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Original Article

Protective effect of propolis from Tigzirt on epirubicin-induced cardiotoxicity and nephrotoxicity

[Efecto protector del propóleo de Tigzirt sobre la cardiotoxicidad y nefrotoxicidad inducidas por epirrubicina]

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Abstract

Context: Epirubicin (EPI), belonging to the anthracycline family, is one of the most effective chemo-therapeutic agents used in the treatment of a variety of solid and hematologic malignant tumors but Its anti-tumor efficacy is dose-dependent, but its clinical use is limited by the development of cardiac, hepatic and nephrotic toxicities.

Aims: To evaluate the protective effect of ethyl acetate extract of propolis (EAP) native to Tigzirt on selected antioxidant status parameters and biomarkers of epirubicin-induced cardiotoxicity and nephrotoxicity in rats.

Methods: Thirty male Wistar albino rats were divided into five groups. It orally dosed with (EAP) or quercetin during 15 days before being subjected to toxicity by injection (*i.v*) of a cumulative dose of 9 mg/kg of epirubicin to repair cardiac and renal damage.

Results: Injection of epirubicin to rats induced cardiac and renal dysfunction as evidenced by a significant increase ($P<0.05$) in serum levels of biochemical markers (CKmb, BNP, LDH, troponin, urea, creatinine and uric acid). This toxicity thus caused lesions, necrosis, and inflammatory infiltrate in the heart and kidneys. The administration of 250 mg/kg of EAP allowed restoring these functions by lowering the level of these parameters. Thus, a balance of oxidative stress was demonstrated with a decrease of +50% in the level of malondialdehyde and nitric oxide and an increase in superoxide dismutase, catalase and glutathione peroxidase. Therefore, significant restoration was observed on organ architecture.

Conclusions: These results show that propolis is rich in phenolic substances, which gave the protective and curative effects against epirubicin-induced cardiotoxicity and nephrotoxicity in Wistar rats.

Keywords: cardiotoxicity; epirubicin; nephrotoxicity; oxidative stress; propolis.

Resumen

Contexto: La epirrubicina (EPI), que pertenece a la familia de las antraciclinas, es uno de los agentes quimioterapéuticos más eficaces utilizados en el tratamiento de una variedad de tumores malignos sólidos y hematológicos, pero su eficacia antitumoral depende de la dosis, pero su uso clínico es limitado por el desarrollo de toxicidades cardíacas, hepáticas y nefróticas.

Objetivos: Evaluar el efecto protector del extracto de acetato de etilo de propóleo (EAP) nativo de Tigzirt sobre parámetros seleccionados del estado antioxidante y biomarcadores de cardiotoxicidad y nefrotoxicidad inducida por epirrubicina en ratas.

Métodos: Treinta ratas albinas Wistar macho fueron divididas en cinco grupos. Se dosificó por vía oral con (EAP) o quercetina durante 15 días antes de ser sometido a toxicidad por inyección (i.v) de una dosis acumulada de 9 mg/kg de epirrubicina para reparar el daño cardíaco y renal.

Resultados: La inyección de epirrubicina a ratas indujo disfunción cardíaca y renal evidenciada por un aumento significativo ($p<0.05$) en los niveles séricos de marcadores bioquímicos (CKmb, BNP, LDH, troponina, urea, creatinina y ácido úrico). Esta toxicidad provocó así lesiones, necrosis e infiltrados inflamatorios en el corazón y los riñones. La administración de 250 mg/kg de EAP permitió restaurar estas funciones al disminuir el nivel de estos parámetros, por lo que se demostró un equilibrio del estrés oxidativo con una disminución de +50% en el nivel de malondialdehído y óxido nítrico, y un aumento de superóxido dismutasa, catalasa y glutatión peroxidasa. Por lo tanto, se observó una restauración significativa en la arquitectura del órgano.

Conclusiones: Estos resultados muestran que el propóleo es rico en sustancias fenólicas que dieron efectos protectores y curativos contra la cardiotoxicidad y nefrotoxicidad inducida por epirrubicina en ratas Wistar.

Palabras Clave: cardiotoxicidad; epirrubicina; estrés oxidativo; nefrotoxicidad; propóleos.

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INTRODUCTION

Chemotherapy is among the most effective cancer treatments. It involves the use of chemical agents in order to stop the growth and thus eliminate cancer cells, even those at remote locations from the primary tumor origin. Anthracyclines are as well among the most effective drugs currently available for the treatment of neoplastic diseases (Chang et al., 2011; Shivani et al., 2014).

Epirubicin (EPI) of the anthracycline family is one of the most effective chemo-therapeutic agents used in the treatment of a variety of solid and hematological malignancies (Judson et al., 2014). It is an isomer of doxorubicin (4'-epi-doxorubicin), it binds rapidly to the nuclear structures of the cell, blocking the synthesis of DNA and RNA, it is an intercalating agent at the DNA level (Conte et al., 2000). Its anti-tumor efficacy is dose-dependent, but its clinical use is limited by the development of cardiac and hepatic toxicities.

