Biochemical Evaluation of the Cardioprotective Effect of Ellagic acid and Resveratrol Against Methotrexate induced Cardiotoxicity in Male Rats

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Abstract

The current work is aimed at exploring the Cardioprotective effect of Ellagic acid (EA) and resveratrol (RES) against methotrexate (MTX), which causes cardiotoxicity. Rats were allocated into five groups as follows: Control, MTX group, EA protective group (30 mg EA/kg b.w.), RES protective group (25 mg RES/kg b.w.), EA plus RES group for 4 weeks. On the 22nd day of EA and RES administration, all groups were injected with MTX (20 mg kg bw i.p.). 4 weeks later, serum and cardiac tissues were collected for cardiac biomarkers, lipid profile and CRP measurement, cardiac histopathological picture, and immunoexpression of cardiac IL-6 and IL-1β. MTX-induced cardiotoxicity, as confirmed through causing histopathological abnormalities of cardiac tissue, decreases cardiac GSH content and SOD activity in addition to significant elevation in cardiac biomarkers Treponin I, CKMB, and LDH beside cardiac inflammatory cytokines IL-6, IL-1β, and CRP. Similarly, MTX significantly increased serum AST, ALT activities, creatinine, and urea levels. On the contrary, oral supplementation of EA or/and RES attenuated alterations in the above-mentioned biomarkers and cardiac histopathological changes. The present results summarized that EA and RES treatment showed Cardioprotective effects against MTX toxicity. Combining EA and RES gives the best result, as both

increase total antioxidant capacity, protecting cardiac tissue from MTX toxicity.

Keywords: Cardiotoxicity, Ellagic acid, Methotrexate, Resveratrol

Introduction

Cardiovascular diseases (CVD) constitute a major health concern, being the most common cause of disability, reduced quality of life, and mortality **(*Mc et al., 2019*)**. The most recent data from the World Health Organization (WHO) reveals that in 2019, around 17.9 million individuals died from cardiovascular diseases (CVDs), comprising 32% of total global deaths. heart attacks and strokes contributed to 85% of all fatalities **(*WHO, 2019*)**. By 2030, cardiovascular disease will account for over fifty percent of the deaths in Africa **(*Yuyun et al., 2020*)**.

Methotrexate (MTX) (2,4-diamino-N10-methyl propyl glutamic acid) is a highly regarded and widely used pharmaceutical agent for multiple malignancies, including leukaemia, lymphoma, breast cancer, hepatocarcinoma, osteosarcoma, and gastric cancers (***Koźmiński et al., 2020***), in addition to its application in rheumatology, particularly for certain autoimmune conditions. It may function by competitively inhibiting dihydrofolate reductase. That involved in the production of tetrahydrofolate (***Pountos and Giannoudis, 2017***). MTX's cytotoxic effects extend beyond tumor cells, impacting important organs such as the heart (***Perez et al., 2005***). MTX treatment stimulates free radical formation and oxidative stress, which increases cellular damage and causing inflammation, necrosis, and fibrosis in tissues (***Erdogan et al., 2015***).

A number of materials possessing antioxidant abilities has been evaluated as prospective therapeutic and protective agents. In recent years, polyphenols have been receiving considerable attention as prophylactic medicines against oxidative cardiac damage to cells or molecules **(*Derong et al., 2016*).**

Ellagic acid (EA) (2,3,7,8-tetrahydroxy-phenomenon [5,4,3-cde] chromene-5,10-dione) is a polyphenolic phytonutrient incorporated in multiple fruits such as blackberries, raspberries, strawberries, cranberries, grapes, pomegranate, and walnuts **(*Kannan et al., 2012; Abe et al., 2012*)**. It possesses a wide range of biological properties. EA demonstrated free radical scavenging activity, chemopreventive effects, anti-apoptotic properties, anti-inflammatory capabilities, anti-cataractogenic effects, gastroprotective attributes, ulcer healing potential, antifibrotic characteristics, antidiabetic properties, hypolipidemic effects, anti-atherosclerotic actions, and

estrogenic/antiestrogenic properties, along with cardioprotective effects **(*Priyadarsini et al., 2002 and Fujimura et al., 2004*).**

Resveratrol (RES) (3,5,4-trihydroxystilbene) is a natural phytoalexin present in numerous plant species, including grapes and nuts. Resveratrol's extensive therapeutic potential, including anticancer, anti-inflammatory, antidiabetic, nephroprotective, neuroprotective, antiobesity, cardioprotective, antioxidant, and anti-aging activities, makes it an issue of significant interest for future clinical research. Multiple experimental studies indicate that RES interrupts various pathogenic processes in diverse cardiovascular disorders ***(Bonnefont, 2016; Zhao et al 2017).***

The objective of this investigation was to assess the potential cardio-protective effects of EA and RES against MTX-induced cardiotoxicity in male rats

Material and Methods

Chemicals

Methotrexate (MTX, MW: 454.5) ($C_{20}H_{22}N_8O_5$) was acquired from Hikma Pharmaceuticals, Egypt (50 mg MTX/2 mL). Ellagic acid ($C_{14}H_6O_8$, MW: 302.19) hydrate, 5 g powder, 97%, could include up to 12% water, was obtained from Alfa Aesar, Germany. Resveratrol ($C_{14}H_{12}O_3$, MW: 228.25) was purchased from the USA, FG-116147.

