



Original Article

Chemical composition of propolis extract and its effects on epirubicin-induced hepatotoxicity in rats



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ABSTRACT

Propolis is a natural substance, produced by honeybees from the resin of various plants. The purpose of this study was to determine the chemical composition and evaluate the hepatoprotective effect of ethyl acetate extract of propolis from Tigzirt, against the toxicity induced by epirubicin which is a anti-cancer agent, and belongs to the family of anthracyclines. The study included thirty male Wistar albino rats divided into five groups. The rats received the extraction of propolis or the quercetin orally for 15 days. The hepatotoxicity was promoted by injection epirubicin (*i.v.*) with a cumulative dose of 9 mg/kg. Several biological parameters were measured. Oxidative status was also assessed by evaluating antioxidant enzyme and histological study of some organs. Epirubicin caused oxidative stress by a significant decrease in hepatic antioxidant enzymes (glutathion peroxidase, catalase, superoxide dismutase), increased malondialdehyde and liver parameters (ASAT, ALAT, γ GT, ALP) compared to the control. The histological study revealed major damage to the liver. Perturbations in this liver function, antioxidant status and damage to the liver by epirubicin have been repaired by the administration of propolis. Furthermore, epirubicin showed inflammatory effects induced by an increase in TNF- α and PGE2. Pretreatment with propolis to rats restored these inflammatory parameters. The chemical identification of extract of propolis by HPLC/UV shows the presence of polyphenolic compounds and many flavonoids. The propolis extract showed a significant reduction in oxidative damage from oxidative stress and a very important protective effect against epirubicin-induced hepatotoxicity.

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Introduction

The epirubicin (EPI) is one of the effective chemotherapeutic agents. It belongs to the anthracyclines family (Judson *et al.*, 2014). It is widely used for the treatment of various breast cancers (Jing Wu *et al.*, 2015). EPI is derived from the doxorubicine and it was metabolized in the liver to give metabolites such as the épirubicinol and EPI glucuronide (Weenen *et al.*, 1984). It has been demonstrated that the doxorubicine and the epirubicin have high toxicity for the liver (Weenen, 1984; Le Bot *et al.*, 1988). The mechanism of action of epirubicin appears to be related to its ability to bind to nucleic acids (Chang *et al.*, 2014). It is fixed quickly on the nuclear struc-

tures of the cell by intercalating in the DNA between base pairs, blocking the synthesis of the DNA and RNA (Jain *et al.*, 2005).

Several hypotheses were suggested regarding the mechanisms of toxicity induced by anthracyclines. It has been reported that the oxygen free radicals and lipid peroxidation play an essential role in the hepatic damages caused by the epirubicin (Singal and Iliskovic, 1998). In addition, it has been clearly shown that the culture of the hepatocytes in the presence of epirubicin decreases the cellular viability by disturbances of the cellular membranes. This process has been found to be related also to the generation of the oxygen free radicals produced during EPI metabolism (Germain *et al.*, 2003). Moreover there is a correlation between EPI and the activity of serum transaminases among cancer patients having received this drug (Ganzina, 1983; Le Bot *et al.*, 1988; Twelves *et al.*, 1992).

Despite the discovery of new synthetic compounds that have antioxidant effects, natural sources remain the main supplier for obtaining antioxidant molecules which can be used to prevent the

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oxidative damages and the toxicity generated by chemotherapy. A large amount of researches therefore have been done on the natural products especially for those rich in polyphenols and flavonoids which have shown interesting antioxidant biological properties (Kurek-Górecka et al., 2014; Georgiev et al., 2014). In this context, many antioxidant molecules have been proposed as protective against oxidant toxicity (Du and Lou, 2008; Vincent et al., 2013).

The propolis is one of these antioxidant products (Segueni et al., 2016). It is a series of resinous, gummy and balsamic substances of viscous consistency collected by the bees on certain parts of plants (primarily buds and barks of certain trees) (Harfouch et al., 2016). Its extracts contain high amounts of polyphenols, flavonoids, and ascorbic acid. Thus it has potent antioxidant properties (Boufadi et al., 2014; Machado et al., 2016). Many studies have shown that propolis has a number of biological roles as analgesic-anesthetic activity (Orsatti and Sforzin, 2011), antiallergic activity (Mehmett Yasar et al., 2016), antibacterial activity (Boufadi et al., 2016), anti-cancer activity (Zabaoui et al., 2017), immunomodulatory activity (Soltani et al., 2017), and anti-inflammatory activity (El-Guendouz et al., 2017).

The aim of this study is to evaluate the protective effect of ethyl acetate extract of propolis (EAP) obtained from Tizirt (Tizi Ouzou, Algeria) by the method of grids on hepatotoxicity induced by the epirubicin *in vivo*.

Materials and methods

Reagents and chemicals

Epirubicin (EPI, 50 mg) was purchased from the laboratory Pharmazie Thymoorgan GmbH, Allmagne. The solvents CH₂Cl₂, MeOH, EtOH, EtOAc, and CHCl₃ were obtained from Sigma–Aldrich (St Louis, MO). formic acid, trifluoroacetic acid (TFA), acetonitrile, 1.1.3.3-tetramethoxypropane, thiobarbituric acid, Tris–HCl, EDTA, sucrose, bovin serum albumin, MnCl₂, mercaptoethanol, NADPH, Ellman's reagent – 5,5'-dithiobis(2-nitrobenzoic acid), MDA, SOD, H₂O₂, quercetin and formaldehyde is an aldehyde were also purchased from Sigma–Aldrich.

Collection and extraction of the propolis

Propolis was collected according to the method of grids in June 2015 from a region of Tizirt (Tizi Ouzou, Algeria), and was stored at –18 °C. This propolis has antioxidant effects (Boufadi et al., 2014, 2017). It was made by a breed of bees (*Apis mellifera intermissa*).

The crude propolis (10 g) was divided into small pieces, crushed and extracted three times with ethanol 95% (100 ml) in ultrasonic water during 1.30 h. The suspension was then filtered on paper Whatman No. 1. The solvent was evaporated to dryness under reduced pressure at 60 °C. The filtrate represents the ethanolic extract of propolis (EEP).

