorubicin induced cardiomyopathy in rats

[Caracterización de los efectos antihipertensivos y cardioprotectores del aceite de oliva virgen extra contra la miocardiopatía inducida por doxorrubicina en ratas]

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Abstract

Context: The medicinal uses of olive fruit oil (*Olea europaea*) are clearly documented as an anti-inflammatory, anti-diabetic (type-2 DM), and to protect against heart diseases.

Aims: To evaluate the therapeutic influence of extra virgin olive oil (EVOO) as an antihypertensive and cardioprotective against doxorubicin (DXR) induced cardiomyopathy in rats.

Methods: Wistar rats were divided into five groups as group 1 (normal control), group 2 (disease control i.e., DXR-treated) and groups 3, 4 and 5 as therapeutic groups (i.e., DXR- treated rats plus 2.5%, 5%, and 10% EVOO in diet respectively). Cardiac injury was induced by the administration of DXR. Cardioprotective, anti-hypertensive and antioxidant potential of EVOO were studied by measuring different blood, serum, and tissue biomarkers, enzymes and hemodynamic parameters in disease control and experimental rat groups.

Results: Hemodynamic parameters were significantly reduced in rats administered with 2.5%, 5%, and 10% EVOO as compared to group 2 rats at p<0.05, p<0.01, and p<0.001 respectively. Serum levels of creatinine kinase, troponin T, CK-MB and LDH were found markedly reduced in rats fed with 10% dietary EVOO as compared to disease control rats (p<0.01). Serum levels of antioxidant enzymes like GSH and SOD were found significantly higher in group 5 rats as compared to group 2 at p<0.01, and p<0.001, respectively.

Conclusions: Dietary administration of EVOO has the potential to reduce drug-induced cardiomyopathies and sustained use of EVOO in diet could be cardioprotective and useful against high blood pressure.

Keywords: antihyperlipidemic; blood pressure; cardiac diseases; drug-induced cardiac injury; monounsaturated fatty acids.

Resumen

Contexto: Los usos medicinales del aceite de oliva (*Olea europaea*) están claramente documentados como antiinflamatorios, antidiabéticos (DM tipo 2) y para proteger contra las enfermedades del corazón.

Objetivos: Evaluar la influencia terapéutica del aceite de oliva virgen extra (AOVE) como un antihipertensivo y cardioprotector contra la miocardiopatía inducida por doxorrubicina (DXR) en ratas.

Métodos: Ratas Wistar se dividieron en cinco grupos como grupo 1 (control normal), grupo 2 (control de la enfermedad) y grupos 3, 4 y 5 como grupos terapéuticos (es decir, tratamiento con AOVE) respectivamente. La lesión cardíaca fue inducida por la administración de DXR. El potencial cardioprotector, antihipertensivo y antioxidante de diferentes concentraciones de AOVE (es decir 2,5%; 5% y 10% en la dieta) se estudió midiendo/evaluando diferentes biomarcadores de sangre, suero y tejido, enzimas y parámetros hemodinámicos en los tratados con DXR ratas alimentadas con diferentes concentraciones de AOVE en la dieta.

Resultados: Los parámetros hemodinámicos se redujeron significativamente en ratas administradas con 2,5%; 5% y 10% de AOVE en comparación con las ratas del grupo 2 a p<0,05, p<0,01 y p<0,001 respectivamente. Los niveles séricos de creatinina quinasa, troponina T, CK-MB y LDH se encontraron notablemente reducidos en ratas alimentadas con 10% de AOVE en la dieta en comparación con las ratas de control de la enfermedad (p<0,01). Los niveles séricos de enzimas antioxidantes como GSH y SOD se encontraron significativamente más altos en ratas del grupo 5 en comparación con el grupo 2 a p<0,01 y p<0,001, respectivamente.

Conclusiones: La administración dietética de aceite de oliva virgen extra tiene el potencial de reducir las cardiomiopatías inducidas por fármacos y el uso sostenido de AOVE en la dieta podría ser cardioprotector y útil contra la presión arterial alta.

Palabras Clave: ácidos grasos monoinsaturados; antihiperlipidémico; enfermedades cardiacas; lesión cardíaca inducida por fármacos; presión sanguínea.

