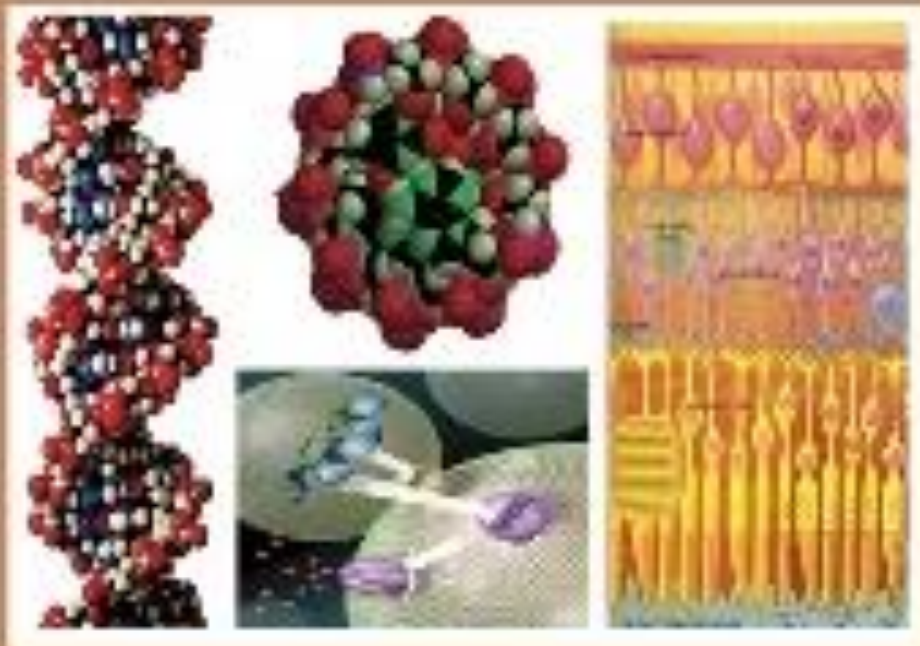




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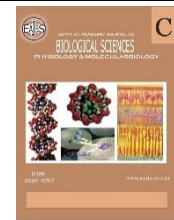
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Protective Role of Nano-Quercetin and Different Natural Extracts against Cardiotoxicity Induced by Glucocorticoids (Anti-COVID-19 Drugs): Molecular and Biochemical Studies

Rasha A. Ebiya* and Marwa T. Hassen

Department of Zoology, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo 11757, Egypt

E-mail: Rasha.ebiya@women.asu.edu.eg* ; marwa.hassen@women.asu.edu.eg

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ABSTRACT

Background New health crisis coronavirus COVID-19 infection was announced as a pandemic disease in 2020. Different recommended protocols of treatment included antiviral drugs and glucocorticoids such as azithromycin and dexamethasone. The study was designed to demonstrate the cardiotoxic consequences of anti-COVID-19 drugs and to clarify the protective role and mechanisms of different dietary natural extracts. **Methods** Forty-eight rats were divided into eight groups; Control, groups II-VI received 30 mg/kg azithromycin, 30 mg/kg dexamethasone and anti-COVID-19 protocol (30 mg/kg azithromycin, 30 mg/kg dexamethasone, 6.7 mg/kg vitamin C and 0.26 mg/kg zinc) orally for fourteen days, groups V-VIII received co-treatment of anti-COVID-19 protocol plus orally administration of 7.5 mg/kg Arabic gum, 4 ml/kg *Nigella sativa* oil, 83 μ mol/kg nano-quercetin, and 500 mg/kg *Thymus vulgaris* extract, respectively for 14 days. **Results** anti COVID-19 drugs showed significant elevation in cardiac enzymes, malondialdehyde, nuclear factor-kappa B and interleukin-1 Beta besides a decrease in superoxide dismutase level. In addition, significant upregulation in expression levels of tumor necrosis factor-alpha, caspase-3 genes and alpha-smooth muscle actin in myocardial cells. However Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extract co-treatments significantly improved these alterations and attenuated the expression of caspase-3, TNF- α genes and α -SMA protein. **Conclusion:** combined treatment with the examined dietary natural extracts especially nano quercetin established ameliorative effects against cardiotoxicity induced by anti-COVID-19 drugs through anti-inflammatory and anti-apoptotic effects.

INTRODUCTION

The year 2020 started with the health crisis of the novel coronavirus (COVID-19) pandemic disease that was reported by WHO. It is also defined as Coronavirus 2 (SARS-CoV-2) (Guo *et al.*, 2020). The earliest spread of this pandemic infection was reported in January 2020, Centre of Disease Control and Prevention informed the existence of coronavirus in China (Munster *et al.*, 2020). There were 223 countries at the risk of this infection. Up to now, there are about 767.97 million infections and 6.95 million deaths have been reported across the world (Iqbal *et al.*, 2020).

Till now, there has no drug has been approved for COVID-19 treatment. Despite different protocols for treatment were recommended worldwide. Based on previously reported drugs with antiviral effects including, Remdesiver, Azithromycin (AZ), Ritonavi, Lopinavir, Ribavirin, Flavipiravir, Hydroxychloroquine, Chloroquine, Interferons, Tocilizumab and Corticosteroids were used (Matthay *et al.*, 2020; Sanders *et al.*, 2020; Zhai *et al.*, 2020). Despite many of these drugs being associated with cardiotoxicity; cardiac arrhythmia is the consequence of cardiac injury and metabolic dysfunction (Adeyemi, 2020; Yu *et al.*, 2020; Saleh *et al.*, 2020).