Then the ethanolic dry extract (EEP) was suspended in 200 ml of distilled water and extracted with 200 ml of chloroform. The organic layer was eliminated and the aqueous phase was extracted with 200 ml of ethyl acetate (EtOAc) three times. The organic phase of EtOAc was after complete evaporation of solvent the ethyl acetate fraction was denominated EAP.

HPLC/UV analysis of phenolic compounds from extract propolis

Chromatographic analysis of ethyl acetate extract of propolis (EAP) was performed on a high performance liquid chromatography (Agilent 1100). Separation was done on an Agilent poroshell 120EC column (100 mm × 2.1 mm, 2.7 μm), using mobile phases: water/TFA/formic acid (99:0.25:0.75) (A) and acetonitrile (B). The elution was carried out at a flow rate of 0.6 ml/min with a 10 μl

aliquot and at a temperature of 55 °C. Using a gradient method as following (t/min,% B): (0, 0), (1, 10), (2, 12.5), (3, 15), (9, 80), (10, 100), (11, 100), (14, 0) with post 5 min. Chromatograms were recorded at 270 and 320 nm.

The sample was prepared by diluting EAP with methanol 1:100 (v/v). The propolis components were identified by comparing their retention times and their UV spectra with different commercially phenolic standards (*trans*-cinnamic, gallic acid, benzoic acid, ferulic acid, *m*-coumaric acid, caffeic acid, rosmarinic acid, and ellagic acid), flavonoids (catechin, hesperidin, thymol, galangin, tectochrysin, pinocembrin, acacetin, rutin, chrysin, apigenin, kaempferol, and quercetin) and others compounds (ascorbic acid, menthol). The standards were dissolved in methanol to give stock solutions at 1 mg/ml. The quantification of constituents was determined using standard curves expressed in mg per 1 g of crude propolis.

Animals and accommodation conditions

Thirty male Wistar rats (100–150 g) (provided by the Pasteur Institute in Algeria) were used in the present experiment. The animals were housed in metallic cages and subjected to an adaptation period of two weeks at an ambient temperature and a photoperiod of 12/12 h. The rats have a free access to the food and water *ad libitum*. The protocol was consistent with the guidelines of the National Institutes of Health (NIH). 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).

Animal treatment protocol

After two weeks of acclimatization, the rats were divided into five groups, then they were treated daily by gastric gavage for 15 days as follows: the animals of group G1 and G2 received 1 ml of physiological saline, the rats of group G3 and G4 received 1 ml of 100 and 250 mg/kg of EAP respectively, and the group G5 received 50 mg/kg quercetin.

At day 15, G2–G5 rats received three intravenous injections (*i.v.*) of epirubicin at 48 h intervals for one week to reach a cumulative dose of 9 mg/kg (Dobbs et al., 2003). All animals were observed and body weights were recorded daily.

Twenty-four hours after the last epirubicin injection, the animals are fasted for 12 h before blood collection. In order to avoid any risk of changing the biochemical parameters by administering a general anesthesia, the animals were kept under light anesthesia chloroform just before the collection of blood samples by cardiac puncture protocol on either dry or heparinized tubes. Plasma and serum samples were obtained by centrifugation at 112 × *g* for 20 min and the remaining erythrocytes were washed with physiological saline three times, lysed by addition of ice-cold distilled water, and incubated for 15 min on ice. Cell debris was removed by centrifugation at 5000 t/min for 5 min, and the lysate was recovered to assay for antioxidant enzymes in erythrocytes. The peritoneal fluid was collected in sterile phosphate buffered saline (PBS). The liver was immediately removed, washed with saline and divided into two portions. One portion was stored in 10% formaldehyde at room temperature.

The hepatic tissue homogenate was prepared according to the method of Tang et al. (2012) with some modifications. The second part of liver pieces was homogenized in an ice-cold buffer (250 mM sucrose, 10 mM Tris–HCl, 0.5 mM EDTA and 0.5% bovin serum albumin, pH 7.4). The supernatant was recovered by centrifugation at 112 × *g* for 15 min at 4 °C. Washing and centrifugation were repeated twice, the mitochondria pellet was re-suspended in

PBS buffer (0.1 ml of EDTA 10 mM, 0.1 ml of sodium azide 35%, pH 7.4), for various assays.

Assessment of hepatic function

The activity of aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) were determined with kits from bio-Merieux (bioMerieux SA France). Total bilirubin in plasma was measured using the method of Pearlman and Lee (1974). The activity of 5' nucleotidase (5'NUC, EC 3.1.3.5) and alkaline phosphatase (ALP, EC 3.1.3.1) were determined with kits from BioVision (BioVision, USA). The activity of gamma-glutamyl transferase (γ -GT; EC 2.3.2.2) was determined with kits from GenWay Biotech (GenWay Biotech, USA).

Estimation of oxidative stress markers

The lipid peroxidation of the liver homogenate is measured by the method of Yagi et al. (1976), by measuring thiobarbituric acid reacting through spectrophotometric at 535 nm using 1,1,3,3-tetramethoxypropane as a standard. The results were expressed as equivalents of malondialdehyde (MDA) in tissue homogenate in nmol/g tissue.

The enzymatic activity of catalase in tissue homogenate from liver was determined by the method of Luck (1963) and Aebi (1974) which depends on evaluation of decomposition rate of hydrogen peroxide by a spectrophotometric at 240 nm. The results were expressed as unit/mg tissue. The enzymatic activity of superoxide dismutase was measured according to the method of Elstner et al. (1983), which is based on the chemical reaction that generates the superoxide ion ($O_2^{\cdot-}$) from molecular oxygen in the presence of EDTA, $MnCl_2$ and mercaptoethanol. The enzymatic activity of glutathione peroxidase (GPx) of liver was performed according to the method described by Paglia and Valentin (1967). The principle of the test is based on the conversion of $NADPH + H^+$ into $NADP^+$ as a result of a series of reactions. Results were given as U/mg tissue. The determination of the level of thiols in the liver was carried out according to the method of Riddles et al. (1979) using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB), which is a chemical used to quantify the number or concentration of thiol groups in a sample. It was developed by George L. Ellman.