INTRODUCTION

Doxorubicin (DXR) classified as an anthracy-cline antibiotic is widely recommended for cancer treatment such as breast cancer and carcinoma of small cells of lungs and leukemias (Hsu et al., 2014). However, the use is limited due to potential cause of acute and chronic toxicities in treated individuals (Pathan et al., 2010). The acute toxicity cause myelosuppression, nausea, vomiting, and cardiac arrhythmias, which are reversible; however, the chronic toxicities are irreversible and unmanageable producing cardiomyopathy and heart failure (Pathan et al., 2010; Hsu et al., 2014). The complete and exact mechanisms of these toxicities are still poorly understood but assumed by the various researchers to be involved the production of oxidative stress (Soga, 2006), induction of apoptosis (Ferreira et al., 2007), activation of renin angiotensin system (RAS) (Nazeyrollas et al., 1999), oxidative stress induced by DNA damage (Ferreira et al., 2007), lipid peroxidation and creatinine kinase enzyme impairment (Toko et al., 2002; El-Shitany et al., 2008). Several drugs (e.g., dexrazoxane, amifostine, fullerenol, aspirin) and natural products (e.g., vitamin A, vitamin E) have been investigated to counter DXR-induced cardiomyopathy with promising clinical effects (Nazeyrollas et al., 1999), but still need to fully elucidate their therapeutic benefits. In parallel to that, alternate regimens with minimum adverse effects on cardiac tissue are urgently needed.

Being the main component of the Mediterranean diet, the olive oil (produced from the olive fruit of *Olea europaea* L., *Oleaceae*) contain monounsaturated fatty acids as well as an elevated content of antioxidant agents (Núñez-Córdoba et al., 2009). Although the health benefits of dietary fats are controversial, but a diet rich in monounsaturated fatty acids like olive oil reduces inflammation and pose beneficial effects on genes link to cancer (Martín-Peláez et al., 2017). Similarly, a diet adhesion to olive oil could contribute to prevent age-related hypertension (Núñez-Córdoba et al., 2009). A study published by the PREDIMED (Mediterranean diet prevention) demonstrated that individuals consuming a diet with EVOO reported lower diastolic blood pressure (DBP) than those who consumed a low-fat one (Toledo et al., 2013; Guasch-Ferré et al., 2014). Oleic acid, the main monounsaturated fatty acid of olive oil significantly lowers the level of C-reactive protein (CRP; an important inflammatory marker) (Lockyer et al., 2017). Similarly, the antioxidant content of olive oil with modest amount of oleocanthal, vitamin E and K are biologically active to reduce the risk of chronic diseases as well as to fight against inflammation by inhibiting some genes and protect blood cholesterol from oxidation (Lockyer et al., 2017) . Both these effects significantly lower the chances of heart disease progression. In addition, several investigations revealed the use of olive oil to fight against Alzheimer's disease, rheumatoid arthritis and to prevent strokes (Gorzynik-Debicka et al., 2018). From digestive point of view, olive oil decreases the cholelithiasis risk by decreasing the gastric secretory function and pancreatic exocrine secretions in response to food (Casas et al., 2018). Furthermore, olive oil heals gastric ulcer and offers a high barrier to resistance against non-steroidal anti-inflammatory drugs (NSAIDs)-induced gastric ulcerogenesis (Casas et al., 2018).

Cardiovascular diseases (CVDs) are much prevalent in Saudi Arabia and their treatment demands higher use of medication and costs in treating individuals (Hersi et al., 2012; Koura et al., 2012). Similarly, the large consumption of fatty food, smoking, stress and lack of exercise may propagate or worse the heart diseases and cardiac myopathies. In contrast, the use of healthy diet enriched with unsaturated fatty acids may reduce and prevent the risk of CVDs, however; still need to explore their full therapeutic benefits and underlying mechanism of actions (Hersi et al., 2012; Koura et al., 2012).