Animals

The study used 40 male rats, each weighing 120± 10 grams, acquired from the Faculty of Veterinary Medicine at Suez Canal University. Rats were allowed to acclimatize to the experimental environment for one week. Rats were kept in separate metal cages (4 rats per cage) under controlled atmospheric conditions (20º - 24ºC), humidity (50%), and a typical light-dark cycle. Rats had unrestricted access to their usual meals and water. The experimental design of this work was approved by the Suez Canal University Faculty of Veterinary Medicine's scientific research ethics committee (no. 2020087).

Experimental design

Forty male rats were subjected to a four-week experiment and randomly assigned to five treatment groups, with eight rats in each group. Group I: normal control rats were administered 1 ml of saline orally every day. Group II: MTX group, rats were administered 1 ml of saline orally every day and received injections of Methotrexate (20 mg/kg body weight). Group III: rats were administered Ellagic acid (30 mg/kg body weight) **(*Hemmati et al., 2018*)** orally once daily. Group IV: rats were administered Resveratrol (25 mg/kg body weight **(*Manjunatha et al., 2020*)** orally once a day. Group V: Rats were administered Ellagic acid and Resveratrol orally once daily at the previously established dosage. On the 22nd day of the experiment, all rats in groups II, III, IV, and V

received a single intraperitoneal injection of Methotrexate (20 mg/kg body weight) **(*Mahmoud et al., 2021*)** to cause acute cardiac toxicity.

Sampling

After four weeks from EA and RES administration, animals in all groups fasted overnight before being decapitated. Blood samples were drawn from the medial canthus of the eye and deposited in clean, dry, screw-capped centrifuge tubes to clot at room temperature. The samples were then centrifuged at 3000 r.p.m. for 15 minutes to separate clear serum, which was subsequently used for biochemical analysis. Heart samples were dissected and separated longitudinally into two pieces; the first was homogenized in 4 ml of phosphate-buffered saline before centrifugation at 8000 x g for 10 minutes. The supernatant was transferred to a fresh tube for measurement of GSH, SOD, and MDA. The second part was preserved in 10% formalin at room temperature. Histopathological and immunohistochemical examination of TNFα, IL-1β, and IL-6 were performed on sections embedded in paraffin.

Assessment of cardiac biomarkers

Serum cardiac troponin-I levels were quantified using an immunodiagnostic kit obtained from Monobind Inc. (Lake Forest, California, USA). The serum level of creatine kinase isoenzyme MB (CK-MB) was measured using a standard diagnostic kit from Pars Azmun Co. (Iran). Serum LDH levels were assessed using a reagent kit, all of them measured according to methods of ***Larue et al., 1993; Morisok and Clayson, 1988; Chan et al., 1985 and Buhl et al., 1978.***

Quantification of Nitric Oxide (NO), C-reactive Protein (CRP), and Homocysteine

Nitric oxide (NO) was quantified via an ELISA kit that used the Sandwich-ELISA methodology **(*Moshage et al., 1995*)**. The Rat High-Sensitive CRP ELISA is a very sensitive two-site enzyme-linked immunoassay for the quantitative determination of C-reactive protein in rat biological **s**amples (Kim et al., 2010). The quantitative measurement of endogenous rat homocysteine (Hcy) was conducted using the Rat Homocysteine (Hcy) ELISA Kit **(*Fu et al., 2001*)**. Assays were performed in accordance with the kit instructions.

Assessment of lipid profile

Reagent kit for the quantitative assessment of cholesterol, triglycerides, and HDL-c concentrations in serum via an enzymatic colorimetric technique according **to *Richmond in (1973), Allain et al. (1974), and Lopez-Virella et al. (1977)*.** LDL-c was computed using the formula established by ***Friedewald et al. (1972).***

LDL-c (mg/dl) = Total cholesterol – ([triglycerides ÷ 5] – HDL-c)

Assessment of Hepatic and Renal Function Tests

Reagent kit for the assessment of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in serum, adhering to IFCC guidelines **(*Bergmeyer and Horder, 1980*)**. Reagent kit for measuring creatinine levels in serum and urine. A colorimetric approach utilizing alkaline picrate (Jaffé) **(*Bartel, 1972*).** Agent kit for the quantification of urea content in serum and urine. Enzymatic ultraviolet technique **(*Taylor et al., 1992*)**

Cardiac Glutathione, Superoxide Dismutase, and Malondialdehyde Assay

The levels of GSH in cardiac tissue homogenates were determined using a standard diagnostic kit from ZellBio GmbH, Germany (***Baker et al., 1990***). The Superoxide Dismutase (SOD) Activity Assay Kit (ZellBio GmbH, Germany) was used to evaluate SOD (***Oyanagui, 1984***). MDA was measured using the Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (ZellBio GmbH, Germany) (***Botsoglou and N.A. 1994***). Assays were carried out in line with the kit instructions.