Azithromycin, an azalide antibiotic, has powerful antiviral activity and anti-inflammatory against SARS-CoV-2. Also, it is reported to have a safety profile against severe COVID-19 by reducing cytokine levels. Additionally, used as a medication for pulmonary disease in AIDS patients (Kavacs and Masur, 2000; Rao, 2014; Oldenburg, 2021). Moreover, sudden death was recorded due to ventricular arrhythmia in patients after AZ treatment (Ray *et al.*, 2013). Also, cardiotoxicity in rats was recorded due to AZ use (Atli *et al.*, 2015; El-Shitany, and El-Desoky, 2016). Gyongyosi *et al.*, (2017) stated that the main cause of myocardial fibrosis resulting in AZ use is myofibroblasts proliferation which causes cardiac dysfunction, arrhythmias and increased myocardial wall stiffness.

Using high-flow oxygen, mechanical ventilation and circulatory support was suggested for critical COVID-19 patients with hypoxemia (Haase *et al.*, 2021). Also, the World Health Organization recommended dexamethasone (DX) for controlling severe COVID-19 to reduce the mortality rate by using systemic glucocorticoids (Lamontagne, 2020; Sterne, 2020). Other studies demonstrated a decrease in mortality rate by using 6 mg/d DX for 10 days (Horby *et al.*, 2021; Munch *et al.*, 2021).

Dietary natural antioxidant extracts found in fruits, vegetables, and seeds

enhanced protective effects, in addition to their use in alternative medicine (Hasona *et al.*, 2017). Some of these natural extracts possess antioxidant and anti-inflammatory effects and show a safety profile against cardiovascular complications. *Nigella sativa* (NS) was reported to induce anti-inflammatory properties (Houghton *et al.*, 1995; Burits and Bucar, 2000; Kanter *et al.*, 2006). It was found to reduce cardiotoxicity and attenuate myocardial infarction by decreasing the oxidative stress state, pro-inflammatory mediators, and cardiac tissue injury (Murugesan *et al.*, 2012; Ahmed and Hassanein, 2013). Arabic gum (AG), a dried extract of *Acacia senegal* plant, is widely used in some traditional therapies and as a food additive. It was shown to improve cardiovascular risk indicators and prevent lipid peroxidation in kidney tissue after gentamicin use (Al-Majed *et al.*, 2002; Gado and Aldahmash, 2013). *Thymus vulgaris* extract was found to have an impact on controlling the development of disorders such as cancer, chronic inflammation, hepatotoxicity, and oxidative stress initiated by dexamethasone (Tabari *et al.*, 2017). Quercetin(Q) is widely reported for its multiple health benefits due to its anti-inflammation, antioxidant properties and increasing endothelium-dependent vasodilation. It is found in apples, peppers, and onions (Murakami *et al.*, 2008; Jatap *et al.*, 2009; Shen *et al.*, 2012). Recently in 2020, (Ahmed, 2020) stated that quercetin improved the compromised vascular reactivity stimulated by metabolic syndrome (MetS), nano quercetin elevated histopathological indices of cardiac damage stimulated by MetS (Ahmed *et al.*, 2021)

Therefore, the current study tested the hypothesis that the use of different dietary natural extracts would prompt better control of cardiotoxicity induced by anti-COVID-19 drugs. Further, the research was extended to determine the mechanisms regarding anti-apoptotic, anti-fibrotic, and anti-inflammatory reactions of co-treatment of Arabic gum, *Nigella sativa* oil, nano-

quercetin, and *Thymus vulgaris* extract.

MATERIALS AND METHODS

Experimental Animals:

All experimental protocols were approved by the Faculty of Women for Arts, Science and Education, Ain Shams University (Cairo, Egypt) and the Ethical Committee for Animal Use. Our research used forty-eight adult male albino rats (150–200 g). Animals were housed in cages (six rats/cage), under natural light, temperature (20–22°C) and kept in a properly ventilated room. They were kept for a week for adaptation before the beginning of the experiment.

Materials:

Nigella sativa oil (Pharco-Company, Cairo, Egypt), Azithromycin (Novartis Company, Cairo, Egypt) and dexamethasone (Pfizer company, Cairo, Egypt) were purchased from pharmacy. *Thymus vulgaris* was purchased from Sekem Company (Cairo, Egypt). The plant collection and use were in accordance with all the relevant guidelines. Arabic gum was purchased from Haraz company for herbs and medicinal plants (Cairo, Egypt). Nano-quercetin was purchased from One Planet Nutrition company (Florida, USA).

Preparation of Extracts:

The aqueous extract of *Thymus vulgaris* was performed as follows: 60 grams of dried leaves were infused in 120 ml distilled water for 24 hours then filtered and used fresh (Shati and Elsaid, 2009). The aqueous extracts of Arabic gum were prepared by dissolving a 10-gram fine powder of Arabic gum in 100 ml distilled water, left for 3 days in the refrigerator then filtered and used fresh (Ayaz, 2017). These extracts were preserved at -20 for 3 days only.

Experimental Design:

Forty-eight rats were distributed randomly to 8 groups (6/group). Group I, Control (C): rats received distilled water. Group II (DX): rats were orally administered dexamethasone (30 mg/kg/day) (Sterne *et al.*, 2020). Group III (AZ): rats received azithromycin at a dosage of 30 mg/kg/day orally (Oldenburg, 2021). Group IV (P): anti-

covid-19 treatment protocol (Covid-19 treatment protocol used in Egypt) group: rats received azithromycin (30 mg/kg), dexamethasone (30 mg/kg), vitamin C (6.7 mg/kg) and zinc (0.26 mg/kg). Groups V (P+AG), VI (P+NS), VII (P+Q) and VIII (P+TV): rats received treatment protocol in addition to Arabic gum (7.5 mg/kg), *Nigella sativa* oil (4 ml/kg) nano-quercetin (83 µmol/kg) (Ahmed *et al.*, 2021) and *Thymus vulgaris* extract (500 mg/kg) co-treatment. All treatments were orally administered for fourteen days.