Considering the above-mentioned health benefits of dietary olive oil, the present study was designed to aim the ameliorative potential of EVOO's as an antihypertensive and cardioprotective at different doses (i.e., 2.5%, 5% and 10%) in dietary intake against DXR-induced cardiomyopathies in Wistar-strain rats. The study objectives were

unique and novel to find out the cardioprotective effects of EVOO against drug induced cardiac injuries in rats, which are not previously reported; although some studies demonstrate antihypertensive effects of dietary olive oil in spontaneously hypertensive rats (Rodriguez-Rodriguez et al., 2009; Villarejo et al., 2015; Vazquez et al., 2019). Different biochemical parameters including hemodynamic measurements and biochemical estimation of different cardiac biomarkers and enzymes with standard procedures and protocols were used to access the beneficial effect of EVOO's as an anti-hypertensive and cardioprotective. The study findings demonstrated that the dietary intake of EVOO's could revert the DXR-induced cardio myopathies in rats. In parallel to that, the further investigations could be initiated in humans to elucidate the EVOO beneficial impact as an anti-hypertensive and to protect against DXR-induced cardiotoxicities and associated comorbidities when used as an adjunct therapy or as a dietary supplement with other anti-hypertensive drugs.

MATERIAL AND METHODS

Experimental animals

The adult albino female rats (i.e., Wistar strain) weighing 170 - 200g were housed in standard cage (six rats per cage) under optimal laboratory conditions at room temperature 25 ± 2°C with 12 hours light/dark cycle. The rats had free access to normal pellet diet water *ad libitum* and acclimatized for 7 days on a standard maintenance diet. The normal control rats in group 1 and DXR-treated rats were adopted to the standard maintenance diet through the whole study period while the therapeutic rats in groups 3, 4 and 5 were acclimatized with normal standard diet enriched with different concentrations of EVOOs for first 7 days and after that continued for the whole study period. In each group 6 rats were included for the experimental studies. The institutional research ethics committee of pharmacy college (IRECPC), Umm Al-Qura University, Makkah, Saudi Arabia (approval No. UQ-12678/2019) approved the study and animal experiments were conducted in accordance with the appropriate guidelines of the

Declaration of Helsinki. All the research protocols were followed as per guidelines of the kits, reagents and machine's manufacturers' recommendations.

Drugs and reagents

DXR used for analytical and diagnostic purpose only was obtained from Dabur® (Ghaziabad, India) and highly pure grade extra virgin olive oil was purchased from the local market of Makkah, Saudi Arabia. The quantitative characterization of the olive oil administered to the rats were determined by following the same procedure as previously described by the Olmo-García et al. (2018). The acidity value in functional oil was measured as 0.12% and peroxide value 8.5 meq/kg. The main fatty acids contents of the oil were evaluated as palmitic (C16) 9.05%, stearic (C18) 12.67%, oleic (C18:1) 78.69%, and linoleic (C18:2n6) 3.71%. Similarly, total ethyl esters and total sterols were calculated as 11 mg/kg and as 1296 mg/kg, respectively. The other reagents, chemicals and kits were of analytical grade and company specified.

Experimental protocol and treatment line

Group 1 rats were fed with normal diet during the whole study and group 2 rats were administered to DXR (1.25 mg/kg, i.p.) in 16-divided doses/month to induce cardiac injury and were fed with normal standard diet until the last DXR dose was administered. We followed the same DXR-treatment line to induce cardiac toxicity in groups 3, 4 and 5 rats as previously described by Andreadou et al. (2007) with slight modification in treatment. Briefly instead of 20 mg/kg, i.p. of DXR to induce acute cardiotoxicity as reported by Andreadou et al. (2007), we divided this dose into 16 equal doses; i.e., 1.25 mg/kg body weight (BW) of experimental rats in group 2, 3, 4 and 5 and administered for one month to induce persistent cardiac injury. Groups 3, 4 and 5 rats were fed with EVOO 2.5%, 5% and 10% concentrations in standard maintenance diet respectively while acclimatized for 7 days to this EVOO enriched diet. The criteria to evaluate the therapeutic potential of three different EVOO doses at 2.5%, 5% and 10% concentrations were in accordance with studies of