Assessment of inflammation markers

Prostaglandin E2 (PGE2) levels at the peritoneal fluid were quantified using the Prostaglandin E2 ELISA kit (Abcam Discover More, UK) according to the manufacturer's instructions. The level of TNF- α in the liver was evaluated using the ELISA kit (Thermo Fisher Scientific, USA).

Histological studies

Liver portions stored in 10% formaldehyde were embedded in paraffin, and cut with a rotating microtome (Leica, Germany) at 5 μ m thickness, spread on microscope slides and stained with hematoxylin and eosin, and the slides were observed by light microscopy (Drury and Wallington, 1967).

Statistics

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

Results

EAP chemical composition

The HPLC chromatograms obtained at 270 and 320 nm for the propolis extract (EAP) from Tizirt are shown in Fig. 1A and B, respectively. We were able to identify flavonoids: catechin (1), quercetin (2), rutin (3), acacetin (4), chlorogenic acid (5), apigenin (6), pinocembrin (7), chrysin (8), kaempferol (9), thymol (10); phenolic acids: ferulic acid (11), gallic acid (12), caffeic acid (13), ellagic acid (14), *m*-coumaric (15), rosmarinic acid (16), *trans*-cinnamic (17); and ascorbic acid (18). We have also been able to observe benzoic acid, galangin and tectochrysin in our propolis extract. The phenolic acid and flavonoid content of tizirt propolis is shown in Table 1. The main phenolic acid found is caffeic acid (0.85 mg/g at 2.38 min), while pinocembrin is the main flavonoid (0.82 mg/g at 6.73 min). Rutin and apigenin was found in low levels.

Rat body weight evolution and clinical observations

In this study, body weight was measured weekly (Table 2). No animal mortality was observed during the administration of the epirubicin dose. A very significant decrease in the weight of the rats that received only injections of epirubicin (G1) and those treated with quercetin (G5) was noted. Normal weight values were observed in rats treated with 100 and 250 mg/kg of EAP (G3 and G4).

Clinical signs in rats in each group were followed continuously after injection of epirubicin, to detect the effects of epirubicin and the protective action of ethyl acetate propolis extract EAP.

The rats in G1 (control) and G4 (rats treated with 250 mg/kg EAP) did not show any change in the behavior indicating that propolis extract inhibited epirubicin hepatotoxicity. However, there are serious changes in the physical activity and behavior of rats in G2 (which received only epirubicin) such as: drowsiness, hypoactivity, isolation, straightening of hair, tachycardia, difficulty breathing and loss of balance. Animals of G3 and G5 show only tachycardia and difficulty breathing.

Hepatic function

Several hepatic parameters were measured to evaluate the effect of Tizirt propolis on the treatment of epirubicin-induced hepatotoxicity. Table 3 illustrates these hepatic parameters. Markers of liver function, AST, ALT, Bil T, ALP, 5'NUC and γ GT were significantly elevated in the epirubicin-treated group compared with control. On the other hand, the group of rats treated with 250 mg/kg of EAP (G4) showed significant improvement in the hepatic status compared to G2. However, giving to rats (G5) one of the pure compounds orally (quercetin) had no significant hepatic function and antioxidant effect.

Oxidative stress status

Table 4 demonstrates the level of parameters and enzymes related to oxidative stress status in the liver tissues of the different groups. These results indicate that epirubicin (G2) decreased antioxidant enzyme activities (SOD, CAT and GPx) and thiol-containing compounds in the liver. In addition, the level of lipid peroxidation in the liver was significantly increased (5.84 nmol/l) in the group that received only epirubicin (G2) compared with the control group. G4 did not show significant changes in oxidative parameters comparing to the control group. The rats in G3 (treated with 100 mg/kg EAP) and G5 (treated with 50 mg/kg quercetin) showed a reduction in hepatic antioxidant status parameters (SOD, CAT, GPx, and thiols) and increased MDA rate.

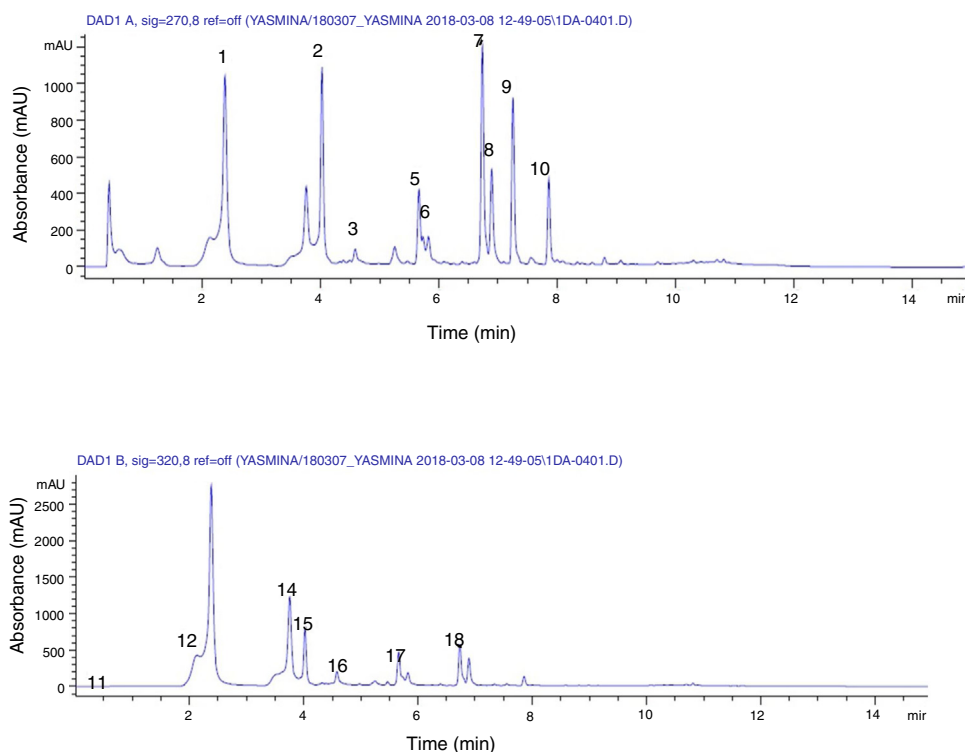


Fig. 1. Chromatograms by HPLC/UV analysis of EAP at a wavelength of 270 (A) and 320 nm (B): 1, catechin; 2, quercetin; 3, rutin; 4, acacetin; 5, chlorogenic acid; 6, apigenin; 7, pinocembrin; 8, chrysin; 9, kaempferol; 10, thymol; 11, ferulic acid; 12, gallic acid; 13, caffeic acid; 14, ellagic acid; 15, *m*-coumaric acid; 16, rosmarinic acid; 17, *trans*-cinnamic acid; 18, ascorbic acid.