Collection of Samples:

Twenty-four hours after to last administration, blood samples were assembled using microcapillaries from the retro-orbital plexus, serum was stored at -80 °C for biochemical analysis. Then, rats were dissected (via cervical dislocation), and heart tissues were kept at -80 °C for molecular and biochemical analysis. All methods were carried out in accordance with relevant guidelines and regulations.

Assessment of Gene Expression:

Real-time PCR (qRT-PCR) analysis was done on TNF- α and Caspase-3 genes to assess changes in their expression levels using GAPDH as an internal control. Total RNA was extracted from heart tissues following the supported manufacture of Direct-zol RNA Miniprep Plus kits (ZYMO RESEARCH CORP. USA). The purity and concentration of RNA were assessed using a spectrophotometer (Beckman dual, USA) at wavelength 260 nm. The integrity of extracted RNA was explored using (1%) agarose gel. Both cDNA synthesis and qRT-PCR were done according to the manufacture of SuperScript IV One-Step RT-PCR kit (Thermo Fisher Scientific, Waltham, USA), then were applied in real-time PCR (Applied Biosystem, Foster, USA). Final volume of 50 µl: 10 µl RNA (0.5 µg), 0.5 µl RT Mix, 25 µl of 2X RT-PCR Master Mix, 2.5 µl of primers, and 9.5 µl of nuclease-free water. Sequences of primers were TNF- α (F) 5'-TAGCTCCCAGAAAAGCAAGC -3' and (R) 5'-TTTCTGGAGGGAGATGTGG -3'; Caspase-3 (F) 5'-AGGACTCTAGA CGGC

ATCCA -3' and (R) 5'- CAGTGAGACTT GGTGCAGTGA -3'; GAPDH (F)TGGATT TGGACGCATTGGTC 3 and (R) 5- TTTGC ACTGGTACGTGTTGAT - 3. The PCR reaction steps were: first 55 °C for 10 min (1-cycle, reverse Transcription reaction), 95 °C for 2 min then 45 cycles including 95 °C for 10 s, 56 °C for 10 s, 72 °C for 30 s and 72 °C for 5 min for 1 cycle. Amplification was specified using melting curve analysis. The obtained data were explored using the method of $2^{-\Delta\Delta CT}$ and presented as the number of fold differences relative to the negative control (Ebiya *et al.*, 2016; Montaser *et al.*, 2017). The relative expression of each gene was normalized to the housekeeping gene.

Western Blotting Analysis:

Protein quantification was done for the heart tissues of the studied groups using a protein extraction kit (Bio-Rad Inc., US) according to the recommended manufacturer. Protein concentration was detected following manufacture instructions of Bio Basic Inc kit (Markham Ontario, Canada). Then protein (20 µg) were loaded in 2x Laemmli sample buffer (equal volume containing 2.1% SDS, 26.3% glycerol, 0.001% bromophenol blue and 65.8 mM Tris HCl, pH 6.8 and 10% 2-mercaptoethanol), which were boiled (5 min at 95°C); proteins were separated on SDS-polyacrylamide gels (20 µg protein/lane) using acrylamide Kit (Bio-Rad Laboratories Inc. UA). The separated protein is transmitted from the gel to the membrane (PVDF) in 1x transfer buffer (25 mM Tris, 190 mM glycine and 20% methanol). The blot was run using Bio-Rad Trans-Blot Turbo for 7 min at 25 V. Blocking of membrane was done in tris-buffered saline (TBST, pH 7.5 with 0.1 % Tween 20) and bovine serum albumin (BSA, 3 %) at 22°C for 1 hr. The mouse anti- α SMA (Sigma-Aldrich, St. Louis, UA) and rabbit anti-Actin (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies in TBST were incubated with sample α SMA overnight at 4°C according to manufactured instructions. Then the blots were rinsed 5 times with TTBS for 5-min washes and incubated with horseradish peroxidase-conjugated (secondary goat anti-rabbit IgG, Amersham,

NA934) at 22°C for 1 hr. The blot was washed 4 times for 3 min using TBST. The expressed proteins were detected by applying the chemiluminescent kit (Clarity™ Western ECL substrate Bio-Rad) following the supported manufacturer. The Image analysis software (ChemiDoc MP image J program) was used to value the α SMA intensity and normalized it to beta-actin. The evaluation was recurring in triplicate from three individuals for each group.

Biochemical Study:

Measurement of Cardiac Enzymes:

Serum lactate dehydrogenase (LDH) and creatine kinase MB isoenzyme (CK-MB) concentration were measured using ELISA assay kits, Enzyme-linked Immunosorbent Assay Kit (organism species: *Rattus norvegicus* (Rat) for creatine kinase MB isoenzyme) and for Lactate Dehydrogenase (LDH) (Cloud-clone crop, USA). LDH and CK-MB concentrations were analyzed following supplied manufacturers, expressed as nanograms per milliliter (ng/ml) (Pesce, 1987; Azhar and El-Bassossy, 2014).

Oxidative Stress Markers:

Enzyme Activity of superoxide dismutase (SOD) and malondialdehyde (MDA) were measured in myocardial muscle using Bio-diagnostic kits (Egypt) according to supplied manufacturers (Nishikimi, *et al.*, 1972; Uchiyama and Mihara, 1978).

Assessment of Interleukin-1 Beta (IL-1 β):

Concentration of interleukin-1 Beta (IL-1 β) was conducted in myocardial muscle using ELISA assay kits For Interleukin 1 Beta (IL-1 β) (Cloud-clone crop, USA). The IL-1 β concentrations were calculated and expressed in picogram per milligram (pg/mg) tissue according to the supplied manufacturer.