Andreadou et al. (2007), who reported the successful treatment of acute DXR-induced cardiotoxicity in rats with 200 mg/kg BW olive oil rich diet. Likewise, Rodriguez et al. (2007; 2009) also demonstrated the beneficial impact of 15% pomace olive oil rich diet in hypertensive rats. Recently, Vazquez et al. (2019) also explicated the cardioprotective effect of EVOO at a concentration of 750 mg/kg BW in spontaneously hypertensive rats. After 24 h of last dose administered (i.e., at day 30), blood samples were drawn from retro-orbital plexuses under light ether anesthesia from all experimental rats. The biochemical parameters including lactate dehydrogenase (LDH), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and electrolyte (i.e., Na^+, K^+ and Ca^{++}) were estimated using standard kits. For LDH analysis, the kit was purchased from AAT Bioquest® (Sunnyvale, CA, USA) and for serum SGPT and SGOT estimation, the kits were procured from Crescent diagnostics® (Jeddah, Saudi Arabia). The blood troponin-T levels were measured by following the standard kit procedure purchased from Wako pure chemical industries® (Osaka, Japan) while creatine phosphokinase (CPK) and creatine phosphokinase MB (CPK-MB) were measured by following the procedure of a kit purchased from life diagnostics® Inc. (Pennsylvania, USA). The heart was excised and washed in an ice-cold physiological saline solution. A portion of cardiac tissue was weighed and homogenized by using a Heidolph 50110 R2R20 homogenizer (Heidolph Instruments GmbH®, Germany) for serum enzyme estimations including malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase acyltransferase (CAT) while following the standard kit protocol and procedures. The assays were performed on the supernatant preparation in a Sorvall RC-2B centrifugation (BioSurplus®, USA) of the homogenate at 14,000 rpm for 30 min at 4°C.

Hemodynamic measurements

Hemodynamic measurements (e.g. systolic, diastolic, mean blood pressure, and heart rate) were performed by using tail cuff method through Biopac® non-invasive blood pressure recording instrument (Biopac® systems, USA). First, the rats under study were adopted to the procedure for 10-15 days while putting in the restrainer for 15 min/day prior to the measurement of hemodynamic parameters and then the final measurements were done. After 24 h of last dose administered (i.e., at day 30), hemodynamic parameters were recorded in each group consisting of 6 rats.

Biochemical analysis in serum

Troponin-T, CPK, CK-MB, LDH, SGPT, SGOT, Na^+, K^+, and Ca^{++} ions in cardiac tissue and rat serum were measured by specific diagnostic enzyme kits as mentioned above by using biochemistry semiautoanalyzer, Nicholas Piramal 5010 (DiaSys®, India) machine.

Biochemical assays in cardiac tissues homogenates

The lipid peroxidation in rat cardiac tissue homogenates was performed by following the method as previously described by Ohkawa et al. (1979). The antioxidant enzymatic activity of GSH, SOD and CAT in cardiac tissue were determined by using the standard protocol as previously demonstrated by Ellman, Marklund, and Claiborne respectively (Ellman, 1959; Marklund and Marklund 1974; Claiborne, 1985).

Statistical analysis

The data were expressed as mean ± standard error of mean (S.E.M.). Each experiment was performed in triplicate. The data among different groups were compared by the analysis of variance (ANOVA) followed by Tukey's post-test. The p values were considered statistically significant at $p < 0.001$, $p < 0.01$, and $p < 0.05$ where applicable by using the Minitab software, version 17.0 (Minitab Inc, State College, PA, USA).

RESULTS

Ameliorative influence on hemodynamic parameters

An increase in systolic, diastolic, mean blood pressure, and heart rate were observed in disease

control group (group 2) after the administration of DXR as compared to the normal control group (group 1) and were found statistically significantly at p<0.01 as expressed in Table 1. However, the DXR-treated rats in groups 3, 4, and 5 fed with different concentrations of EVOO's (i.e., 2.5%, 5 %, and 10% in diet) showed marked reduction in the systolic, diastolic, mean blood pressure and heart rate as compared to DXR-treated rats at p<0.05; p<0.01; p<0.001 as depicted in Table 1.

Creatinine kinase, troponin T, CK-MB and LDH levels in serum

DXR administration at a dose of 1.25 mg/kg, i.p. in 16 divided doses/month in group 2 rats caused a significant increase in their serum CK, troponin T, CK-MB and LDH enzyme activities as compared to their respective values in normal control (i.e., group 1) and found statistically significant at p<0.01 (Table 2). The higher estimation of these enzymes possibly indicated cardiac injuries in DXR treated rats. Dietary administration of EVOO's at different doses (i.e., 2.5%, 5%, and 10% in diet) significantly reduced their elevated levels and revert to be like the normal ones when their estimation in groups 3, 4, and 5 rats was compared with group 2 animals and found statistically significant at p<0.01 and p<0.001 (Table 2). However, this effect was noted more pronounced in rats feeding on 10% dietary EVOO as shown in Table 2.