Table 1
Composition of propolis extracts from Tizgirt by HPLC/UV (mg/g).

Peak number	Compound	Retention time (min)	Amount (mg/g EAP)
1	Catechin	2.38	0.78
2	Quercetin	5.73	0.76
3	Rutin	4.58	0.01
4	Acacetin	5.25	0.17
5	Chlorogenic acid	5.66	0.2
6	Apigenin	6.08	0.01
7	Pinocembrin	6.73	0.82
8	Chrysin	7.25	0.55
9	Kaempferol	6.14	0.31
10	Thymol	7.85	0.21
11	Ferulic acid	0.63	0.05
12	Gallic acid	2.13	0.29
13	Caffeic acid	2.38	0.85
14	Ellagic acid	3.75	0.6
15	<i>m</i> -Coumaric acid	4.02	0.41
16	Rosmarinic acid	2.13	0.18
17	<i>trans</i> -Cinnamic acid	5.66	0.34
18	Ascorbic acid	6.73	0.38

Inflammation markers

We measured the concentration of PGE2 in peritoneal exudates of rats treated or not treated with EAP or quercetin and those suffering from toxicity caused by epirubicin. Administration of EAP at concentrations of 100 and 250 mg/kg (G3 and G4) respectively decreased PGE2 production by 37 and 69% (Fig. 2) compared to the control group. In G2 group, the level of PGE2 (2066.66 pg/ml) increased significantly in relation to G1 (750.3 pg/ml). In the same way, in G2 group (Table 4), the level of TNF- α in the liver (164 pg/g) increased significantly in relation to G1 (63.66 pg/g). This increase was reversed by pretreatment with 250 mg/kg of EAP (G4) which showed a significantly lower level of this parameter compared to the group G2 (the rats that were injected with epirubicin).

Histopathological studies

Histological studies of the liver of rats treated with propolis or not before induction of toxicity by epirubicin are illustrated in Fig. 3.

The rats in the control group (G1) show a normal histological structure of the hepatic parenchyma, with sinusoids and a centrilobular vein that are essentially normal (Fig. 3A). However, in the

Table 2
The body weight measured every three days of Wistar rats exposed to epirubicin (EPI).

Groups	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 17	Day 19
G1	124 \pm 1.54	129 \pm 1.23	133 \pm 1.84	136 \pm 0.76	143 \pm 1.63	148 \pm 0.94	153 \pm 1.45	159 \pm 1.52
G2	123 \pm 1.76 ^a	132 \pm 1.82 ^a	137 \pm 2.85	145 \pm 1.54 ^a	153 \pm 1.95	156 \pm 3.01 ^b	144 \pm 1.80	124 \pm 2.69 ^b
G3	117 \pm 0.67	126 \pm 1.34	131 \pm 1.17	142 \pm 1.02	156 \pm 1.20	160 \pm 1.36	163 \pm 1.39 ^a	163 \pm 1.32
G4	119 \pm 2.61 ^b	130 \pm 1.57	141 \pm 2.34	148 \pm 3.43 ^b	160 \pm 1.10	163 \pm 1.18 ^a	167 \pm 1.00	169 \pm 1.05
G5	125 \pm 1.22	134 \pm 1.93	143 \pm 1.93	151 \pm 1.43	154 \pm 2.53	159 \pm 1.77 ^a	152 \pm 1.79	146 \pm 1.61

The values are expressed as mean \pm SD ($n = 5$).

^a Significant difference from the control group ($p < 0.05$).

^b Significant difference from the EPI hepatotoxicity group ($p < 0.05$).

Table 3
Effect of ethyl acetate extract of propolis (EAP) from Tizgirt on hepatic function parameter values of Wistar rats exposed to epirubicin (EPI).

Parameter	G1	G2	G3	G4	G5
ALAT (U/l)	40 ± 3.22	122.33 ± 6.28	50.66 ± 3.14	39 ± 3.57	61.66 ± 4.03
ASAT (U/l)	38.33 ± 1.03	130 ± 10.31 ^b	53.33 ± 2.87	35 ± 4.09	63.66 ± 9.39 ^a
Bil T (mg/l)	05 ± 0.89	58 ± 6.75 ^a	08.33 ± 0.51	06 ± 0.89 ^a	15.33 ± 2.58
5'Nucléotidase (U/l)	2.5 ± 0.89	12.33 ± 2.25 ^a	04.50 ± 0.44	03 ± 0.44 ^b	7.33 ± 1.12 ^a
PAL (U/l)	49 ± 3.57	288.33 ± 17.91 ^b	73.33 ± 10.81 ^b	50.33 ± 5.95 ^a	114.66 ± 13.09 ^a
γGT (U/l)	27 ± 3.57	106 ± 16.17	40.33 ± 1.86	35 ± 2.68	55.33 ± 4.58

The values are expressed as mean ± SD (*n* = 5).

^a Significant difference from the control group (*p* < 0.05).

^b Significant difference from the EPI hepatotoxicity group (*p* < 0.05).

Table 4
Effect of ethyl acetate extract of propolis (EAP) from Tizgirt on malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), thiol and tumor necrosis factor alpha (TNF-α) in the liver of Wistar rats exposed to epirubicin (EPI).