Assessment of Nuclear Factor-Kappa B (NF- κ B):

NF- κ B concentration was measured in myocardial muscle following the manufacture of ELISA kits for nuclear factor-kappa B (My Biosource, USA). NF- κ B content is expressed in picogram per milligram (pg/mg) tissue.

Statistical Analysis:

Obtained data was analyzed using

SPSS (version 25, USA) and was applied for summarizing and comparing data with one-way ANOVA. Data were presented in the form of mean \pm SE and considered significant at $p \leq 0.05$.

Ethical Compliance:

All procedures performed in this study involving animal experiments were in accordance with the ethical standards of the institutional and national research committee and authorized by the Ethical Committee (Faculty of Women for Arts, Science and Education, Ain Shams University, Code number: Sci 1332307007). All methods included in this study were in accordance with ARRIVE guidelines.

RESULTS AND DISCUSSION

Gene Expression Analysis:

TNF- α and Caspase-3 genes were investigated as molecular biomarkers. The changes in their mRNA expression levels were detected by applying real-time PCR. Anti-apoptotic effects of *Arabic gum*, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extract co-treatment with anti-Covid-19 drugs recommended in Egyptian protocol for COVID-19 treatment were detected in heart tissues. Expression levels of caspase-3 gene showed a significant ($P \leq 0.05$) increase after administration of dexamethasone, azithromycin and anti-covid-19 treatment protocol compared to the control group (9.05 ± 0.65 , 8.84 ± 0.67 , 6.71 ± 1.60 and

1.01 ± 0.20 , respectively). Oral Co-treatment with Arabic gum, *Nigella sativa* oil, nano-quercetin and *Thymus vulgaris* extract in separate tested doses showed a protective effect through significant downregulation ($P \leq 0.05$) of caspase -3 expression level as compared to groups that received tested drugs only (4.58 ± 0.5 , 4.39 ± 1.03 , 3.43 ± 0.03 , 4.62 ± 0.12 and 1.01 ± 0.20 respectively). The best protective effect was demonstrated after nano-quercetin treatment. The inflammatory molecular biomarker TNF- α showed a significant ($P \leq 0.05$) increase in mRNA expression level of TNF- α gene in response to oral administration of dexamethasone, azithromycin and anti-covid-19 treatment protocol compared to the control group (8.56 ± 1.46 , 7.63 ± 0.82 , 6.09 ± 1.20 and 0.99 ± 0.14 , respectively). Significant improvement was detected in expression levels of mRNA of TNF- α gene resulting in co-treatment with *Arabic gum*, *Nigella sativa* oil, nano-quercetin and *Thymus vulgaris* extraction in separate doses after heart toxicity occurred in groups II-IV as compared to the control group (5.60 ± 0.15 , 5.66 ± 1.13 , 3.79 ± 0.27 , 5.29 ± 0.86 and 0.99 ± 0.14 respectively). As shown in Figure (1) highly protective effect is recorded in group VIII that received nano-quercetin co-treatment induced highly significant ($P \leq 0.01$) downregulation of TNF- α expression level as compared to groups II-IV that received tested drugs only.

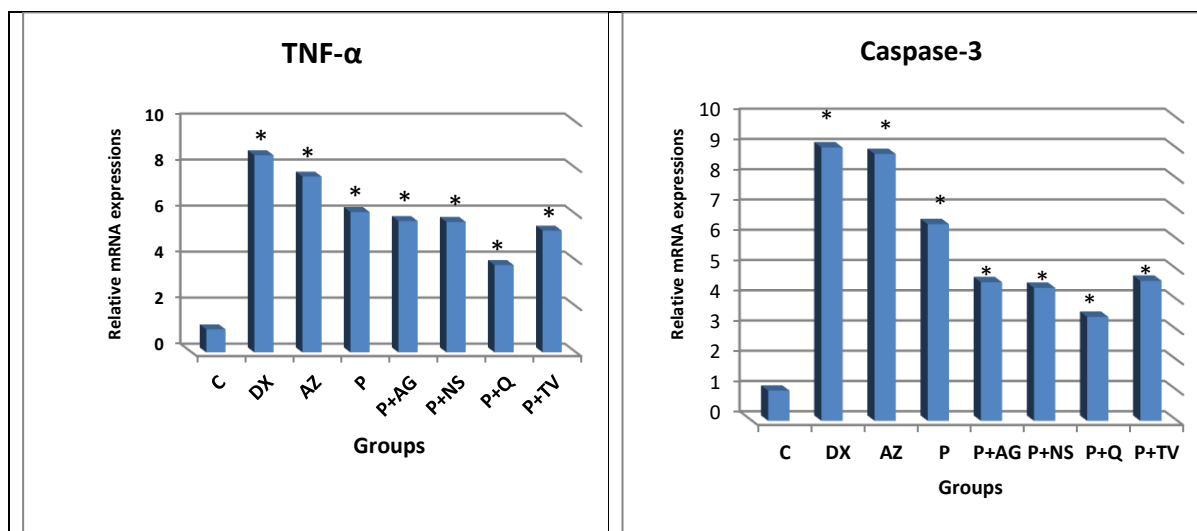


Fig 1. Effect of Arabic gum, *Nigella sativa* oil, nano- quercetin and *Thymus vulgaris* extract on TNF- α and caspase-3 mRNA expression in cardiac tissues of rat treated with DX, AZ, and anti- COVID-19 using real time PCR. Data are stated as mean \pm SE . Values with (*) are significantly different at ($p \leq 0.05$) compared to control.