Table 1. Effect of different doses of dietary EVOO on rat hemodynamic measurements.

Group	SBP (mm Hg)	DBP (mm Hg)	MBP (mm Hg)	HR (beats/min)
Normal control	140.2 ± 1.22	94.5 ± 1.01	115.35 ± 0.89	310.10 ± 3.2
Doxorubicin control	195.1 ± 2.32[B]	120.45 ± 1.09[B]	160.12 ± 1.4[B]	425.14 ± 3.97[B]
DXR + EVOO 2.5%	178.6 ± 1.98[c]	110.01 ± 1.05[b]	150.21 ± 0.69[b]	408.13 ± 3.12[b]
DXR + EVOO 5.0%	165.6 ± 1.65[c]	106.93 ± 1.19[c]	138.02 ± 1.01[a]	374.24 ± 3.18[b]
DXR + EVOO 10.0%	150.1 ± 2.22[b]	102.35 ± 1.12[b]	125.10 ± 0.74[c]	344.58 ± 3.48[b]

Each experiment was performed in triplicate where n = 6 in each group. Data were expressed as mean ± SEM. Treatments not sharing the same letters in the same row were significantly different by ANOVA followed by a Tukey's test where [a]p<0.05; [b]p<0.01; [c]p<0.001 compared to doxorubicin control and [B]p<0.01 compared to normal control. DXR: doxorubicin; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HR: heart rate.

Table 2. Effect of different doses of dietary EVOO on rat troponin T, CPK, CPK-MB, and LDH enzyme levels.

Experimental groups	Troponin T (ng/mL)	CPK (U/L)	CPK-MB (IU/L)	LDH (U/L)
Normal control	0.32 ± 0.03	158.37 ± 1.49	7.9 ± 0.89	150.41 ± 0.92
Doxorubicin control	1.80 ± 0.14[B]	300.05 ± 3.59[B]	25.3 ± 2.01[B]	295.21 ± 3.11[B]
DXR + EVOO 2.5%	1.01 ± 0.05[c]	230.24 ± 1.48[b]	20.1 ± 0.98[c]	245.16 ± 1.02[b]
DXR + EVOO 5.0%	0.51 ± 0.04[b]	190 .48 ± 1.60[c]	14.6 ± 0.57[b]	205.02 ± 0.90[b]
DXR + EVOO 10.0%	0.39 ± 0.04[c]	167.38 ± 1.59[b]	10.2 ± 0.48[c]	180.11 ± 0.86[b]

Each experiment was performed in triplicate where n = 6 in each group. Data were expressed as mean ± SEM. Treatments not sharing the same letters in the same row were significantly different by ANOVA followed by a Tukey's test where [a]p<0.05; [b]p<0.01; [c]p<0.001 compared to doxorubicin control and [B]p<0.01 compared to normal control. DXR: doxorubicin; CPK: creatine phosphokinase; CPK-MB: creatine phosphokinase MB; LDH: lactate dehydrogenase.

Table 3. Effect of dietary EVOO on SGPT, SGOT, calcium, sodium and potassium levels in rat serum.

Experimental groups	SGPT (ALT) (U/L)	SGOT (AST) (U/L)	Calcium (Ca++) (mg/dL)	Sodium (Na+) (mmol/L)	Potassium (K+) (mmol/L)
Normal control	48.28 ± 0.51	97.27 ± 0.46	9.45 ± 0.25	146.89 ± 0.25	8.01 ± 0.67
Doxorubicin control	80.25 ± 0.73[B]	185.34 ± 0.82[B]	12.05 ± 0.14[B]	158.01 ± 0.26[B]	7.32 ± 0.24 [B]
DXR + EVOO 2.5%	68.34 ± 0.88[a]	157.48 ± 0.75[b]	11.03 ± 0.22[b]	153.66 ± 0.21[c]	7.64 ± 0.24 [c]
DXR + EVOO 5.0%	59.51 ± 0.41[b]	130 .58 ± 0.60[b]	10.37 ± 0.18[c]	152.68 ± 0.32[b]	7.99 ± 0.79 [b]
DXR + EVOO 10.0%	51.38 ± 0.49[c]	102.47 ± 0.46[c]	10.02 ± 0.39[b]	149.40 ± 0.47[c]	8.12 ± 0.56 [b]

Each experiment was performed in triplicate where n = 6 in each group. Data were expressed as mean ± SEM. Treatments not sharing the same letters in the same row were significantly different by ANOVA followed by a Tukey's test where [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ compared to doxorubicin control and [B]$p < 0.01$ compared to normal control. DXR: doxorubicin; SGPT: serum glutamic pyruvic transaminase; ALT: alanine aminotransferase; SGOT: serum glutamic oxaloacetic transaminase; AST: aspartate aminotransferase.