Anthracyclines can induce various toxicities, including hematological toxicity, nephrotoxicity, hepatotoxicity and cardiotoxicity (Shivakumar et al., 2012; Elsherbiny and El-Sherbiny, 2014). Among these toxicities, cardiotoxicity is one of the major concerns limiting the effectiveness of treatment or altering the quality of life of patients treated with anthracyclines.

Dose reduction protocols have been proposed to avoid the risk of delayed cardiac toxicity, but this could decrease the effectiveness of the cytotoxic activity of anthracyclines (Elsherbiny and El-Sherbiny, 2014).

Renal injury is a dose-dependent side effect of epirubicin, limiting its clinical application in tumor chemotherapy (Wu et al., 2017).

Epirubicin-induced nephrotic damage is related to renal tubular cell injury and death, inflammation and oxidative stress, which are considered to be the essential mechanisms of nephrotoxicity (Lin et al., 2010; Abdelraouf et al., 2012).

Several authors agree that epirubicin toxicity is associated with deteriorating antioxidant status, resulting from increased levels of malondialdehyde (MDA) and decreased activities of antioxidant enzymes (GSH, SOD) (Kebieche et al., 2009; Marrington et al., 2011; Prado et al., 2011).

To defend against free radical attacks, the organism has a protective enzyme system, namely superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which are capable of directly trapping pro-oxidant radicals and can prevent oxidative stress-related diseases (Otunola et al., 2014).

Several types of research have therefore been carried out on natural products, particularly those rich in polyphenols and flavonoids, which have shown interesting biological antioxidant properties (Georgiev et al., 2014).

Among these protective substances, it was interested in propolis, which is a natural resinous substance collected by bees either from tree buds such as poplar, oak, alder, etc. or from conifers, amalgamated with a salivary secretion of the bees (Sheng et al., 2007). The forty or so flavonoids present in propolis give it the activity of "free radical scavengers" (Boufadi et al., 2017). It is, after tea and red wine, the richest element in flavonoids.

This action has been demonstrated where phenolic compounds, flavonoids, and especially artepillin C, oppose lipid peroxidation and prevent free radical damage (El-Guendouz et al., 2017).

Propolis has shown several biological activities such as antioxidant activity (El-Guendouz et al., 2017), anti-inflammatory activity (Batista et al., 2018; Guzmán-Gutiérrez et al., 2018), immunomodulatory activity (Soltani et al., 2017), anticancer activity (Brihoum et al., 2018), antibacterial activity (Kasiotis et al., 2017; Jiyeon and Kim, 2018), hepatoprotective activity (Chaa et al., 2019).

In view of these data, it decided to investigate the protective effect of Tigzirt propolis against cardiotoxicity and epirubicin-induced nephrotoxicity in Wistar rats.

MATERIAL AND METHODS

Chemicals

The epirubicin (EPI, 50 mg) was purchased from Pharmazie Thymoorgan laboratory GmbH, Germany. Sigma-Aldrich provided the solvents MeOH, EtOH, ethyl acetate, CH_2Cl_2, and CHCl3 (St Louis, MO). Formic acid, acetonitrile, 1.1.3.3-tetra-methoxypropane, thiobarbituric acid, trifluoroacetic acid (TFA), Tris–HCl, sucrose, bovine serum albumin, $MnCl_2$, EDTA, NADPH, 5,5'-dithio-bis-(2-nitrobenzoate), mercaptoethanol, MDA, H_2O_2, SOD, as well as quercetin and formaldehyde aldehyde.

Origin of propolis

In June 2016, in the Tigzirt region (Tizi Ouzou, Algeria) in Latitude: 36° 53' 59.99" N, Longitude: 4° 06' 60.00" E, propolis was harvested by a bee breed (*Apis mellifica intermissa*). The grid method was used to harvest this raw propolis, which was then placed in the freezer at -18°C before the study.

A recent study reported that propolis from Tigzirt (Algeria) contains the highest amount of antioxidant substances compared to several other types of Algerian propolis (Boufadi et al., 2014). This propolis has shown several antioxidants (Boufadi et al., 2014; 2017) and antimicrobial activities (Boufadi et al., 2016). Nine flavonoids have been identified in propolis from Tigzirt: catechin, quercetin, rutin, acacetin, chlorogenic, apigenin, pinocembrin, chrysin, kaempferol; seven phenolic acids: thymol, ferulic acid, gallic acid, caffeic acid, ellagic acid, rosmarinic acid and trans-cinnamic acid; and two organic acids: m-coumaric and ascorbic acid (Chaa et al., 2019).

Preparation of propolis extract

It was carried out the extraction according to the protocol of Boufadi et al. (2014), which consists of cutting the raw propolis into small pieces, grinding them before extracting the active ingredients with 95% (v/v) ethanol (in the proportions raw propolis/solvent = 1/10: w/v) in a cold water ultrasonic bath (Fisherbrand, USA), for 1 hour and 30 min. It was performed this extraction operation 3 times. After that, the suspension was filtered on Whantman N°1 paper before evaporating the solvent dry under reduced pressure at a temperature of 60°C. This filtrate represents the ethanolic extract of propolis (EEP).

This dry ethanolic extract (EEP) was then suspended in 200 mL of distilled water and extracted with 200 mL of chloroform. The organic layer was removed and extracted from the aqueous phase with 200 mL ethyl acetate (EtOAc) three times. The organic phase of EtOAc was collected after complete evaporation of the ethyl acetate solvent, which was noted as EAP.