Cardiac Histopathology by H & E stain

Heart tissue samples were maintained in a 10% formalin solution for 24 hours at 37°C. The samples were then fixed in paraffin and cut into tissue block slices. After staining with hematoxylin and eosin, the 5 mm thick sections were observed under a light microscope at 10x, 40x, and 100x magnification to determine the histological changes **(*Bancroft and Gamble, 2008*)**

Expression of TNF-α, IL-β, and IL-6 in cardiac immunohisto-chemistry

Immunohistochemical expression utilizing TNF-α Rabbit polyclonal antibody, Abclonal (A11534), at a dilution of 1:100. Conduct high-pressure antigen retrieval using 10 mM citrate buffer at pH 6.0 before initiating the IHC staining process using Anti-IL-1 β Mouse mAb (service, GB12113) diluted at 1:200 and IL-6 Rabbit pAb (A0286 ABclonal) diluted at 1:100. ***Feldmann and Maini, 2001*** *and* ***Taga and Kishimoto, 1997.***

Statistical examination

Statistical analyses were performed using SPSS Inc. Released, 2009, to assess if variables varied across groups. Before doing statistical analysis, the Shapiro-Wilk test was used to ensure that the data was normal. The variance was analysed using one-way ANOVA, and the means were compared using Duncan's multiple-range test. Probability values less than 5% ($P < 0.05$) were considered significant.

Results

Effect of EA and RES on Cardiac biomarkers, inflammatory markers levels and cardiac antioxidant status in all groups

MTX group (II) revealed significant elevation ($P< 0.05$) in cardiac biomarkers Troponin I, CKMB and LDH as seen in table (1) as well

elevation (P< 0.05) in cardiac related inflammatory markers NO, CRP and HCY as illustrated in table(2) while table (5) shown significant reduction (P< 0.05) in cardiac activity of GSH and SOD with significant increase in MDA cardiac levels. Treated groups with EA or/and RES showed significant improvement (P< 0.05) in previous mentioned parameters especially group (V) which treated with both EA and RES\

Effect of EA and RES on lipid profile in all groups

Table (3,4) shown effect of MTX group revealed significant elevation (P< 0.05) in TG, TC and LDL while significant reduction (P< 0.05) in HDL level beside damage effect on kidney and liver which appeared as significant (P< 0.05) elevation in creatinine, urea levels and ALT&AST activities. Treated groups with EA or/and RES showed significant improvement (P< 0.05) in previous mentioned parameters especially group (V)

Effect of EA and RES on cardiac histopathological picture

Histopathological examination of cardiac tissue stained with H&E (Fig.1); control negative group (A), showed normal syncytium of myofibers; branched and anastomosed myofibers with central nuclei and abundant acidophilic sarcoplasm. Cardiac myofibers were separated by loose connective tissue with small blood capillaries. Administration of methotrexate in control positive group (B) was displayed disorganized cardiac myofibers with faded areas, blood vessels were encouraged with blood with focal localization of immunocompetent cells. Whereas the treatment groups with EA (C), RES (D) and EA with RES (E); showed significant reduction in the histopathological changes; reorganization of the cardiac myofibers was noticed. Sections were devoid of congestion. (C, D & E). Furthermore, amelioration of the immunocompetent cell distribution was apparent.

Effect of EA and RES on cardiac cytokines immunoexpression

Histological evaluation of the immunostained tissues with TNF-α, IL-1β and IL-6 monoclonal antibody of the experimental groups as seen in (fig.2,3,4) exhibited had less to absent immunopositivity in the control negative group (A). Whereas it was strong immunoreactivity in MTX group (B). the treated groups (C, D & E) displayed faint expression.

Table (1): *Effect of EA and RES on Cardiac biomarkers levels:*

Groups / Biomarkers	Control (group I)	MTX (group II)	EA (group III)	RSV (group IV)	EA + RSV (group V)
Troponin-I (ng/ml)	0.21±0.02^{e}	0.88±0.01^{a}	0.53±0.03^{c}	0.61±0.04^{b}	0.37±10.01^{d}
CK-MB (U/L)	14.72 ± 0.24^{d}	23.56± 0.58^{a}	19.33± 0.69^{b}	20.66± 0.59^{b}	16.84±0.20^{c}
LDH (U/L)	236.17± 1.34^{d}	428.48± 17.84^{a}	350.20± 17.47^{b}	338.20± 26.11^{b}	286.55± 7.75^{c}

Data are expressed as M±SE, P≤0.05 implies that the means with varied superscripts are substantially different.