Table 4. Effect of dietary EVOO on lipid peroxidation and antioxidant enzymes.

Experimental groups	MDA (nM/mg protein)	GPx (µM of GSH/min/mg protein)	CAT (µM of H_2O_2/min/mg protein)	SOD (U/mg protein)
Normal control	3.25 ± 0.41	28.48 ± 0.29	115.69 ± 1.50	8.49 ± 0.74
Doxorubicin control	5.67 ± 0.27[B]	15.99 ± 0.49[B]	148.58 ± 0.69[c]	4.70 ± 0.67[B]
DXR + EVOO 2.5%	4.59 ± 0.22[a]	19.35 ± 0.46[b]	132.34 ± 0.56[c]	5.45 ± 0.70[b]
DXR + EVOO 5.0%	3.99 ± 0.57[b]	21 .01 ± 0.35[c]	123.51 ± 0.46[b]	6.75 ± 0.52[a]
DXR + EVOO 10.0%	3.50 ± 0.52[c]	22.99 ± 0.79[b]	119.51 ± 0.73[b]	7.85 ± 0.21[c]

Each experiment was performed in triplicate where n = 6 in each group. Data were expressed as mean ± SEM. Treatments not sharing the same letters in the same row were significantly different by ANOVA followed by a Tukey's test where [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ compared to doxorubicin control and [B]$p < 0.01$ compared to normal control. DXR: doxorubicin; MDA: malondialdehyde; GSH: glutathione reductase; CAT: catalase acyltransferase; SOD: superoxide dismutase.

Serum levels of SGPT, SGOT, Na+, K+, and Ca++ ions

As shown in Table 3, SGPT and SGOT levels were found significantly increased in serum of the disease control rats (i.e., group 2) as compared to normal controls (group 1) at $p < 0.01$. However, the dietary intake of EVOO caused significant reduction of SGOT and SGPT serum levels in groups 3, 4 and 5 animals fed with different concentrations of EVOOs at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (Table 3). Interestingly, no significant alterations were found in the serum levels of Na+, K+ and Ca++ in normal control, disease control and therapeutic control groups. A significant reduction in serum SGOT and SGPT levels were demonstrated in rats fed with 10% EVOO in diet (Table 3).

Lipid peroxidation evaluation by measuring malondialdehyde (MDA) levels in tissue homogenates

Myocardial MDA contents were measured to evaluate the lipid peroxide levels of the cardiac tissue and were found significantly higher ($p < 0.01$) in DXR treated rats (group 2) as compared to normal control (group 1) as shown in Table 4. MDA levels were decreased in all dietary EVOO fed rats in groups 3, 4, and 5. However, statistically much significant decrease was measured in rats fed with 10% dietary EVOO (i.e., group 5) at $p < 0.001$ as illustrated in Table 4.

Evaluation of antioxidant enzymes

DXR treated rats in group 2 showed higher levels of CAT and marked reduction in GSH and SOD

levels as compared to group 1 normal rats (p<0.01). It indicated much significant role of these enzymes to modulate reactive oxygen species, free radicals and oxidative stress in DXR treated animals (George et al., 2018). However, dietary intake of EVOO at all doses markedly restored the levels of antioxidant enzymes in groups 3, 4 and 5, where significant elevation was noticed at 10% EVOO intake in diet at p<0.01; p<0.001 when compared to group 2 DXR-treated animals (Table 4).

DISCUSSION

Therapeutic use of DXR is well documented and well known as an important chemotherapeutic agent against breast and lung cancers as well as the sarcomas of the soft tissues (Hsu et al., 2014). However, the emergence of severe adverse events like cardiomyopathy and cardiac failure limits it's administration in cancer patients (Ferreira et al., 2007).