Parameter	G1	G2	G3	G4	G5
MDA liver (nmol/ml)	0.96 ± 0.11	5.84 ± 0.99	2.20 ± 0.13 ^b	0.89 ± 0.06	4.18 ± 0.11 ^a
SOD liver (U/cg Hb)	48.80 ± 2.30	15.94 ± 2.33 ^a	23.70 ± 1.93	50.74 ± 1.87 ^b	20.76 ± 1.70
CAT liver (U/mg Hb)	90.38 ± 3.49	29.72 ± 1.9 ^b	53.81 ± 1.87 ^a	96.69 ± 1.6 ^b	38.05 ± 1.24
GSH-Px liver(U/g Hb)	76.08 ± 2.42	12.78 ± 1.4 ^b	46.04 ± 1.43 ^a	86.51 ± 2.11 ^a	36.26 ± 2.43
Thiol liver (mmol/g)	1.57 ± 0.16	0.25 ± 0.02 ^b	1.27 ± 0.06	1.89 ± 0.07 ^a	0.84 ± 0.04
TNF-α (ng/g)	63.66 ± 3.72	164 ± 7.79 ^a	104.3 ± 5.81 ^a	68.33 ± 2.73	127.66 ± 5.39

The values are expressed as mean ± SD (*n* = 5).

^a Significant difference from the control group (*p* < 0.05).

^b Significant difference from the EPI hepatotoxicity group (*p* < 0.05).

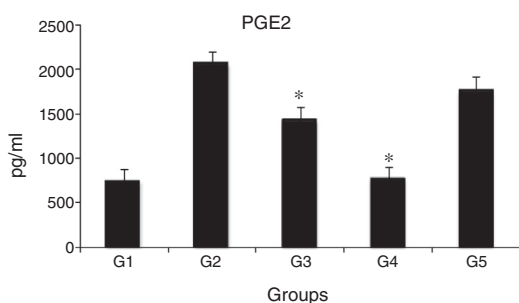


Fig. 2. Effects of treatment with EAP on Prostaglandin E2 in peritoneal exudates of rats. Bars represent the mean ± S.E.M. of five repetitions. *Significant inhibition in relation to the control group, *p* < 0.05.

G2 group (rats treated with epirubicin only), the biopsy showed major damage in the liver, very severe cell necrosis with hepatic degeneration, dilation of sinusoids with a very dense lymphocyte inflammatory infiltrate, associated dilation and congestion of central veins filled with debris (Fig. 3B and C). The treatment of rats with 100 mg/kg of propolis shows a less severe focal necrosis localized around congestion of central veins (Fig. 3D).

The histological study of the liver (Fig. 3E) of rats received 250 mg/kg of EAP (G4) revealed architecture similar to that observed in the control group (G1). Fig. 3F shows that quercetin (G5) was unable to maintain a normal liver structure, where an injured parenchyma or degenerate hepatocytes responsible for focal necrosis, dilation of sinusoids, congestion of centrilobular veins surrounded by lymphocytic inflammatory infiltrate.

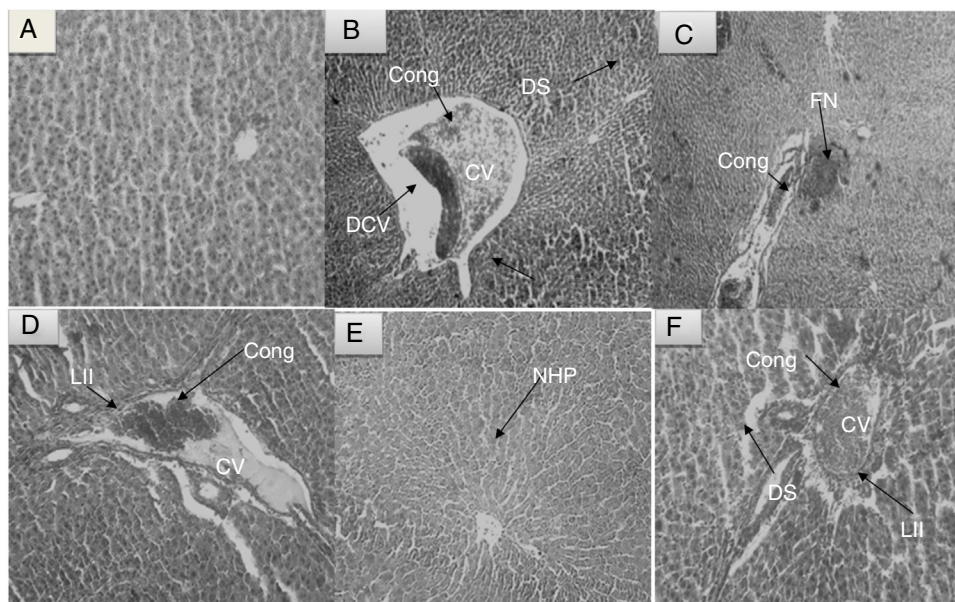


Fig. 3. Histological cuts of rat's liver of groups: G1 (A); G2 (B, C); G3 (D); G4 (E) and G5 (F). Abbreviations: CV, central vein; Cong, congestion; FN, focal necrosis; LII, lymphocytic inflammatory infiltrate; NHP, normal hepatic parenchyma; DS, dilation of sinusoids.

Discussion

The anthracyclines are chemotherapy drugs. They are used alone or in combination with many cancers' treatment. The doxorubicin and the epirubicin which belong to anthracyclines family are considered as the most toxic chemotherapy drugs for the liver (Weenen, 1984; Le Bot et al., 1988).

Our results revealed a very significant increase in the enzymatic activity of ALT, AST, ALP and γ -GT in the rats of the group G2 that received an injection of epirubicin (9 mg/kg). The increase in hepatic enzyme activity in the blood may be due to tissue damage in the liver, changes in membrane permeability, increased synthesis or decreased aminotransferase catabolism (Farag et al., 2010).

In addition, AST, ALT, and ALP are the primary enzymes used to assess the status of liver function (Wallace and Meyer, 2008). These are the most sensitive biomarkers, directly involved in the extent of cellular damage and toxicity, as they are cytoplasmic and released into the circulation after cell injury (Soudani et al., 2011).

Decrease in antioxidant activity of the tested polyphenols and flavonoids purs (curcumin, quercetin) *in vivo* could be explained by the poor absorption of these compounds from the gastric intestinal tract (Rasoanaivo et al., 2011). It has been shown that crude plant extracts sometimes have greater biological activity than isolated molecules at an equivalent dose (Rasoanaivo et al., 2011).