Animals and experimental design

Thirty male Wistar albino rats (100 to 115 g) were used for this experiment provided by Pasteur Institute (Algiers, Algeria). The protocol complies with the guidelines of the National Institute of Health (NIH-USA). All animal experiments were approved by the local ethical committee for animal care of the institution (University Abdelhamid Ibn Badis, Mostaganem) (rat/mouse 20% maintenance, RN-01-20K12; Carfil Quality).

Upon receipt, the rats were randomly divided into 5 experimental groups containing 6 rats each, subjected to a two-week adaptation period at room temperature (22 ± 3°C), humidity (45-50%), and a 12/12 h photoperiod.

The rats were placed in polyethylene cages lined with litter made of wood shavings. These were cleaned, and their litter changed daily. The animals were provided with food and water as well (kibbles from the feed production company, Bouzaréa, Algiers).

After the adaptation period, the rats were orally dosed of the first and second group (G1 and G2) 1 mL of physiological water daily, while those of groups 3 and 4, respectively, received 1 mL of 100 and 250 mg/kg of ethyl acetate extract of Tigzirt propolis (EAP), and those of group G5 were given 1 mL 50 mg/kg of quercetin. The administration was by repeated doses for 15 days.

On day 15, the groups G2 to G5 were intravenously injected with epirubicin at 48 h intervals for

one week to reach a cumulative dose of 9 mg/kg (Dobbs et al., 2003). All were under observation, and their body weight was recorded daily.

Twenty-four hours after completion of treatment, prior to blood collection, it held the rats unfed for 12 hours. The following day, it kept them under light chloroform anesthesia before termination in order to avoid any risk of biochemical parameter change prior to blood samples collection by cardiac function, which was immediately performed in four different tubes: EDTA, dry, heparinized and citrated.

Biochemical and antioxidant assays

Assessment of cardiac function

Creatine kinase MB, total troponin was determined with the BioVision kits (BioVision, USA), whereas lactate dehydrogenase (LDH) and type B natriuretic peptide (BNP) with the Biolab kits (France).

Assessment of renal function

Urea was determined with the Kit Chronolab System, whereas creatinine and uric acid with bioMerieux (BioMerieux SA, France).

Estimation of oxidative stress markers

The mitochondrial matrix was prepared according to the method described by Rustin et al. (1994). The pieces of the different organs (kidneys and heart) were placed in a mitochondrial isolation ice buffer (10 mM tris-HCl, pH 7.4, 250 mM Sucrose, 0.5 mM EDTA, and 0.5% bovine serum albumin). After homogenization of the fragments, the homogenate obtained was centrifuged at 10000 rpm/10 min at 4°C. The mitochondrial pellets were washed twice with the isolation buffer and then resuspended in the same buffer solution. The mitochondrial matrix was extracted by freezing and thawing, followed immediately by repeated homogenization of the fresh mitochondrial preparations in order to burst the mitochondria. After centrifugation at 10 000 rpm/10 min, the supernatant obtained was stored at 4°C in order to determine the parameters of antioxidant status (Lück, 1965), superoxide dismutase (Elstner et al., 1983), glutathione peroxidase (Rotruck et al., 1973), malondialdehyde (Yagi, 1976), nitric oxide (Alam et al., 2013) and thiols (Riddels et al., 1979).

Histopathological study

It carried out the histological study on the different organs (kidney and heart) according to the protocol of Drury and Wallington (1967), stored renal and cardiac portions in 10% formaldehyde aldehyde embedded in paraffin, and cut with a rotating microtome (Leica, Germany) at 5 µm thickness, spread on microscope slides and stained with hematoxylin and eosin, which it was observed by light microscopy.

Statistical analysis

SigmaStat software (SPSS, 3.0, SPSS, Inc., Chicago, IL) was used for the analysis data presented as mean ± SD and evaluated by one-way ANOVA, with Dunnett's post hoc test. When appropriate, ANOVA was used on Rank with Dunnett's post hoc test.

RESULTS

Clinical observations and the evolution of rat body weight

Rats in group G1 (control) and group 4 (rats treated with 250 mg/kg PEA), showed no signs of toxicity or mortality. However, serious changes were noted in the physical activity and behavior of group 1 rats poisoned by epirubicin (tachycardia, difficulty in breathing, or hypoactivity, among others).

In the same study (Chaa et al., 2019), the change in body weight was observed, where we noticed no mortality during the experiment period. Normal development was observed in the rats of groups 1, 3 and 4. In contrast, a significant decrease was observed in the rats of group 2 (treated with epirubicin alone).

Table 1. Effect of ethyl acetate extract of propolis (EAP) native to Tigzirt on cardiac function parameter values of Wistar rats exposed to epirubicin (EPI).