Western Blotting Analysis:

To assess whether treatment with azithromycin, dexamethasone and anti-covid-19 treatment protocol correlates with extreme proliferation of myofibroblasts, as an indicator of fibrosis and consequently cardiovascular complication, cardiac cells were used to detect the expression level of α -SMA protein using western blot analysis. Figure (2) shows a significant increase in the expression level of Alpha smooth muscle

actin (α -SMA) correlated with administration of the above drugs. At the same time, a significant decrease in α -SMA expression resulted in co-treatment with all tested natural extracts as compared to the control group, referring to that the most beneficial ameliorative effect appeared in a decline of α -SMA expression was correlated to nano-quercetin co-treatment as a strong indicator of powerful protective role.

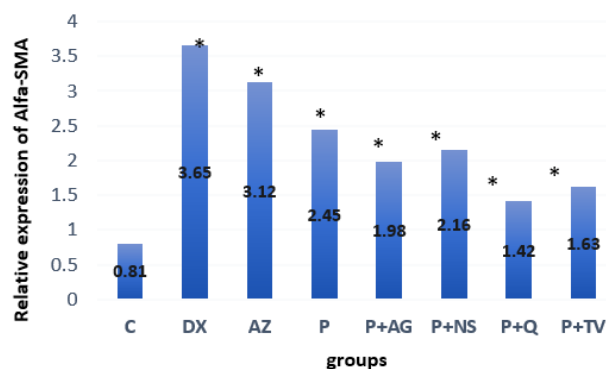


Fig. 2: Effect of Arabic gum, *Nigella sativa* oil, nano- quercetin and *Thymus vulgaris* extract on α -SMA protein expression in cardiac tissues of rat treated with DX, AZ, and anti- COVID-19 using western blot analysis. Data are stated as mean \pm SE of protein intensity normalized to beta-actine. Values with (*) are significantly different at ($p \leq 0.05$) compared to control.

Biochemical Results:

Assessment of Cardiac Enzymes:

Serum CK-MB and LDH were assessed in tested groups (Fig. 3). Significant ($p \leq 0.05$) elevation in both CK-MB and LDH were recorded after administration of DX, AZ, and anti-COVID-19 drugs for 14 days

compared to control group. Co-treatment with Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extraction showed a significant ($p \leq 0.05$) reduction of the levels of these enzymes compared to groups that received drugs only.

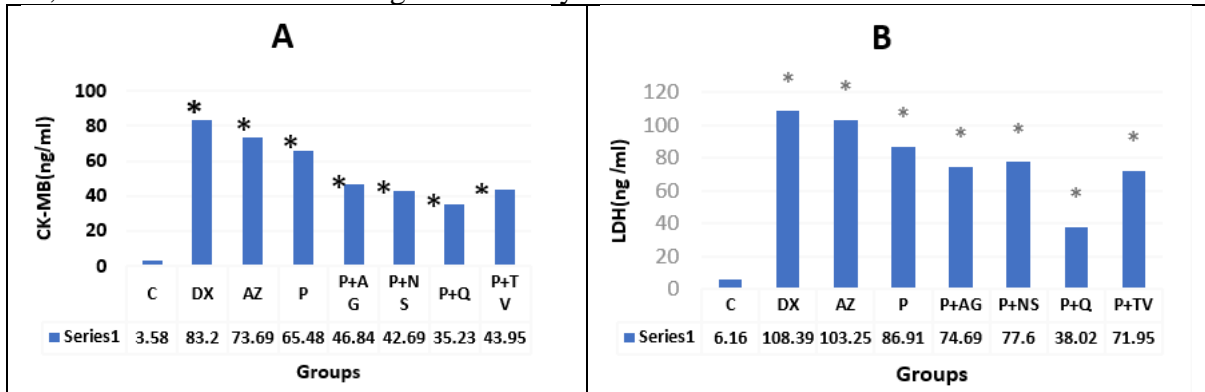


Fig. 3: Effect of Arabic gum, *Nigella sativa* oil, nano-quercetin and *Thymus vulgaris* extract on cardiac enzymes activity in serum of rat treated with DX, AZ, and anti-COVID-19. (A) CK-MB and (B) LDH. Data are stated as mean \pm SE. Values with (*) are significantly different compared control group at ($p \leq 0.05$).

Assessment of Oxidative Stress:

MDA and SOD levels were measured in the heart tissues of tested groups to evaluate the oxidative stress state (Fig. 4). Administration of DX, AZ, and anti-COVID-19 drugs induced a significant increase in MDA level compared to control. However, co-treated groups with Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extraction proved a

significant reduction in MDA level, indicating the best ameliorative effect in the group co-treated with nano-quercetin. Conversely, there was a significant ($p \leq 0.05$) decrease in the SOD level of DX, AZ, anti-COVID-19 drug groups compared to control group. Despite this, great improvement in SOD level was observed caused by co-treatment with examined natural extracts, especially nano-quercetin.