Table (2): *Effect of EA and RES on serum levels of NO, CRP and Homocysteine (HCY)*

Groups / Parameters	Control (group I)	MTX (group II)	EA (group III)	RSV (group IV)	EA + RSV (group V)
NO(µmol/L)	26.11±0.14^{a}	17.72±0.2^{e}	22.13±0.66^{d}	20.60±0.59^{c}	24.45±0.28^{b}
HCY(nmol/ml)	5.24± 0.1^{e}	10.90±0.19^{a}	7.90±0.66^{c}	8.71±0.56^{b}	6.29±0.1^{d}
CRP(ng/ml)	6.34±0.1^{e}	16.85±0.23^{a}	11.59±0.82^{c}	13.19±0.81^{b}	7.70±0.26^{d}

Data are expressed as M±SE, P≤0.05 implies that the means with varied superscripts are substantially different.

Table (3): *Effect of EA and RES on lipid profile determination*

Groups / Parameters	Control (group I)	MTX (group II)	EA (group III)	RSV (group IV)	EA + RSV (group V)
TC(mg/dL)	55.37±0.72^{d}	82.76±0.96^{a}	71.99±2.11^{b}	75.09±1.66^{b}	61.50±0.4^{c}
TG(mg/dL)	63.50± 0.7^{d}	101.83±0.92^{a}	82.90±4.72^{b}	86.90±3.21^{b}	72.10±0.4^{c}
HDL(mg/dL)	14.67±0.22^{d}	20.37±0.12^{a}	17.69±0.9^{bc}	18.35±0.59^{b}	16.13±0.14^{cd}
LDL(mg/dL)	28.0±0.81^{d}	42.02±0.66^{a}	37.72±0.28^{b}	39.36±0.431^{b}	30.95±0.18^{c}

Data are expressed as M±SE, P≤0.05 implies that the means with varied superscripts are substantially different.

Table (4): *Effect of EA and RES on liver and kidney function biomarkers*

Groups / Biomarkers	Control (group I)	MTX (group II)	EA (group III)	RSV (group IV)	EA + RSV (group V)
ALT(U/L)	90.64±1.26^{e}	173.75±2.11^{a}	146.11±2.05^{c}	154.96±2.16^{b}	126.63±1.14^{d}
AST(U/L)	24.96±0.36^{e}	52.27± 0.72^{a}	43.38±1.08^{c}	46.0± 0.88^{b}	33.79± 0.79^{d}
Creatinine (mg/dL)	0.40±0.01^{e}	1.00± 0.05^{a}	0.60±0.03^{c}	0.71± 0.01^{b}	0.51± 0.01^{d}
Urea(mg/dL)	15.91±0.13^{d}	28.59± 0.7^{a}	20.35±0.56^{b}	21.79± 0.5^{b}	17.87± 0.19^{c}

Data are expressed as M±SE, P≤0.05 implies that the means with varied superscripts are substantially different.

Table (5): *Levels of GSH, SOD and MDA activity in cardiac tissue*

Groups / measurement	Control (group I)	MTX (group II)	EA (group III)	RSV (group IV)	EA + RSV (group V)
GSH(µg/100mg)	51.93±0.86[a]	31.27± 0.78[d]	39.39±1.19[c]	36.87±0.83[c]	46.29± 0.72[b]
SOD(U/mg)	1.19±0.02	0.64± 0.01	0.82± 0.01	0.76± 0.01	0.94± 0.01
MDA(nmol/mg)	1.42±0.02[e]	2.83±0.02[a]	2.13± 0.02[c]	2.29± 0.02[b]	1.74± 0.03[d]

Data are expressed as M±SE, P≤0.05 implies that the means with varied superscripts are substantially different.