Thus, cell necrosis, destruction of the hepatic parenchyma, or increased membrane permeability of hepatocytes may cause these enzymes to flow into the bloodstream and thereby increase their serum levels (Adeneye et al., 2006; Jodynis-Liebert et al., 2010). On the other hand, ALP is a ubiquitous enzyme, especially in the liver, bile ducts, kidneys, bones and placenta, where it is found in high concentration. Their increase in circulation usually indicates intrahepatic biliary obstruction, primary biliary cirrhosis, or disruption of hepatic architecture (Epstein et al., 1986; Sharma et al., 2013).

Our study has shown that epirubicin causes severe liver damage agreeing with the results obtained by Iwakiri et al. (2007), Kebieche et al. (2009), Prado et al. (2010) and Hwan Shin et al. (2014) who also demonstrated the hepatotoxic effect of epirubicin.

The results also showed that the administration of Tizgirt propolis to rats exposed to epirubicin significantly reduced the enzymatic activity of ALT, AST and ALP. This reveals the propolis ability to ameliorate chemotherapy-induced liver injury (epirubicin) indicating that propolis is effective at preventing liver damage caused by epirubicin.

According to Boufadi et al. (2017), the extract propolis from Tizgirt has anti-oxidant and anti-radical effects. These authors confirmed that propolis can protect the body from damage caused by oxidative stress.

In the present study, epirubicin increased markers of oxidative stress in the liver (lipid peroxidation) while decreasing the level of endogenous antioxidative agents such as GSH, catalase and superoxide dismutase activity. Propolis from Tizgirt has successfully reversed this oxidative stress in liver tissues. This antioxidant effect is certainly due to the presence of phenolic acids and flavonoids in propolis, as well as their ability to trap free radicals. The oxidative stress induced by anthracyclines has been incriminated in the initiation of its multi-organ toxicity, because it undergoes a bio-reducing activation by redox cycle *via* its unique chemical structure favoring the formation of free radicals (Ravi and Das, 2004). Another previous study (Koka et al., 2010) suggested that the anticancer efficacy of epirubicin is related to its pro-oxidative properties. This fact can explain that the administration of antioxidants (such as propolis) does not affect the chemotherapeutic effectiveness of epirubicin (Koka et al., 2010). The protection provided by fruits and vegetables against degenerative diseases (cancer and cardiovascular diseases), has been attributed to various antioxidants contained in these foods, including flavonoids. Flavonoids, such as quercetin and

kaempferol, cause damage to nuclear DNA and lipid peroxidation in the presence of transition metals (Rahal et al., 2014).

The lipid peroxidation of biological membranes may cause a loss of membrane fluidity as well as an increase in membrane permeability and an impairment of the function of the receptors (Nehru and Anand, 2005). The increase in lipid peroxidation is largely due to inhibition of the superoxide dismutase (SOD) and the catalase (Newairy et al., 2009).

It is also known that propolis generally acts by different mechanisms (Araujo et al., 2012) as inhibitor of cyclooxygenase, prostaglandins PGE2 and pro-inflammatory cytokines (Mirzoeva and Calder, 1996; Hu et al., 2005). It has been demonstrated that propolis extracts have high effect on inflammatory cell activity (cell migration, macrophage activation) (Bueno-Silva et al., 2013). Propolis from Tizgirt inhibited PGE2 and TNF- α production during epirubicin-induced toxicity. This may explain the anti-inflammatory effects of propolis extract, with the inhibition of neutrophil mobilization in the peritoneal cavity.

The source of propolis from Tizgirt is buds of poplar, eucalyptus, birch, willow, chestnut, and fruit trees, which are rich in flavonoids, phenolic acids and their esters. This propolis is dark brown and has been harvested in a wetland and a Mediterranean climate (Boufadi et al., 2014).

Conclusion

In our study, we have demonstrated that propolis extracts can prevent the toxic effects of epirubicin resulting from the oxidative damages induced by the chemotherapy by this agent. Our results have showed that propolis effects are not limited to the prevention of oxidative stress but also it can reverse the oxidative damages resulted from epirubicin. These findings make propolis a promise agent to treat and prevent the most important side effect of anthracyclines.

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Authors contribution

This article is part of SC's doctoral thesis work. SC contributed to the collection and extraction of propolis, as well as in experimental conception. MYB and PVA performed the HPLC analysis of the propolis extract. SK and AR contributed to the liver function analysis. MYB and JS assisted in oxidative stress analyzes and biomarkers of inflammation. HAC and SC conducted the histopathological study. MYB and SC were the coordinator of the project (conception et rédaction du manuscrit).

Conflicts of interest

The authors declare no conflicts of interest.

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References

- Adeneye, A.A., Ajagbonna, O.P., Adeleke, T.I., Bello, S.O., 2006. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. *J. Ethnopharmacol.* 105, 374–379.
- Aebi, H., 1974. Catalase. In: Bergmeyer, H.U. (Ed.), *Methods of Enzymatic Analysis*. Verlag Chemie/Academic Press Inc, Weinheim/NewYork, pp. 673–680.