Parameter	G1	G2	G3	G4	G5
CKmb (UI/L)	5.16 ± 1.03	37.83 ±3.31	9.5 ± 2.87	8.66 ± 4.09	17.33 ± 2.39
LDH (UI/L)	208.66 ± 6.22*	790.33 ± 12.28*	291.66 ± 18.14**	245.33 ± 9.57**	612.51 ± 17.33*
Troponine T (µg/L)	0.14 ± 1.01	0.76 ± 0.09*	0.29 ± 0.04*	0.17 ± 0.05	0.41 ± 0.02
BNP (pg/mL)	6.66 ± 3.57*	58 ± 4.9*	11.33 ± 2.81*	6.66 ± 2.95*	19.66 ± 4.04*

The values are expressed as mean ± SD (n = 5). *Significant difference from the control group ($p<0.05$). **Significant difference from the epirubicin toxicity group ($p<0.05$). G1: control; G2: 9 mg/kg of epirubicin (7 days); G3: 100 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G4: 250 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G5: 50 mg/kg of quercetin then 9 mg/kg of EPI (7 days).

Table 2. Effect of ethyl acetate extract of propolis (EAP) native to Tigzirt on renal function parameter values of Wistar rats exposed to epirubicin (EPI).

Parameter	G1	G2	G3	G4	G5
Urea (g/L)	0.15 ± 0.03	1.63 ± 0.20**	0.29 ± 0.02*	0.18 ± 0.02	0.73 ± 0.05
Uric acid (mg/L)	36.66 ± 5.13*	276.66 ± 37.58**	60.33 ± 4.04**	46.66 ± 6.65*	87 ± 11*
Creatinine (mg/L)	9.33 ± 2.51	37.66 ± 3.05*	11.66 ± 2.51**	10.33 ± 1.54*	17.66 ± 3.05*

The values are expressed as mean ± SD (n = 5). *Significant difference from the control group ($p < 0.05$). **Significant difference from the epirubicin toxicity group ($p<0.05$). G1: control; G2: 9 mg/kg of epirubicin (7 days); G3: 100 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G4: 250 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G5: 50 mg/kg of quercetin then 9 mg/kg of EPI (7 days).

Cardiac function

The findings proved the existence of acute cardiotoxicity induced by epirubicin. Very significant elevation was observed in cardiac biomarkers (LDH, CK-MB, troponin T, BNP) (Table 1) in the group cured with epirubicin (G2) compared to the control group (G1). In contrast, rats in G3 and G4 (which received 100 and 250 mg/kg of EAP before the injection of epirubicin) showed a decrease in these biomarkers compared to those in (G2). Rats cured with quercetin (G5) showed a slight decrease compared to (G2).

Renal function

Injection of epirubicin in rats at a dose of 9 mg/kg (G2) induced renal dysfunction, as evidenced by a significant increase in serum levels of biochemical markers (Table 2) (urea, creatinine and uric acid) compared to the control group, while treatment with 100 and 250 mg/kg of EAP (G3, G4) restores renal function by lowering the level of these parameters compared to (G2). However, orally dosing (G5) with one of the purest compounds (quercetin) had no significant effect on renal function.

Oxidative stress status

Table 3 shows the oxidative stress parameter level in heart and kidney tissues in rats of the different groups. These results indicate a significant decrease in antioxidant markers (SOD, CAT, GSH-Px and thiols) in (G2) (cured only with 9 mg/kg of epirubicin) in comparison to the control group (G1).

The activity of these antioxidant enzymes significantly improved ($p<0.05$) in the rats cured with propolis, especially in that of G4 group (250 mg/kg of EAP) in comparison to G2 group.

Moreover, it was recorded a strong lipid peroxidation and an increase in the nitric oxide (NO) level in the heart and kidneys in G2 in comparison to G1. In contrast, a very significant decrease was

observed in these markers in G4 (predosed with 250 mg/kg of EAP) in comparison to G2. G3 (predosed with 100 mg/kg of EAP) and G5 (dosed with 50 mg/kg of quercetin) showed a reduction in cardiac and renal antioxidant status parameters (SOD, CAT, GPx and thiols) and an increase in MDA and nitric oxide (NO) levels.

Histopathological study

Histopathology of the heart

The heart microscopic study in the control group (G1) showed a normal histological structure of the heart tissue (Fig. 1A). The histological heart section in G2 (dosed with 9 mg/kg of PPE) revealed the presence of edema, very important congestion with areas of necrosis and plasmocyte inflammatory infiltrates (Fig. 1B-C). Treatment with 100 mg/kg of propolis (G3) showed slight congestion with no inflammation (Fig. 1D). The heart architecture of the rats in G4 (those dosed with 250 mg/kg of EAP) was similar to that observed in the control group (Fig. 1E). G5 shows inflammatory infiltrations, capillary congestion, and some areas of necrosis (Fig. 1F).

Histopathology of the kidneys

The kidneys histological section of the control group (G1) showed normal architecture with a regular distribution of cells, preserved glomeruli, together with the absence of congestions and inflammatory infiltrates (Fig. 2A). Epirubicin showed damaged kidney tissue, swollen and undefined glomeruli, capillary congestion and hemorrhages, and even the presence of inflammatory infiltrates (Fig. 2B-C). Slight capillary congestion of the renal parenchyma was observed in G3, which was dosed 100 mg/kg of propolis before the injection of epirubicin (Fig. 2D). In contrast, administration 250 mg/kg of EAP to G4 showed a similar nephrotic architecture to that of the animals in the control group (G1). The renal parenchyma is highly conserved with intact glomeruli and a total absence of granular congestion (Fig. 2E).