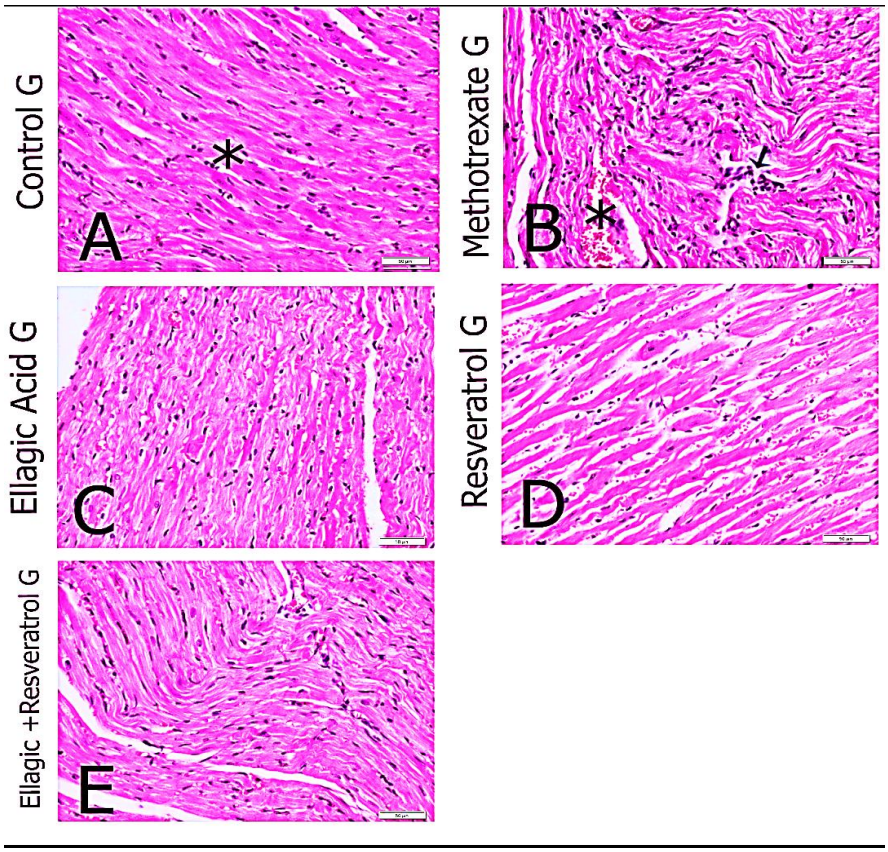

Fig. (1) Photomicrographs of the heart stained with H&E. Normal control group of (A) showing normal cardiac myofibers (*). Control positive (methotrexate group) (B); showing disorganized myofibers with encouraged blood vessels (*) and aggregation of inflammatory cells (arrow). Treated groups with Ellagic acid (C), resveratrol (E) and ellagic acid with resveratrol (E) group showed lacking congestion and inflammatory cell localization. scale bar 50 µm

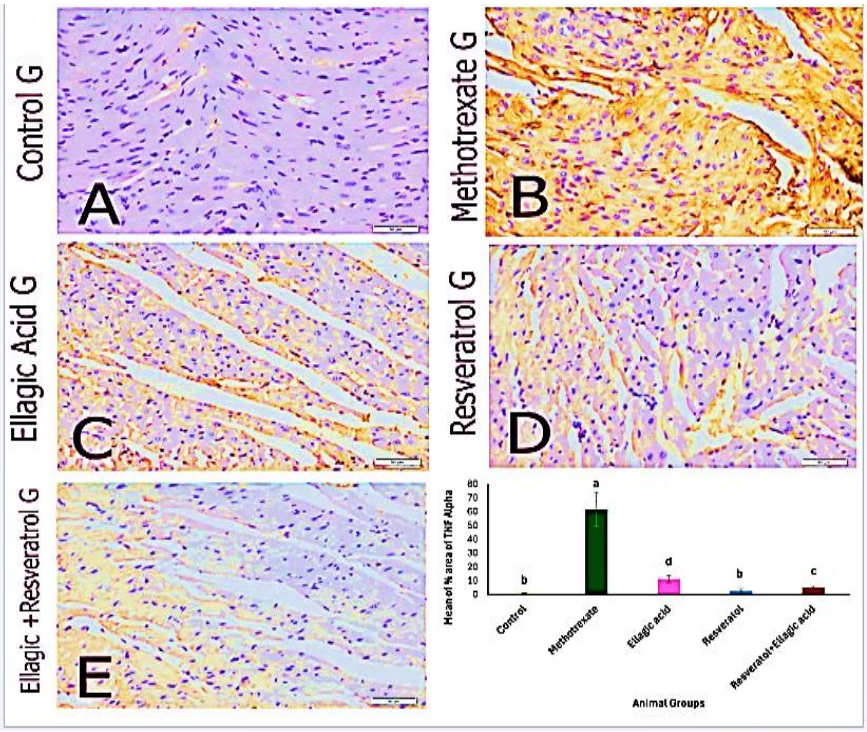

Fig. (2) Photomicrographs of the heart immunostained with TNF-α monoclonal antibody displayed the highest immunoreactivity in MTX group (B). the minimal reaction was in control (A) and ellagic acid with resveratrol (E) group. Meanwhile, weak reaction was noticed in ellagic acid (C) and resveratrol (D) groups. Mean area % of immunoreaction in cardiac myofibers immunostained with TNF-α (F). All values were reported as mean ± S.E.M. Superscript letters point to a significant difference at $P \leq 0.05$ using one-way ANOVA and Bonferroni's test for multiple comparisons. Scale bar: 50 µm.