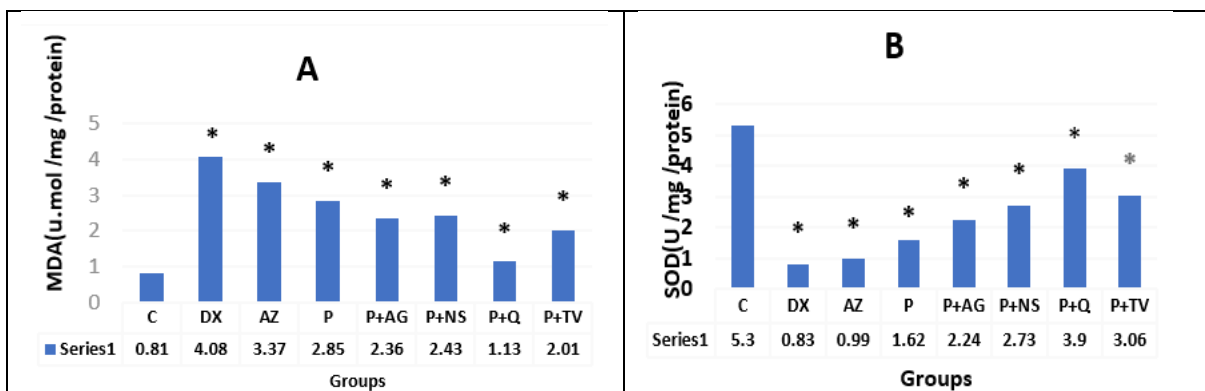


Fig. 4: Effect of Arabic gum, *Nigella sativa* oil, nano-quercetin and *Thymus vulgaris* extract on the antioxidant activity and oxidative stress state in cardiac tissues of rat treated with DX, AZ, and anti-COVID-19. (A) MDA and (B) SOD. Data are presented as mean \pm SE. Values with (*) are significantly different at ($p \leq 0.05$) compared to control.

Nuclear Factor-Kappa B (NF- κ B):

DX, AZ, and anti-COVID-19 drug administration induced a significant increase in NF- κ B level (six folds) compared to control group. However, Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extraction co-treatment significantly reduced NF- κ B level to three folds in both Arabic gum and *Nigella sativa* oil groups and two folds only in group co-treated with nano-quercetin (Fig. 5A).

Assessment of IL-1 β :

Treatments of rats with DX, AZ,

and anti-COVID-19 drugs significantly ($p \leq 0.05$) elevated levels of interleukin-1 Beta (IL-1 β) concentration as compared to the control values in heart tissue (172.12 ± 1.01 , 118.49 ± 1.14 , 93.09 ± 0.85 , and 34.81 ± 0.08 respectively). Co-treatments of these rats with Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extraction significantly decreased IL-1 β content compared to the control values (68.34 ± 0.98 , 74.92 ± 1.00 , 47.95 ± 0.98 , 70.36 ± 0.68 , and 34.81 ± 0.08 respectively) (Fig. 5B).

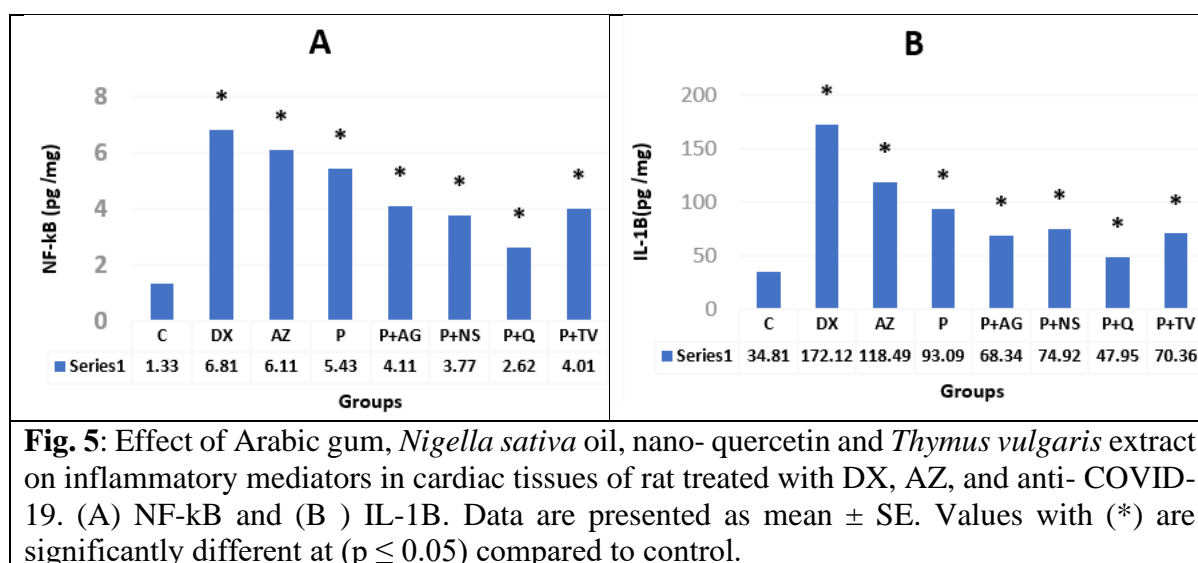


Fig. 5: Effect of Arabic gum, *Nigella sativa* oil, nano- quercetin and *Thymus vulgaris* extract on inflammatory mediators in cardiac tissues of rat treated with DX, AZ, and anti- COVID-19. (A) NF-kB and (B) IL-1B. Data are presented as mean \pm SE. Values with (*) are significantly different at ($p \leq 0.05$) compared to control.

It was the first time to investigate the effect of anti-COVID-19 drugs and their mechanism with the hypothesized protective effect of different dietary natural antioxidant extracts on heart damage and cardiotoxicity. The impact of azithromycin, dexamethasone and anti-covid-19 recommended protocol for COVID-19 treatment for 14 days on

cardiotoxicity were assessed by evaluating cardiac enzymes, expression levels of mRNA for caspase-3 and TNF- α genes, oxidative stress state and α -SMA expressed protein in cardiac cells. Also, the protective role of Arabic gum, *Nigella sativa* oil, nano-quercetin, and *Thymus vulgaris* extract co-treatment was evaluated (Fig. 6).