- Araujo, M.A.R., Libério, S.A., Guerra, R.N.M., Ribeiro, G.M.N.S., Nascimento, F.R.F., 2012. Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis. *Rev. Bra. Farmacogn.* 22, 208–219.
- Boufadi, Y.M., Soubhye, J., Riaz, A., Rousseau, A., Vanhaeverbeek, M., Nève, J., Boudjeltia, K.Z., Van Antwerpen, P., 2014. Characterization and antioxidant properties of six Algerian propolis extracts: ethyl acetate extracts inhibit myeloperoxidase activity. *Int. J. Mol. Sci.* 15, 2327–2345.
- Boufadi, Y.M., Soubhye, J., egrave, N., ve, J., Van Antwerpen, P., Riaz, A., 2016. Antimicrobial effects of six Algerian propolis extracts. *Int J Food Sci Technol.* 51, 2613–2620. <http://dx.doi.org/10.1111/ijfs.13247>.
- Boufadi, Y.M.V., Antwerpen, P., Chikh Alard, I., Nève, J., Djennas, N., Riaz, A., Soubhye, J., 2017. Antioxidant effects and bioavailability evaluation of propolis extract and its content of pure polyphenols. *J. Food Biochem.* 10.1111/jfbc.12434.
- Bueno-Silva, B., Alencar, S.M., Koo, H., Ikegaki, M., Silva, G.V., Napimoga, M.H., Rosalen, P.L., 2013. Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. *J. Agric. Food Chem.* 61, 4546–4550.
- Chang, W.T., Cheng, H.L., Hsieh, B.S., Chiu, C.C., Lee, K.T., Chang, K.L., 2014. Progesterone increases apoptosis and inversely decreases autophagy in human hepatoma HA22T/VGH cells treated with epirubicin. *Sci. World J.* 10.1155/2014/567148.
- Dobbs, N.A., Twelves, C.J., Gregory, W., Cruikshanka, C., Richards, M.A., Rubens, R.D., 2003. Epirubicin in patients with liver dysfunction: development and evaluation of a novel dose modification scheme. *Eur. J. Cancer* 39, 580–586.
- Drury, R.A.B., Wallington, E.A., 1967. *Carleton's Histological Technique*, vol. 151., 4th ed. Oxford University Press, New York, pp. 242–245.
- Du, Y., Lou, H., 2008. Catechin and proanthocyanidin B4 from grape seeds prevent doxorubicin-induced toxicity in cardiomyocytes. *Eur. J. Pharmacol.* 591, 96–101.
- El-Guendouz, S., Al-Waili, N., Azza, S., Elamine, Y., Zizi, S., Al-Waili, T., Al-Waili, A., Lyoussi, B., 2017. Antioxidant and diuretic activity of co-administration of *Capriaris spinosa* honey and propolis in comparison to furosemide. *Asian Pac. J. Trop. Med.* 10, 974–980.
- Elstner, E.F., Youngman, R.J., Obwald, W., 1983. Superoxide dismutase. In: Bergmeyer, H.U. (Ed.), *Methods of Enzymatic Analysis*, 3rd ed, pp. 293–302.
- Epstein, E., Kiechle, F., Artiss, V.L., Zak, J.D.B., 1986. The clinical use of alkaline phosphatase enzymes. *Clin. Lab. Med.* 6, 491–505.
- Farag, A.G.A., Elhalwagy, M.E.A., Farid, H.E.A., 2010. Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats. *Pesticide biochem & physio.* 97, 267–274.
- Ganzina, F., 1983. 4-Epi-doxorubicin, a new analogue of doxorubicin: a preliminary overview of preclinical and clinical data. *Canc. Treat. Rev.* 10, 1–22.
- Georgiev, V., Ananga, A., Tsolova, V., 2014. Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients* 6, 391–415.
- Germain, E., Bonnet, P., Aubourg, L., Grangeponde, M.C., Chajes, V., Bounoux, P., 2003. Anthracycline-induced cardiotoxicity is not increased by dietary omega-3 fatty acids. *Pharm. Res.* 47, 111–117.
- Harfouch, R.M., Mohammad, R., Suliman, H., 2016. Antibacterial activity of Syrian propolis extract against several strains of bacteria in vitro. *World J. Pharm. Pharmaceut. Sci.* 6, 42–46.
- Hu, F.L., Hepburn, H.R., Li, Y.H., Chen, M., Radloff, S.E., Daya, S., 2005. Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. *J. Ethnopharmacol.* 100, 276–283.
- Hwan Shin, D., Seong Hyeok, P., Sung, W., Chun-Woong, P., Kun, H., Youn, B., 2014. Hepatic uptake of epirubicin by isolated rat hepatocytes and its biliary excretion after intravenous infusion in rats. *Arch. Pharm. Res.* 37, 1599–1606.
- Iwakiri, T., Okumura, M., Muneaki, H., Yuki, K., Ichihara, E., Yohei, K., Kazuhiko, A., 2007. Inhibition of carrier-mediated uptake of epirubicin reduces cytotoxicity in primary culture of rat hepatocytes. *J. Appl. Toxicol.* 28, 329–336.
- Jain, M., Barthwal, S.K., Barthwal, R., Govil, G., 2005. Restrained molecular dynamics studies on complex of adriamycin with DNA hexamer sequence d CGATCG. *Arch. Biochem. Biophys.* 439, 12–24.
- Jodynis-Liebert, J., Nowicki, M., Murias, M., Adamska, T., Ewertowska, M., Kujawska, M., Piotrowska, H., Konwerska, A., Ostalska-Nowicka, D., Pernak, J., 2010. Cytotoxicity, acute and subchronic toxicity of ionic liquid, didecyl dimethyl ammonium saccharinate, in rats. *Regul. Toxicol. Pharmacol.* 57, 266–273.
- Judson, I., Verweij, J., Gelderblom, H., Hartmann, J.T., Schöffski, P., Blay, J.Y., Kerst, J.M., Sufliarsky, J., Whelan, J., Hohenberger, P., Krarup-Hansen, A., Alcindor, T., Marreaud, S., Litière, S., Hermans, C., Fisher, C., Hogendoorn, P.C., dei Tos, A.P., van der Graaf, W.T., 2014. European Organisation and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. *Lancet Oncol.* 15, 415–423.
- Kebieche, M., Lakroun, Z., Lahouel, M., Bouayed, J., Meraihi, Z., Soulimani, R., 2009. Evaluation of epirubicin-induced acute oxidative stress toxicity in rat liver cells and mitochondria, and the prevention of toxicity through quercetin administration. *Exp. Toxicol. Pathol.* 61, 161–167.
- Koka, P.S., Mondal, D., Schultz, M., Abdel-Mageed, A.B., Agrawal, K.C., 2010. Studies on molecular mechanisms of growth inhibitory effects of thymoquinone against prostate cancer cells: role of reactive oxygen species. *Exp. Biol. Med.* 235, 751–760.