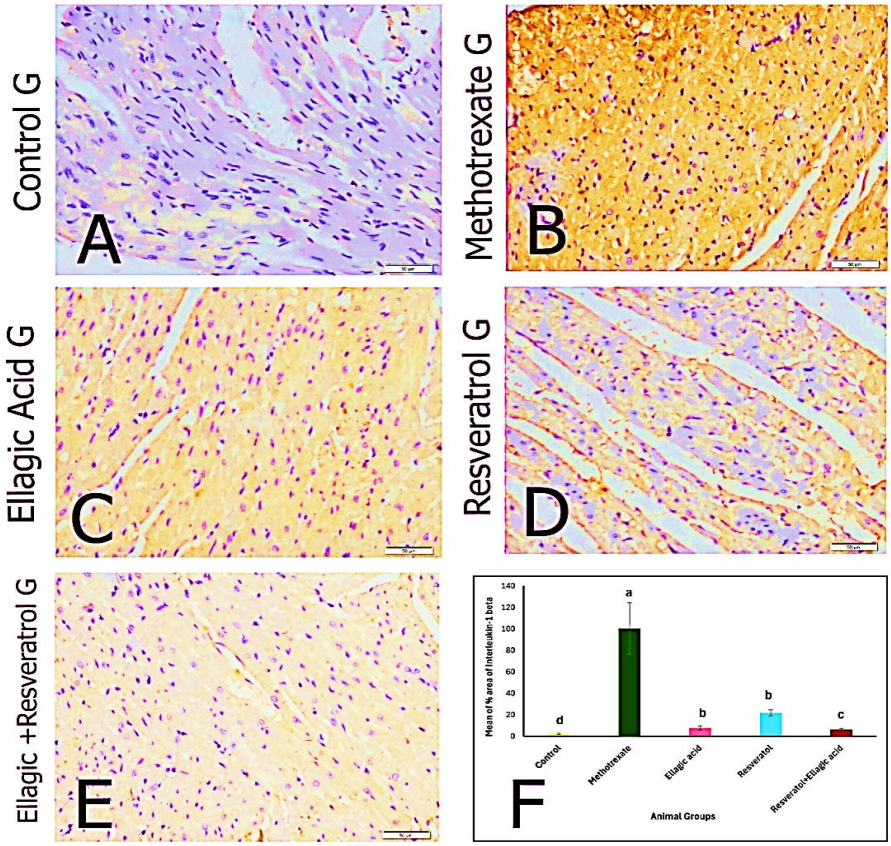

Fig. (3) Photomicrographs of the heart immunostained with IL-1β monoclonal antibody showed intense immunoreactivity in the MTX group (B). Notice the weak reaction of the treated groups (C, D & E). Mean area % of immunoreaction in cardiac myofibers immunostained with IL-1β (F). All values were reported as mean ± S.E.M. Superscript letters point to a significant difference at P≤0.05 using one-way ANOVA and Bonferroni's test for multiple comparisons. Scale bar: 50 µm.

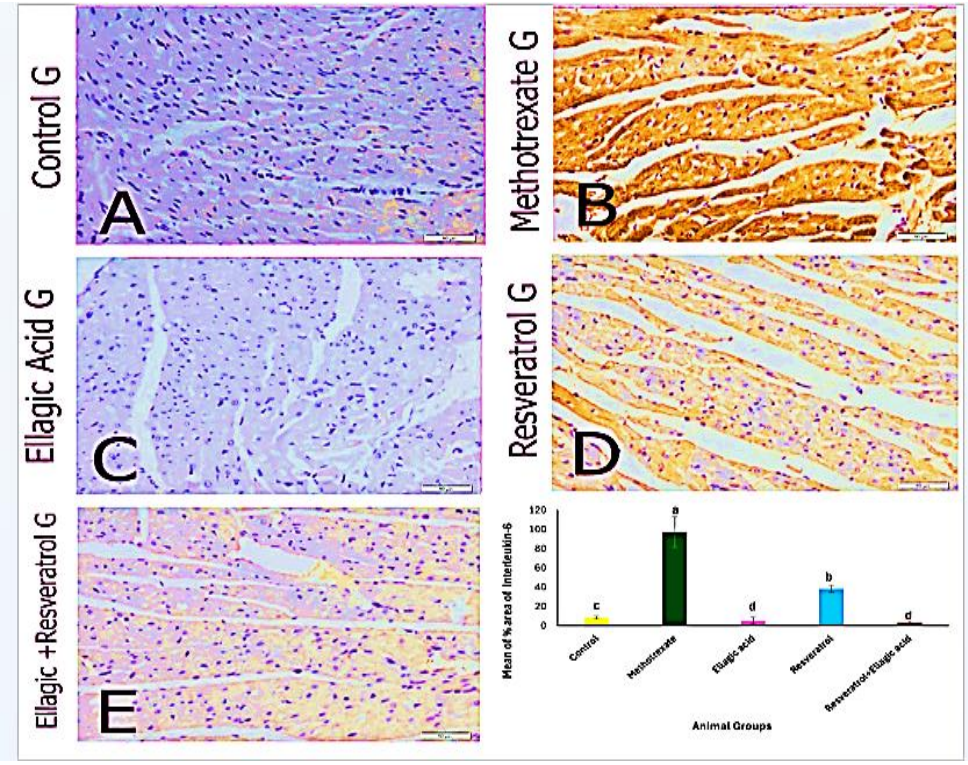

Fig. (4) Photomicrographs of the heart immunostained with IL-6 monoclonal antibody displayed a strong reaction in the MTX group (B). No detectable reaction was in the control (A) and ellagic acid (C) groups. A faint reaction was observed in the resveratrol (D) and ellagic acid with the resveratrol (E) group. Mean area % of immunoreaction in cardiac myofibers immunostained with IL-6 (F). All values were reported as mean ± S.E.M. Superscript letters point to a significant difference at P≤0.05 using one-way ANOVA and Bonferroni's test for multiple comparisons. Scale bar: 50 µm.