Table 3. Effect of ethyl acetate extract of propolis (EAP) native to Tigzirt on malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), thiol in the heart and kidneys of Wistar rats exposed to epirubicin (EPI).

Parameter	G1	G2	G3	G4	G5
Cardiac oxidative stress status					
MDA heart (nmol/mg)	0.13 ± 0.02	0.66 ± 0.70	0.43 ± 0.04	0.13 ± 0.03	0.54 ± 0.04
NO heart (µmol/g)	3.94 ± 0.69	13.71 ± 1.27**	8.16 ± 0.41	4.18 ± 0.25*	10.39 ± 0.93**
SOD heart (U/cg)	16.98 ± 1.27	5.09 ± 1.06**	10.40 ± 0.37*	17.02 ± 1.26*	7.59 ± 0.55*
CAT heart (U/mg)	20.12 ± 0.88	10.36 ± 0.96**	14.57 ± 0.74**	20.86 ± 1.60*	12.75 ± 1.04*
GSH-Px heart (U/g)	31.53 ± 1.45	14.95 ± 1.16	18.66 ± 0.58	31.28 ± 0.94	14.89 ± 1.10*
Thiol heart (mmol/mL)	0.92 ± 0.03	0.12 ± 0.05	0.39 ± 0.02**	0.90 ± 0.04	0.34 ± 0.01*
Renal oxidative stress status					
MDA kidneys (nmol/mg)	0.34 ± 0.06	1.89 ± 0.72*	0.53 ± 0.04**	0.34 ± 0.06	1.27 ± 0.24*
NO kidneys (µmol/g)	6.05 ± 0.32	16.27 ± 1.05**	12.84 ± 1.91*	6.11 ± 0.25**	13.52 ± 0.98**
SOD kidneys (U/cg)	22.79 ± 2.12	6.13 ± 0.91*	15.20 ± 0.66	23.78 ± 1.45**	10.87 ± 0.82**
CAT kidneys (U/mg)	17.89 ± 0.90	5.03 ± 0.65**	13.00 ± 0.94**	18.14 ± 0.53	9.04 ± 0.39**
GSH-Px kidneys (U/g)	45.12 ± 1.74	20.10 ± 1.23**	34.46 ± 0.76*	44.75 ± 1.98**	24.61 ± 2.12**
Thiol kidneys (mmol/mL)	1.28 ± 0.15	0.47 ± 0.08*	0.94 ± 0.05*	1.30 ± 0.09*	0.79 ± 0.05**

The values are expressed as mean ± SD (n = 5). *Significant difference from the control group ($p < 0.05$). **Significant difference from the epirubicin toxicity group ($p<0.05$). G1: control; G2: 9 mg/kg of epirubicin (7 days); G3: 100 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G4: 250 mg/kg of EAP (15 days) then 9 mg/kg of EPI (7 days); G5: 50 mg/kg of quercetin then 9 mg/kg of EPI (7 days).