The exact mechanism for the severe cardiotoxicities is not well known but is proposed to induce cardiac injury, which further leads to cardiac remodeling by various mechanisms including production of reactive oxygen species, by increasing Ca^{++} overload in the cardiocytes, metabolite accumulation, stimulating the generation of prostaglandins and thromboxanes, stimulating histamine secretion, direct interaction with the actin–myosin contractile system in the heart, and by both positive and negative ionotropic effects (Toko et al., 2002; Soga, 2006; El-Shitany et al., 2008; Hsu et al., 2014). The results of this study substantially validate the findings of the previous investigators where DXR in 16 equal cumulative doses (1.25 mg/kg, i.p) has the capability to induce chronic cardiomyopathy in adult albino rats (Nazeyrollas et al., 1999; Toko et al., 2002; El-Shitany et al., 2008; Pathan et al., 2010; Hsu et al., 2014).

Many researchers have been postulated that the administration of DXR markedly alter the hemodynamic measures in treated subjects where a persistent elevation in systolic, diastolic, mean blood pressure, and heart rate have been documented (Sacco et al., 2001; Pathan et al., 2010; Hsu et al., 2014). Likewise, in the present study, the admin-

istration of DXR at a dose of 1.25 mg/kg, i.p significantly increased (p<0.01) the systolic, diastolic, mean blood pressure and heart rate in the disease control rats (group 2) as compared to normal control rats in group 1 (Table 1). It was also evident by the observations when a decrease in body as well as heart weight was noticed in the DXR treated rats. The plausible justification of these findings might be due to the deleterious effects of DXR on rat's intestinal mucus membrane, which might led to decrease food intake and reduced secretions of the intestinal hormones, which ultimately decreased body weight (Pathan et al., 2010; Hsu et al., 2014).

The Mediterranean olive oil rich diet and different derivatives of olive oil have been proven to exert beneficial effects in different cardiovascular diseases and on hypertensive states (Nekooeian et al., 2014; Lopez et al., 2016; Romero et al., 2016; Tsartsou et al., 2019). It has also been documented that long-term administration of EVOO decreased the higher systolic blood pressure and effective to alleviate cardiac hypertrophy (Poudyal et al., 2010). Some studies also suggest the sustained treatment of EVOO and its derivatives as an effective tool alone or in combination with other antihypertensive regimens to reduce blood pressure and cholesterol (Susalit et al., 2011; Storniolo et al., 2017). Similarly, the aqueous extract of olive oil green leaves has been reported to inhibit angiotensin converting enzyme (ACE) as a proposed mechanism to reduce blood pressure *in vitro* (Villarejo et al., 2015). The findings this study demonstrate that the administration of EVOO enrich diet decreased systolic, diastolic and mean blood pressure and heart rate in DXR-treated rats and restored towards normal value after long-term administration of EVOO enriched diet (Table 1). Our results are in accordance with the studies of Villarejo et al. (2015) who found the beneficial influence of olive oil on cardiovascular system in spontaneously hypertensive rats and suggested that the Mediterranean diet rich in olive oil could be an effective tool for the management of hypertension. Our data are also supported by the findings of Vazquez et al. (2019) who demonstrated cardioprotective effect of olive oil with bioactive compounds in hy-

pertensive rats. The results are also in accordance with previous published studies where an oral administration of olive oil leaf extract (15%) enriched with oleuropein decreased blood pressure after two weeks and cardiac hypertrophy after five weeks administration respectively (Poudyal et al., 2010; Nekooeian et al., 2014). In contrast, we accessed the antihypertensive and cardioprotective influence of dietary EVOO against chemotherapeutically treated rats where high blood pressure and cardiomyopathies were usually seen as common severe adverse event. In parallel to that, the therapeutic influence was more pronounced at 10% EVOO enriched diet to decrease blood pressure and revert cardiac hypertrophic changes (Tables 1 and 4). We did not study the impact of lowering cholesterol levels on hemodynamic measures because anti-hyperlipidemic potential of EVOO is not widely studied and limited to decrease the plasma cholesterol levels and not link to decrease blood pressure in experimental animal models (Lockyer et al., 2017). However, Vazquez et al. (2019) recently reported total cholesterol reduction in simultaneously hypertensive rats (SHR) after EVOO enriched diet, which could be a base for further studies to deliberate the role of dietary EVOO as a hypo-cholesterolemic agent against chemotherapy induced hyperlipidemia in animals and humans.