Discussion

The present study inspected the possible protective effects of EA and RES, antioxidant compounds sourced from fruit, against the detrimental impacts of MTX on serum cardiac markers, oxidative stress indicators, and histopathological alterations, employing a rat model of MTX-induced cardiotoxicity. In our study, MTX-induced cardiac tissue damage was seen in MTX-treated rats, as indicated by histological alterations in the cardiac tissues. These results correspond with the findings of ***Tousson et al., 2016*** who revealed that MTX therapy was associated with several histological abnormalities in rat cardiac tissues. This aligns with our histological findings, as the MTX-treated group

displayed disordered cardiac fibers invaded by inflammatory cells, alongside congested blood arteries interspersed throughout the cardiomyocytes. Consistent with previous findings, MTX-treated rats had myocardial damage, evidenced by a significant elevation in cardiac enzymes Troponin-I, CK-MB, and LDH, as well as an overproduction of oxidative stress, reflected by diminished activities of SOD and GSH. Malondialdehyde (MDA), a by-product of lipid peroxidation, provides an indicator of tissue damage and a marker of oxidative stress resulting from methotrexate (MTX), which markedly elevates MDA levels in MTX-treated rats cardiac tissue **(*Abdel-Daim et al., 2017; Al-Abkal et al., 2022*)**. the reduction of NADPH, employed by glutathione reductase, sustains the reduced form of glutathione, which offers protection against reactive oxygen species (*B**abiak et al., 1998***), and the activation of NOX produces reactive oxygen species (***Arab et al., 2018*)**. The present study reveals that MTX-induced oxidative injury to cardiac tissue, marked by a disrupted oxidant/antioxidant balance, is evidenced by a significant increase in cardiac NOX-2 and MDA levels, along with a concurrent decrease in cardiac GSH levels and SOD activity.

MTX treated animals have significant elevation in HCY, NO and CRP levels rather than other groups as results obtained by **(*Mahmoud et al.,2021*)**. Hyperhomocysteinemia is a contributing factor to MTX cardiotoxicity (***Van et al., 2002***). Reduced THF concentrations affect homocysteine metabolism. Homocysteine is converted back to methionine following its formation from S-adenosylhomocysteine. This process mostly depends on 5-CH3-THF in various organs, hence influencing folate metabolism. Hyperhomocysteinemia has been well investigated, revealing its correlation with increased cardiovascular risk. Several likely mechanisms contribute to this phenomenon: the proliferation of vascular smooth muscle cells, reduced endothelial nitric oxide production in the MTX-treated cohort, a marked reduction in NO levels relative to the control group, and alterations in oxidative stress leading to endothelial dysfunction. The elevation of homocysteine swiftly undergoes auto-oxidation, leading to the production of reactive oxygen species (***Welch and Loscalzo, 1998***).

The present study demonstrates that MTX-treated rats displayed a marked increase in CRP levels and overexpression of IL-1 and IL-6 in cardiac tissue, as homocysteine contributes to the atherogenic process by enhancing mRNA and C-reactive protein (CRP) expression in vascular smooth muscle cells, thereby initiating an inflammatory response **(*Pang et al., 2014*)**. At the cellular level, MTX increases total cholesterol, triglycerides, and LDL

levels. Moreover, MTX causes injury to hepatocytes and renal cells, leading to increased levels of ALT, AST, creatinine, and urea due to the overproduction of reactive oxygen species (ROS), which is marked by a decrease in total antioxidant capacity and an elevation in malondialdehyde (MDA) in these tissues **(*Abdel-Daim et al., 2017*).**

The current study reveals that ellagic acid exerts advantageous cardiac effects, as our results suggest its capacity to alleviate methotrexate-induced cardiac abnormalities, is mostly attributable to its antioxidant capabilities. The administration of EA markedly reduced serum levels of troponin I, CK-MB, and LDH, demonstrating its beneficial protective effect on the heart by mitigating cardiomyocyte damage and subsequently lowering the release of these cardiac markers from the myocardial **(*Nejad et al., 2015*).**

Our histology data corroborated the beneficial effects of EA on oxidative stress-induced necrosis of cardiomyocytes, resulting in less cardiomyocyte damage and a reinstatement of normal heart weight.