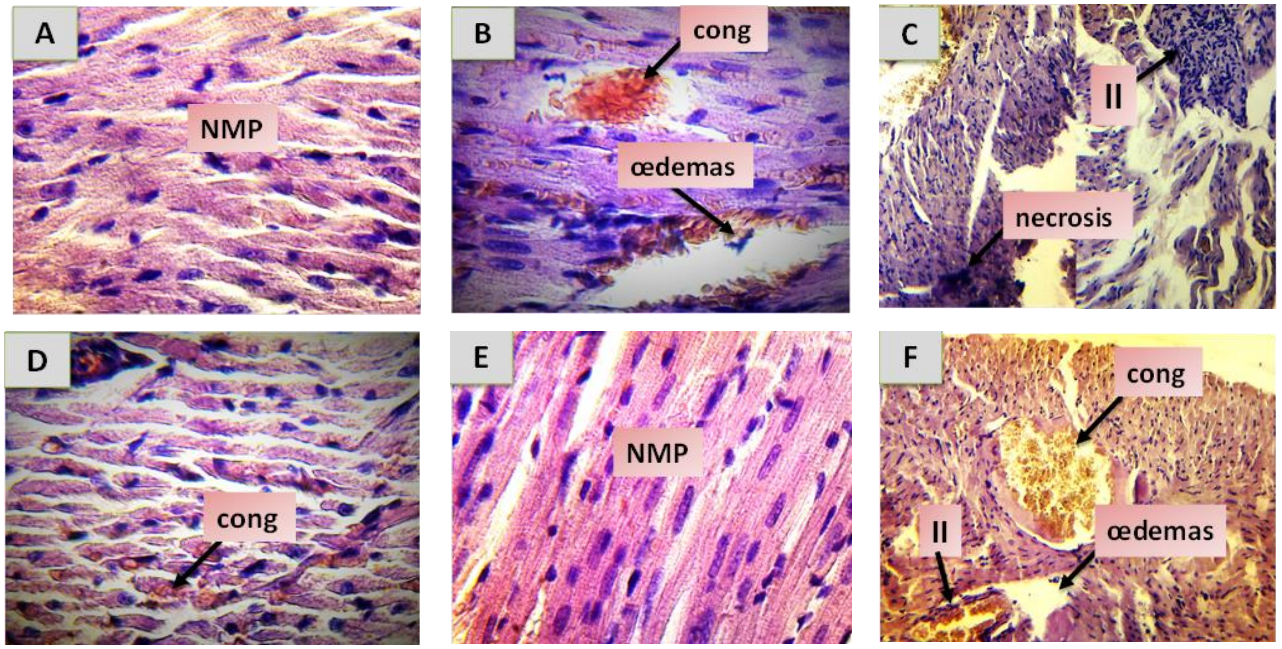

Figure. 1. Histological sections of the hearts of the rats in the group: **(A)** Control; **(B)** and **(C)**: 9 mg/kg epirubicin (7 days); **(D)**: 100 mg/kg EAP (15 days) then 9 mg/kg EPI (7 days); **(E)**: 250 mg/kg EAP (15 days) then 9 mg/kg EPI (7 days); **(F)**: 50 mg/kg quercetin then 9 mg/kg EPI (7 days).

NMP: normal myocardial parenchyma, Cong: congestion, II: inflammatory infiltrate.

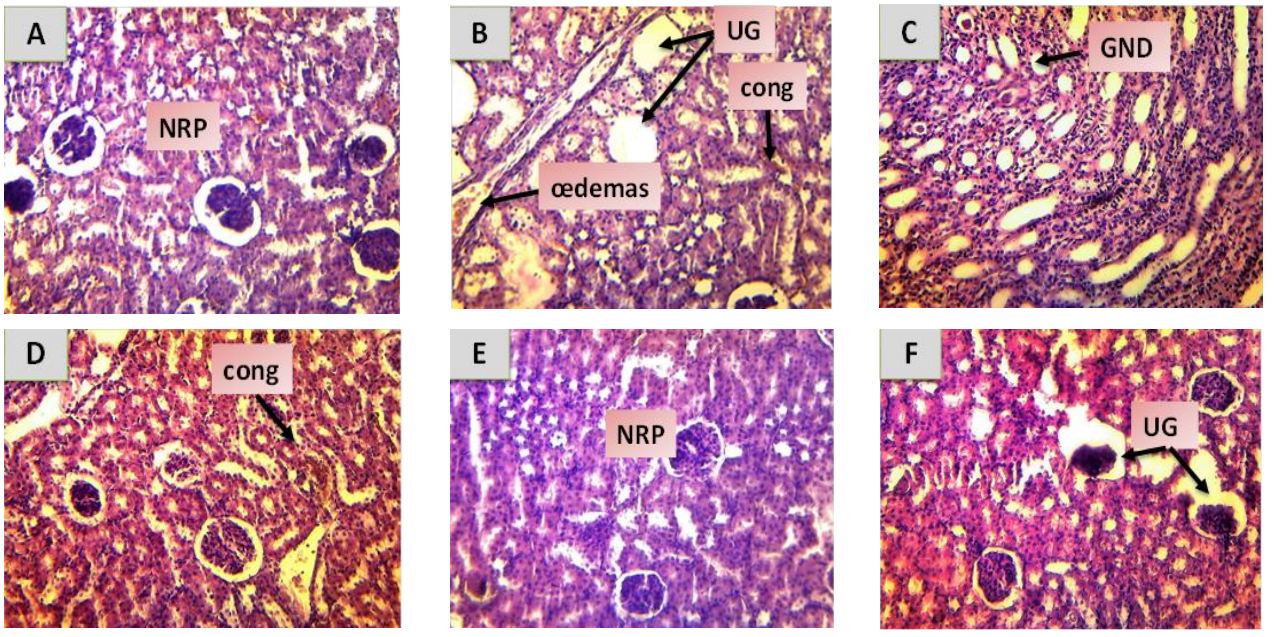

Figure. 2. Histological sections of the kidneys of the rats in the group: **(A)**: Control; **(B)** and **(C)**: 9 mg/kg epirubicin (7 days); **(D)**: 100 mg/kg EAP (15 days) then 9 mg/kg EPI (7 days); **(E)**: 250 mg/kg EAP (15 days) then 9 mg/kg EPI (7 days); **(F)**: 50 mg/kg quercetin then 9 mg/kg EPI (7 days).

NRP: normal renal parenchyma, UG: undefined glomerulus, Cong: congestion.

DISCUSSION

Anthracyclines can induce various toxicities, including hematological toxicity, nephrotoxicity, hepatotoxicity and cardiotoxicity (Shivakumar et al., 2012; Elsherbiny and El-Sherbiny, 2014). Among these toxicities, cardiotoxicity is one of the major concerns limiting the effectiveness of treatment or altering the quality of life of anthracycline-treated patients. Cardiotoxicity is a common and serious adverse effect of anthracycline therapy in breast cancer patients. Current criteria for cardiotoxicity are based on imaging and cardiac biomarkers (Pereira et al., 2020).

Two main types of cardiotoxicity have been identified: acute cardiotoxicity, independent of anthracycline dose, generally mild and reversible, ranging from asymptomatic electrocardiogram (ECG) changes to rare cases of severe acute myocarditis; and delayed, dose-dependent cardiotoxicity leading to chronic, life-threatening, irreversible congestive heart failure (CHF) (Mueller et al., 2004).