- Kurek-Górecka, A., Rzepecka-Stojko, M., Górecki, J., Stojko, M., Sosada, G., Świerczek-Zięba, G., 2014. Structure and antioxidant activity of polyphenols derived from propolis. *Molecules* 19, 78–101.
- Le Bot, M.A., Bégué, J.M., Kernaleguen, D., Robert, J., Ratanasavanh, D., Airiau, J., Riché, C., Guillouzo, A., 1988. Different cytotoxicity and metabolism of doxorubicin, daunorubicin, epirubicin, esorubicin and idarubicin in cultured human and rat hepatocytes. *Biochem. Pharmacol.* 37, 3877–3887.
- Luck, H., 1963. In: Bergmeyer, H.V. (Ed.), *Methods of Enzymatic Analysis*. Academic Press, New York, pp. 885–894.
- Machado, B., Pulcino, T.N., Silva, A.L., Tadeu, D., Melo, R.G.S., Mendonça, I.G., 2016. Propolis as an alternative in prevention and control of dental cavity. *J. Apitherapy* 1, 47–50.
- Mehmetn Yasar, M.D., Yasemin Savranlar, M.D., Hatice Karaman, M.D., Mustafa Sagit, M.D., Sibel Silici, P.D., Ibrahim Ozcan, M.D., 2016. Effects of propolis in an experimental rat model of allergic rhinitis. *American J. Otolaryngology.* 37, 287–293.
- Mirzoeva, O.K., Calder, P.C., 1996. The effect of propolis and its components on eicosanoid production during the inflammatory response Prostaglandins Leukot. Essent. Fatty Acids 55, 441–449.
- Nehru, B., Anand, P., 2005. Oxidative damage following chronic aluminium exposure in adult and pup rat brains. *J. Trace Elem. Med. Biol.* 19, 203–208.
- Newairy, A.S.A., Salama, A.F., Hussien, H.M., Yousef, M.I., 2009. Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. *Food Chem. Toxicol.* 47, 1093–1098.
- Orsatti, C.L., Sforzini, J.M., 2011. Propolis immunomodulatory activity on TLR-2 and TLR-4 expression by chronically stressed mice. *Nat. Prod. Res.* 1, 1–8.
- Paglia, D.E., Valentin, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70, 158–169.
- Prado, C., Isac, S.F., Vickie, E., Baracos, R., Bies, J., Cargar, M., Reiman, T., John, R., Mackey, K., Vijaya, L., Damaraju, B., 2010. An exploratory study of body composition as a determinant of epirubicin pharmacokinetics and toxicity. *Cancer Chemother. Pharmacol.* 67, 93–101.
- Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., Dhama, K., 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed. Res. Int.* 10.1155/2014/761264.
- Rasoanaivo, P., Wright, C., Willcox, M., Gilbert, B., 2011. Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria J.* 10, <http://dx.doi.org/10.1186/1475-2875-10-S1-54>.
- Ravi, D., Das, K.C., 2004. Redox-cycling of anthracyclines by thioredoxin system: increased superoxide generation and DNA damage. *Cancer Chemother. Pharmacol.* 54, 449–458.
- Riddles, P.W., Blakeley, R.L., Zerner, B., 1979. Ellman's reagent: 5,50-dithiobis (2-nitrobenzoic acid) – a reexamination. *Anal. Biochem.* 94, 75–81.
- Segueni, N., Zellaoui, A., Moussaoui, F., Lahouel, M., Rhouati, S., 2016. Flavonoids from Algerian propolis. *Arabian J. Chem.* 9, 425–428.
- Sharma, U., Pal, D., Prasad, R., 2013. Alkaline phosphatase: an overview. *Indian J. Clin. Biochem.* 29, 269–278.
- Singal, P.K., Iliskovic, N., 1998. Doxorubicin-induced cardiomyopathy. *N Engl. J. Med.* 339, 900–905.
- Soltani, E.K., Cerezuela, R., Charef, N., Mezaache-Aichour, S., Esteban, M.A., Zerroug, M.M., 2017. Algerian propolis extracts: chemical composition, bactericidal activity and in vitro effects on gilthead seabream innate immune responses. *Fish Shellfish Immunol.* 62, 57–67.
- Soudani, N., Ben Amara, I., Sefi, M., Boudawara, T., Zeghal, N., 2011. Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats. *Exp. Toxicol. Pathol.* 63, 541–548.
- Tang, D., Kang, R., Coyne, C.B., Zeh, H.J., Lotze, M.T., 2012. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunological reviews.* 249, 158–175.
- Twelves, C.J., Dobbs, N.A., Michael, V., 1992. Clinical pharmacokinetics of epirubicin: the importance of liver biochemistry tests. *Br. J. Cancer* 66, 765–769.
- Vincent, D.T., Ibrahim, Y.F., Espey, M.G., Suzuki, Y.J., 2013. The role of antioxidants in the era of cardio-oncology. *Canc. Chem. Pharmacol.* 72, 1157–1168.
- Wallace, A.D., Meyer, S.A., 2008. Hepatotoxicity. In: Smart, R.C., Hodgson, E. (Eds.), *Molecular and Biochemical Toxicology*, 4th ed. John Wiley and Sons, Hoboken, NJ, pp. 671–692.
- Weenen, H., Van Maanen, J.M.S., De Planque, M.M., Mcvie, J., Pinedo, H., 1984. Metabolism of 4'-modified analogs of doxorubicin. Unique glucuronidation pathway for 4'-epidoxorubicin. *Eur. J. Cancer Clin. Oncol.* 20, 919–926.
- Weenen, H., Van Maanen, J.M.S., De Planque, M.M., Mcvie, J., Pinedo, H., 1984. Metabolism of 4'-modified analogs of doxorubicin. Unique glucuronidation pathway for 4'-epidoxorubicin. *Eur. J. Cancer Clin. Oncol.* 20, 919–926.
- Wu, J., Xue, X., Zhang, B., Jiang, W., Cao, H., Wang, R., Sun, D., Guo, R., 2015. The protective effects of paeonol against epirubicin-induced hepatotoxicity in 4T1-tumor bearing mice via inhibition of the PI3K/Akt/NF-κB pathway. *Chem. Biol. Interact.* 244, 1–8.
- Yagi, K., 1976. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.* 15, 212–216.
- Zabaiou, N., Fouache, A., Trousson, A., Baron, S., Zellaoui, A., Lahouel, M., Lobaccaro, J.M.A., 2017. Biological properties of propolis extracts: something new from an ancient product. *Chem. Phys. Lipids* 207, 214–222.