Our data imply that EA may provide its protective effect via decreasing lipid peroxidation in cardiac tissue. The EA-treated groups had substantial enhancements in GSH and SOD activity, accompanied by a marked reduction in MDA and an elevation in NO levels (***Hemmati et al., 2018***). EA exhibits anti-inflammatory properties by suppressing the pro-inflammatory response via the modulation of cytokines TNF-α, IL-6, and IL-1B production. As a result, it diminished its pro-inflammatory response **(*Karimi et al., 2019*)**. At the cellular level, EA treatment markedly reduced the activity of the enzymes ALT, AST, creatinine, and urea relative to the MTX group **(*Vattem and Shetty , 2005*).** Furthermore, EA markedly enhanced lipid metabolism abnormalities in rats administered MTX.

RES exhibits cardiovascular preventive properties by reducing infarct size, lowering myocardial necroenzyme levels, and reinstating endogenous myocardial antioxidant levels **(Chakraborty *et al., 2015*)**. Furthermore, it reduced the infarcted region and the production of troponin I, CK-MB, and LDH, signifying enhanced cardiac cell viability. The redox status of the hearts was enhanced, as indicated by the levels of SOD, MDA, and GSH (***Cheng et al., 2015***). This elucidates our results and the enhancement of cardiac function in RES-treated rats, as evidenced by the reduction in MDA levels and the decrease in inflammation in RSV-treated hearts (***Ray et al., 1999***). Our findings suggest that the antioxidant activity and elevated NO levels may enhance the cardioprotective effects of RES supplementation, alongside a reduction in plasma levels of inflammatory cytokines such as IL-6, TNF-α and CRP (***Timmers et al.,***

2011). At the cellular level, treatment with RES demonstrated a significant reduction in liver function (ALT, AST) in the MTX-treated group, alongside a notable enhancement in renal function (Creatinine, Urea) attributable to RES's efficacy as an anti-inflammatory agent through the scavenging of free radicals, including superoxide and toxic hydroxyl radicals (***Leonard et al., 2003***). Additionally, EA markedly ameliorated lipid metabolic disorders in MTX-treated rats (***Zhang et al., 2015***), evidenced by improvements in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels.

Conclusion

It Could be concluded that Methotrexate (MTX) which is therapeutic agent that has been widely used for treating cancer and auto-immune diseases has represent toxic effect on health and induce cardiotoxicity. EA and RES administration have cardiac protective effect through alteration cardiac toxicity of MTX on serum cardiac markers, oxidative stress parameters. They decrease inflammation, congestion and fibrosis and prevented the elevation of MDA by activation the antioxidant enzymes.

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الملخص العربي

التقييم الكيميائي الحيوي للتأثير الوقائي للقلب لحمض الإيلاجيك والريسفيراترول ضد السمية القلبية التي يسببها الميثوتريكسات في ذكور الجرذان

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يهدف العمل الحالي إلى استكشاف التأثير الوقائي للقلب لحمض الإيلاجيك (EA) والريسفيراترول (RES) ضد الميثوتريكسات (MTX)ا لذي يسبب السمية القلبية .تم توزيع الفئران في خمس مجموعات على النحو التالي ؛ عملت المجموعة الأولى كمجموعة ضابطة ، وتلقت المجموعة الثانية جرعة من الميثوتريكسات ، والمجموعة الثالثة حمض الايلاجيك 30 مجم /كجم من وزن الجسم ، وتلقت المجموعة الرابعة الريسفيراترول25 مجم /كجم من وزن الجسم ، وتلقت المجموعة الخامسة المادتين معا بنفس الجرعات سابقة لمدة 4أسابيع .في اليوم 22 من إعطاء الايلاجيك والريسفيرترول ، تم حقن جميع المجموعات بالميثوتريكسات وبعد اربعه اسابيع ، تم جمع أنسجة المصل والقلب لقياس المؤشرات الحيوية للقلب ، والصورة النسيجية المرضية للقلب والتعبير المناعي للقلب لانترلوكين 6والانترلوكين 1بيتا. السمية القلبية التي يسببها المثوتريكسات تم تأكيدها من خلال التسبب في تشوهات نسيجية مرضية لأنسجة القلب ، قلة فعالية نظام الدفاع المضاد للأكسدة بالإضافة إلى ارتفاع كبير في المؤشرات الحيوية للقلب بجانب السيتوكينات الالتهابية القلبية .زادت مادة الميثوتريكسات بشكل ملحوظ من انزيمات الكبد في المصل ، ومستويات الكرياتينين واليوريا .على العكس من ذلك ، فإن المكملات الفموية لكل من حمض الايلاجيك والريسفيراترول تخفف من التغيرات في المؤشرات الحيوية المذكورة أعلاه والتغيرات النسيجية القلبية .لخصت النتائج الحالية أن علاج بحمض الايلاجيك والريسفيراترول أظهر تأثيرات وقائية للقلب ضد سمية الميثريكسات و الجمع بين تلك المادتين يعطي أفضل نتيجة حيث يزيد كلاهما من إجمالي القدرة المضادة للأكسدة التي تحمي أنسجة القلب من سمية الميثوتريكسات