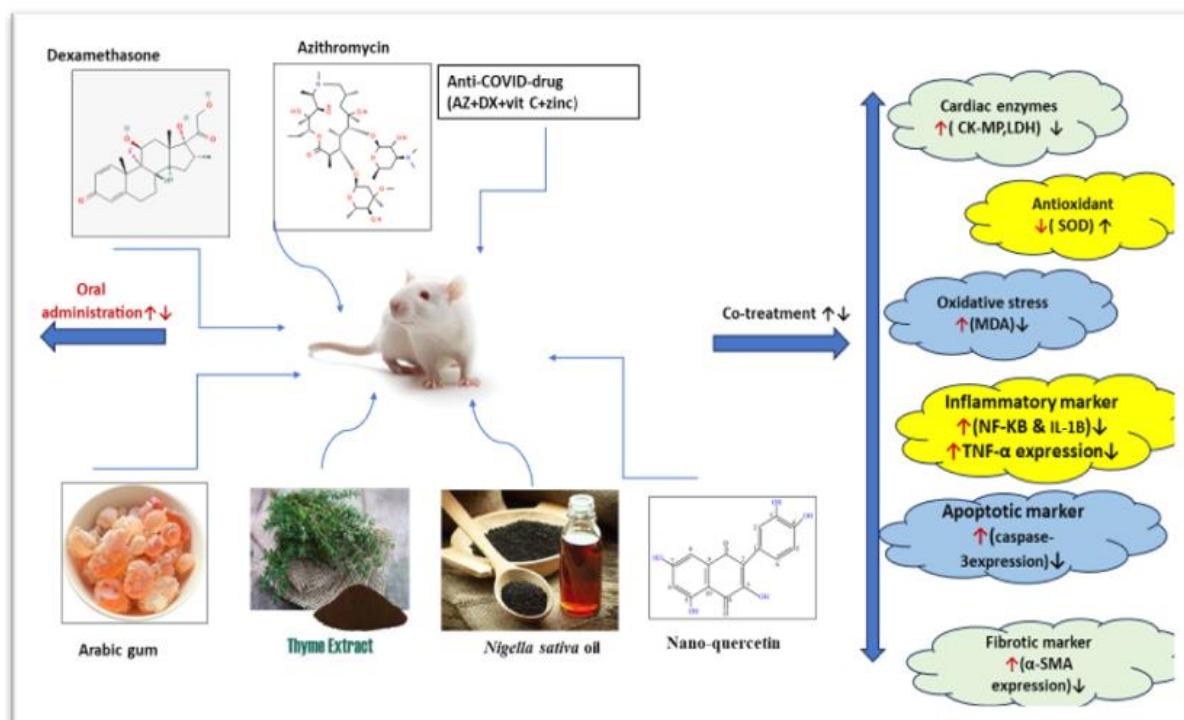


Fig. 6. Schematic diagram illustrating study design and obtained results. ↑elevation of parameter ↓ decrease of parameter. Red arrows represented groups received examined drugs only, black arrows represented groups co treated with natural extracts.

Our findings detected that administration of AZ and DX in separate doses or grouped in the recommended protocol is associated with elevation of caspase-3 and TNF- α mRNA expression levels and increased activity of serum LDH and CK-MB enzymes. These results are matched with data that was previously reported by (Atli *et al.*, 2015; El-Shitany and El-Desoky, 2016) who detected cardiotoxicity and renal failure after AZ and DX use.

In this study, molecular alterations in cardiac tissues were manifested after AZ, DX and anti-COVID-19 protocol through changes in the expression level of both caspase-3 that play an important role in apoptosis and TNF- α , inflammatory mediators enhanced in structural damage (Agosto, 2011; Cai, 2013; Abdel-Wahab, and Metwally, 2015). Significant elevation was detected in the expression level of caspase-3 after two weeks of DX, AZ and anti-COVID-19 protocol administration. Caspase-3 is an important apoptotic marker, so an elevation in

caspase-3 expression level indicates that the use of these drugs could stimulate the propagation of apoptosis in cardiac cells. These findings agreed with (Atli *et al.*, 2015). They documented that azithromycin administration at similar doses caused elevation in caspase-3 expression in rats. However, these effects were modulated by co-treatment with Arabic gum, *Nigella sativa* oil, quercetin and *Thymus vulgaris* extract, accompanied by attenuation in the expression level of caspase-3 in cardiac cells after two weeks of co-treatment of these extracts. The most protective effects were recorded after nano-quercetin use. Similarly, other studies reported an antiapoptotic effect and decline of Caspase-3 expression level after thymoquinone (the active ingredient of *Nigella sativa*) and *Nigella sativa* oil treatment against cardiotoxicity (Adali *et al.*, 2016; Abd El-kader, 2020). Another recent study showed that nano-quercetin improved compromised vascular reactivity and protect from cardiovascular damage associated with metabolic syndrome (Osama, 2021)

Furthermore, our findings demonstrated that administration of DX, AZ and anti-COVID-19 treatment protocol increased inflammatory response as it significantly upregulated the expression level of TNF- α as compared to the control group. These findings were matched to other studies that point out an elevation of TNF- α expression resulted in AZ administration (El-Shitany and El-Desoky, 2016). However, the expression level of TNF- α gene declined after co-treatment with AG, NS, Q and TV against groups that received AZ, DX, and anti-COVID-19 protocol treatment only. These findings matched other studies that revealed the protective role of different natural extracts including Arabic gum, *Nigella sativa* oil, *Thymus vulgaris*, Carvedilol and Vitamin C against cardiotoxicity. These effects may be due to their potential antioxidant effect (Safaeian and Zabolian, 2014; Kashef and Elswaidy, 2022).

An important highlight is that nano-quercetin managed the best protective role in improving the expression levels of both caspase-3 and TNF- α . Quercetin is extensively reported for its anti-inflammatory and antioxidant effects and improved endothelium-dependent vasodilation (Jagtap *et al.*, 2009). Also, there is a noticeable decline in the expression of studied genes in the group that received anti-COVID-19 protocol course against groups that received AZ or DX only. This decline may be due to Vitamin C, incorporated in the protocol course, which possesses antioxidant properties.