The risk of developing cardiotoxicity, particularly CHF, is strongly correlated with the cumulative dose of anthracycline (Peng et al., 2020). In the treatment of breast cancer, two anthracyclines are available: doxorubicin and epirubicin. The probability of developing CHF with epirubicin is related to cumulative doses of 900-1000 mg/m^2 and above.

In addition, epirubicin causes irreversible type I cardiotoxicity characterized by permanent damage associated with cardiomyocyte cell death and ultrastructural change (Plana et al., 2014). It is most often associated with dilated cardiomyopathy. This cardiotoxicity may occur at the time of injection or a few hours after (acute effects), in the first year after the end of treatment (early chronic effects), or a few years after the end of treatment (late chronic effects) (Giantris et al., 1998; Lipshultz et al., 2008).

However, in the present study, there was a disturbance in cardiac function reflected by a very significant increase in cardiac biochemical markers including CKmb, LDH, troponin T, and BNP in rats injected with the cumulative dose of 9 mg/kg of epirubicin (G2) compared with the control group G1.

The significant increase in LDH and CKmb is the first indicator of myopathy. This is a potential for muscle damage as these enzymes have diagnostic values for myocardial (heart muscle) problems (Klein et al., 2020). Thus, cardiac troponin is currently the first-line test for evaluating patients suspected of having an acute infarction (Patibandla et al., 2020).

In addition, B-type natriuretic peptide (BNP) is a marker that belongs to the family of natriuretic peptides. This peptide is synthesized mainly by cardiac myocytes (Maisel et al., 2002). Blood levels of BNP increase in congestive heart failure (Anoop et al., 2020). This explains the increase in plasma BNP in the case of heart failure, as fluid overload and high blood pressure are present (Jourdain et al., 2009).

Indeed, BNP levels increased significantly during high doses of anthracycline-based chemotherapy (Suzuki et al., 1998) and were correlated with diastolic, rather than systolic, dysfunction (Nousiainen et al., 2002).

Evaluation of antioxidant status in cardiac tissue in rats injected with epirubicin alone showed oxidative stress in the heart with an increase in cardiac malondialdehyde (MDA) and nitric oxide (NO) concentrations and a significant decrease in the activity of antioxidant enzymes (SOD, CAT, GSH-Px) and thiols compared to the control group.

Several studies suggest that increased lipid peroxidation and oxygen-free radical-induced damage play a pathogenic role in heart failure (HF) patients (Al-Taher et al., 2020).

Antioxidant therapy could therefore be of potential interest in the treatment of heart failure patients. Knowledge indicates that the combination of several antioxidants could be a therapeutic approach in disorders characterised by increased oxidative stress against myocardial diseases (Sliwa et al., 1998).

Evidently, the administration of Tigzirt propolis (EAP) to rats, notably the concentration of 250 mg/kg of EAP (G4), showed a protective effect against cardiotoxicity while improving the level of cardiac biochemical markers and the antioxidant status of the heart, thus ensuring histologic protection.

According to Chaa et al. (2019), nine flavonoids have been identified in the ethyl acetate extract of Tigzirt propolis (EAP) (catechin, quercetin, rutin, acetine, chlorogenic, apigenin, pinocembrin, chrysin, kaempferol) and seven phenolic acids (thymol, ferulic acid, gallic acid, caffeic acid, ellagic acid, rosmarinic acid, and trans-cinnamic acid) and two organic acids (m-coumaric and ascorbic acid). These identifications were made by the HPLC/UV method.

Indeed, propolis has the capacity to capture and deactivate free radicals produced by the mitochondrial respiratory chain of cardiac cells (Jensen, 2006). The reduction of the O_2- radical in cardiac mitochondria and the direct action against the OH radical, species involved in triggering lipid peroxidation can also be explained by the powerful scavenging effect of propolis. Alternatively, it is also possible that the effect of propolis cytoprotection is achieved by blocking the permeabilization of the internal mitochondrial permeability transition port (mPTP) or by releasing effectors of cell death through the external mitochondrial membrane. Restoration of the mitochondrial membrane potential could also provide a pathway to protect the cell from apoptosis. All these pathways lead to the prevention of cardiotoxicity and degenerative diseases (Mesbah and Zellagui, 2012).

Pretreatment of rats with propolis extract prior to doxorubicin injections significantly reduced peroxidation-induced damage to myocardial tissue and markedly restored catalase and SOD activities. It strongly suggests that propolis extract protects heart tissue from oxidative stress by protecting the mitochondria (Benguedouar et al., 2008).

In addition, ethanol extract from Brazilian propolis may protect rats through its antioxidant power (Nakamura et al., 2012). The *in vivo* antioxidant activity of propolis extracts has shown a decrease in intracellular oxidation at the cellular and mitochondrial proteome levels in yeast cells (Cigut et al., 2011).

Several studies suggest that propolis has a cardioprotective effect against doxorubicin (Chopra et al., 1995; Alyane et al., 2008). Prevention against this cardiotoxicity is based on close cardiac monitoring. In the context of myocardial protection, the use of cardio-protective agents coupled with chemotherapy, such as pharmacological compounds acting on the level of oxidative stress, is currently the subject of intensive research for the optimal use of anthracyclines (Delemasure et al., 2006).