Serum levels of troponin T, CK, CK-MB and LDH are important myocardial biomarkers for the evaluation of cardiotoxicity and congestive heart failure (Martin-Peláez et al., 2017; Sanchez-Rodriguez et al., 2018a). Various scientists found the elevated levels of serum troponin T, CK, CK-MB and LDH during the cardiotoxic events (Martin-Peláez et al., 2017; Sanchez-Rodriguez et al., 2018a). Same as ever, in this study serum levels of these enzymes were also found significantly higher in the disease control group after the administration of DXR, whereas dietary intake of EVOO reversed this increase markedly, where 10% dietary EVOO significantly lowered these enzymes in DXR-administered rats (Table 2) without any significant change in blood pressure. Until now, the knowledge about the underlying mechanisms to reduce the serum levels of these biomarkers is still

lacking for both EVOO and its bioactive components.

The researchers have also been confirmed the involvement of ROS in the generation of myocardial injury and congestive cardiac failure (Hsu et al., 2014). Similarly, an indirect link has been reported to reduce the oxidative stress while treating anti-hypertensive states in animals (Wind et al., 2010). The antioxidant potential of EVOO itself and its bioactive compounds have been described *in vivo*, in experimental animal models, and even in clinical trials where minor components of EVOO (e.g., hydroxytyrosol, oleuropein) have been suggested to mediate antioxidant impacts of EVOO (Romero et al., 2016; Peyrol et al., 2017). We observed that DXR administration led to higher levels of ROS in cardiomyocytes and exhibited a significant ($p<0.01$) rise in the MDA and CAT concentration and a marked ($p<0.01$) reduction in the activity of GSH and SOD in cardiac tissue of rats in group 2. While the rats fed with dietary EVOO found to have decreased MDA and CAT levels and an improvement was seen in their cardiac tissue GSH and SOD levels, which suggest that EVOO might has the antioxidant effect (Table 4). The plausible mechanistic justification of this therapeutic potential is not widely studied in the literature; however, it is thought that the antioxidative influence of EVOO could be either due to its directly antihypertensive effects or mediated by the reduced plasma levels of ACE, which induces the activation of NADPH enzyme (nicotinamide adenine dinucleotide phosphate oxidase) whose blockade reduces oxidative stress (Wind et al., 2010). However, the clear understanding of EVOO antioxidant mechanisms would be a potential future goal of our research. Similarly, a direct link has been reported in oxidative stress and systemic inflammation, which could be a predisposing factor to cardiac injury and cardiomyopathies in humans (Yarla et al 2018; Sanchez-Rodriguez et al., 2019b). A future direction of the work would also be to study the comparative analysis of inflammatory and oxidative biomarkers both in DXR-induced disease animals and EVOO treated animals as no previous studies had reported these findings and pragmatic future studies are required

in this direction to clarify the antioxidant as well as the anti-inflammatory therapeutic benefits of EVOO rich diet in experimental animal models and in humans clinical trials when used as an adjunct therapy or as a dietary supplement with other antioxidant and anti-inflammatory agents.

CONCLUSIONS

The findings of this study substantiate the sustained dietary intake of EVOO as a potential cardioprotective and anti-hypertensive remedy against DXR-induced cardiotoxicities and cardiomyopathies in rats. The hemodynamic measures, blood, plasma and tissue levels of certain cardioprotective biomarkers and antioxidant enzymes levels were efficiently modulated by 10% dietary intake of EVOO in DXR-treated rats. However, further studies in clinical trials are urgently needed to evaluate EVOO's therapeutic benefits in prehypertensive and hypertensive humans. Furthermore, the elucidation and complete understanding of molecular mechanisms responsible for EVOO benefits as an anti-hypertensive, anti-inflammatory, anti-hyperlipidemic and as an antioxidant would be capable to explore novel pharmacological treatments and functional food to better cardiovascular prevention, to sustain high blood pressure and to prevent cardiovascular diseases in humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTION:

Contribution	AlMalki WH	Shahid I
Concepts or ideas	x	
Design	x	x
Definition of intellectual content	x	x
Literature search	x	x
Experimental studies	x	x
Data acquisition	x	x
Data analysis	x	x
Statistical analysis	x	x
Manuscript preparation	x	x
Manuscript editing		x
Manuscript review	x	x