The deposition of extracellular matrix proteins and excessive proliferation of cardiac fibroblasts occurring after cardiomyocytes damage is responsible for heart dysfunction and fibrosis (Gaspard *et al.*, 2014; Wang *et al.*, 2014). Cardiac fibroblasts play an important part in the synthesis of cardioprotective factors and the production of extracellular matrix proteins (Gao *et al.*, 2014). Fibroblasts were reported to be converted into myofibroblasts in pathologic conditions, consequently causing myocardial fibrosis through extreme creation of collagen

and fibronectin, the presence of myofibroblasts in the myocardium is assessed by alpha-smooth muscle actin (α -SMA) recognition (Gava *et al.*, 2016). Similarly, our findings demonstrated that AZ, DX, and anti-COVID-19 treatment protocol showed a significant increase in the expression of α -SMA in cardiac cells against the control group, suggesting intense proliferation of myofibroblasts. In contrast to these groups, animals that received co-treatment with AG, NS, TV, and Q showed large declines in expressed α -SMA of cardiac cells against that of the control group as an indicator of ameliorative effect in suppressing myofibroblasts proliferation and subsequent fibrosis process and cardiac damage.

Our results prove that DX, AZ, and anti-covid-19 drugs resulting in cardiac complications are associated with the initiation of oxidative stress, as evaluated by an elevation in MDA and decrease in SOD, in addition to an increase in activity of serum CK-MB and LDH enzymes. Also, these drugs increase inflammatory response which is accompanied by elevation of NF- κ B content and IL-1 β concentration in cardiac muscle. The increase of NF- κ B content may be attributed to upregulation in TNF- α expression level as TNF- α was reported to attract leukocytes to the inflammatory sites and is responsible for the regulation of NF- κ B production which indicates an inflammatory response to these drugs (Cai, 2013). These results are like that reported by (El-Shitany and El-Desoky, 2016). These effects may be a defense mechanism against toxicity resulting in the administration of DX, AZ. anti-COVID-19 drugs (Salvemini, *et al.*, 1999). Also, decreased activity of SOD in treated groups could be due to enhancement of ROS generation (Atli *et al.*, 2015). This suggests that anti-COVID-19 drugs generated cardiotoxicity could be due to instabilities in the regulation of antioxidant/oxidant state, apoptotic, and inflammatory reactions.

Oxidative stress returns balance to restore the injury or to detoxify the reactive oxygen species (ROS). Free radicals and peroxides formation lead to disturbance of the

cell consequently breaking the DNA strand and numerous pathogenesis of degenerative diseases like cardiovascular disorders (Sinha *et al.*, 2013). Superoxide dismutase (SOD) enzymes performed protective actions against ROS, so a reduction in the capacity of SOD activated ROS formation, which altered cell components (Wang *et al.*, 2013). Natural antioxidants are essential to offer protection to restore the oxidative damage (Iuchi *et al.*, 2003)

Co-treatment of dietary natural extracts, Arabic gum, *Nigella sativa* oil, nano-quercetin and *Thymus vulgaris* extract ameliorated the altered oxidative stress parameters, decreased the elevated activity of cardiac enzymes CK-MB and LDH and attenuated inflammatory marker NF- κ B level, suggesting cardioprotective mechanisms of these dietary natural extracts through their antioxidant and free-radical scavenging activity. Also, these extracts diminished the increased IL-1 β level and indicating anti-inflammatory cardioprotective mechanisms for anti-COVID-19-induced cardiotoxicity. Regarding these observations, the existing findings agreed with (Gado and Aldahmash, 2013; El-Shitany and El-Desoky, 2016; Ahmed *et al.*, 2021). This could be affected by the powerful antioxidant properties of AG. The antioxidant effect of AG was recorded earlier, it had a potential effect in scavenging superoxide radicals induced enzymatically and nonenzymatically and inhibiting lipid peroxidation detected in damaged kidney tissues. Therefore, the protective effect of AG on cardiac tissue by scavenging free radicals induced by tested drugs (Abd-Allah *et al.*, 2002). Additionally, *Thymus vulgaris* extract was reported to delay the development of oxidative stress initiated by dexamethasone. Its metabolites, phenolic monoterpenoids, thymol, and carvacrol possess free radical scavenging properties (Tabari *et al.*, 2017). Moreover, flavonoids such as luteolin, derivatives of apigenin and phenolic acids including cinnamic, and carnosic are responsible for a good impact on the antioxidant effect of thyme (Caprioli *et al.*, 2018).

Conclusion:

Our findings indicate that anti-COVID-19 (glucocorticoids) drugs induced cardiotoxicity appeared by elevation of serum cardiac enzyme activity in addition to increase of oxidative stress state and decrease in antioxidant activity biomarkers. Also, upregulation of Caspase-3 gene expression; apoptotic biomarker, TNF- α gene expression; inflammatory biomarkers and expression of α -SMA; fibrotic biomarker in myocardial cells resulted in anti-COVID-19 drugs administration. Co-administration of AG, NS, Q and TV extracts provided protection against cardiotoxicity, by hindering the free radical-mediated process by providing anti-apoptotic, anti-inflammatory and antifibrosis effects. This co-administration might have an extensive impact on the development of clinical approaches to treat patients with cardiotoxicity.

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Author contribution:

R.A.E: Conceptualization, Methodology, Analysis, Data Analysis, Writing original draft, Project administration. M.T.H. Conceptualization, Methodology, Analysis, Data analysis, all authors reviewed the manuscript.

Declarations

Ethical Compliance:

All procedures performed in this study involving animal experiments were in accordance with the ethical standards following the National Institute of Health general guidelines for the Care and Use of Laboratory Animals in scientific investigations and authorized by the Ethical Committee (Faculty of Women for Arts, Science and Education, Ain Shams University, Code number: Sci 1332307007).

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