These results showed that treatment with ethyl acetate extract of Tigzirt propolis significantly reduced the toxic effect of epirubicin on the heart compared to rats exposed to epirubicin alone.

Thus, nephrotoxicity induced by chemotherapeutic agents can manifest itself as acute or chronic renal failure, tubular dysfunction, proteinuria or even arterial hypertension (Bárdi et al., 2004). Thus, renal monitoring should include a systematic assessment of glomerular filtration rate (GFR), tubular function (urinary pH, proteinuria, electrolytes), as well as blood pressure monitoring (Rodrigues et al., 2017). Renal injury is a dose-dependent side effect of epirubicin, limiting its clinical application in tumor chemotherapy (Wu et al., 2017). Epirubicin-induced nephrotic damage is related to renal tubular cell injury and death, inflammation and oxidative stress, which are considered to be the essential mechanisms of nephrotoxicity (Lin et al., 2010; Abdelraouf et al., 2012).

After injection of 9 mg/kg of epirubicin to rats in the G2 group, a significant alteration was noted in markers of renal function, a significant increase in urea, creatinine and uric acid. Histological examination showed significant damage in the renal structure.

The findings showed a significant increase in MDA and nitric oxide (NO) in the kidney cell homogenate in rats injected with a cumulative dose of 9 mg/kg of epirubicin, as well as a significant

decrease in nephrotic antioxidant enzymes such as SOD, CAT, GSH-Px, and even thiols. This explains why EPI caused an inhibition of the renal antioxidant defense.

The role of ROS in the tubular and glomerular effects of anthracyclines has been clearly demonstrated. *In vivo*, ROS are labeled as mediators of proximal tubular necrosis and acute renal injury (Du and Yang, 1994).

Mitochondria have been defined as one of the targets of PPE-induced subcellular damage in tissues. In addition, it has been shown that PPE may stimulate transmembrane transport of arginine to increase substrate and activate nitric oxide (NO) production mediated by nitric oxide synthase (NOS) (Cendan et al., 1995). In addition, nitric oxide (NO) has a vital function in the local regulation of blood flow in the renal cortex and can influence glomerular filtration. NO can act in different ways, acting as a physiological signal molecule, a protector of cellular functions, and a toxic mediator (Valdivielso and Blantz, 2002). NO-mediated cell damage occurs through several mechanisms, including disruption of mitochondrial respiration, inhibition of enzymes, nitrosylation of proteins, and peroxidation of lipids by mediators such as peroxynitrite produced in the reaction between NO and O_2^-. This reaction occurs when a high concentration of NO becomes available as a result of increased activity of inducible NO synthase (iNOS) (Gordge, 1998). It can assume that an increase in iNOS expression and NO production plays a role in the apoptosis of mesangial cells in vitro (Martinez-Salgado et al., 2004).

Wu et al. (2017) showed that exposure of rats to epirubicin caused severe nephrotic damage, tubular degeneration, necrosis, infiltration of inflammatory cells, and interstitial hemorrhage.

Administration of ethyl acetate extract of Tigzirt propolis preserved the renal architecture while decreasing renal biochemical parameters (urea, creatine and uric acid) and maintaining the antioxidant defense system.

Antioxidants are able to abolish kidney damage by reducing lipid peroxidation and enhancing the trapping capacity of the antioxidant defense system. ROS scavengers have been shown to be beneficial in alleviating kidney damage after epirubicin injection (Randjelovic et al., 2012).

Fadillioğlu et al. (2003) demonstrated that increasing antioxidant status parameters (such as SOD or thiols) prevented the decrease in glomerular filtration induced by anthracyclines. Thus, GSH-Px plays an important role in the detoxification of xenobiotic compounds and in the antioxidation of reactive oxygen and free radical species (Yagmurca et al., 2004).

Several works have confirmed the protective effect of propolis against nephrotoxicity induced by different agents such as gentamicin (Azab et al., 2014; Aldahmash et al., 2016).

The preventive and nephroprotective effect of propolis is certainly due to its chemical composition rich in polyphenols.

CONCLUSIONS

The association of propolis with epirubicin has impressively reduced the level of toxicity of the latter in the heart and kidneys, by modulating the levels of biochemical markers, thus delaying oxidative stress enzymes, reducing the activity of antioxidant enzymes, and inhibiting lipid peroxidation. This combination revealed an improvement in biochemical metabolism. The dose of propolis that has shown to be very effective in preventing toxicity is 250 mg/kg. While the administration of 50 mg/kg of quercetin showed no protective effect against the toxic effects of epirubicin, this confirms that the protective action of propolis is dose-dependent, so this effectiveness is due to all the polyphenols present in this extract (synergistic effect).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Chaa S	Boufadi MY	Keddari S	Benchaib AH
Concepts or ideas		x		
Design	x	x		
Definition of intellectual content	x	x	x	
Literature search	x	x		
Experimental studies	x	x		
Data acquisition	x	x		x
Data analysis	x	x		x
Statistical analysis			x	
Manuscript preparation	x	x		
Manuscript editing	x	x		
Manuscript review	x	x	x	x

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