

Polyphenolic fraction of Algerian propolis reverses doxorubicin induced oxidative stress in liver cells and mitochondria

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Abstract: Doxorubicin (DOX) is a potent anticancer drug; its use has been limited by its hepatotoxicity, which is due to free radicals generation. Propolis, a honeybee product very rich in flavonoids and therapeutic possibilities, has gained popularity as a food and alternative medicine. The present study treats DOX pro-oxidant effect on hepatic cells and mitochondrial functions. The prophylactic effect of propolis ethanolic extract (EEP) against DOX induced mitochondrial oxidative stress has also been investigated. We find that doxorubicin at the amount of 10mg/Kg altered liver mitochondrial functions as attested by the overproduction of superoxide anion (O_2^-) by mitochondrial respiratory chain complex III. The hepatic tissue from DOX treated rats showed also a marked depletion in reduced GSH contents and an inhibition in (Mn-SOD), (Cu-Zn SOD) and (CAT) enzymatic activities. DOX increase cytosolic and mitochondrial lipid peroxidation as attested by the MDA content. These results are reversed after one month *per os* pretreatment by EEP at the amount of 100mg/kg. Propolis polyphenolic fraction protects liver tissue from oxidative stress by protecting mitochondrial functions and reinforcement of enzymatic and non enzymatic antioxidants.

Keywords: Doxorubicin; hepatotoxicity; oxidative stress; Algerian propolis; hepatoprotective effect.

INTRODUCTION

Doxorubicin (DOX) is a chemo-therapeutic agent extensively used for the treatment of many cancers. Cancer therapy with DOX results in cardiotoxicity but also in hepatotoxicity (Weiss, 1992; Takemura and Fujiwara, 2007). To identify DOX toxicity mechanisms and treatments that reduce this adverse response, considerable efforts have been expended. Polyphenols as antioxidant molecules have been used to reduce doxorubicin toxicity (Della *et al.*, 1999).

anthracycline toxicity is essentially due to its induction of mitochondrial oxidative stress. Reactive oxygen species (ROS) are in part generated by Doxorubicin metabolism. Doxorubicin enters mitochondria where it binds to cardiolipin or interacts with mitochondrial DNA inhibiting then the mitochondrial functions (Albercht, 2009).

Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. More than 300 constituents have been identified in propolis (Miguel and Antunes, 2011). Its chemical composition depends on its floral origin with constituents varying widely due to climate and geographical conditions (Seidel *et al.*, 2008). Flavonoids terpenic acids and phenolic compounds are considered as the principal components responsible for propolis biological activities (Kosalec *et al.*, 2004). Nevertheless, its antioxidant activity varies greatly depending on the floral

source (Gardjeva *et al.*, 2007). In our previously studies, we have reported that polyphenolic fraction of Algerian propolis protects kidney, heart and blood tissues in rats treated with anticancer drugs (Lahouel *et al.*, 2004; Boutabet *et al.*, 2011). However, the biochemical mechanisms involved in DOX hepatotoxicity have not been clearly identified. The current study aims to evaluate the implications of mitochondrial oxidative stress in DOX-hepatotoxicity and to investigate *in vivo* the prophylactic effect of propolis ethanolic extract (EEP) on DOX-induced hepatotoxicity in albinos rats.

MATERIAL AND METHODS

Chemicals

Sucrose, EGTA, EDTA, succinate, nitroblue tetrazolium NBT and rotenone were purchased from Sigma. MgCl₂, KCl, DTNB and hydrogen peroxide were purchased from Prolabo.

Plant material

Crude propolis was collected in May-April, 2011 from "Apiocole Coopérative of Kaous, Jijel (Algeria)" and this by scraping hive executives and walls. In order to keep intact all its components, samples were stored at 4°C.

Polyphenols extraction

Cutted into small pieces, propolis was washed twice with 95% ethanol for 2 hours (2x1L), at room temperature. The remaining insoluble, including plant residues, insects and waxes were separated by filtration. Propolis was then dived in 95% ethanol for 15 days (1g for 10mL ethanol),

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the filtrate was then evaporated at 80°C with an (evaporator E100). The residual, called propolis ethanolic extract (PEE), was retaken in 70% methanol and restored at 4°C until analysis (Brunetton, 1993).

Total phenolics and flavonoids content

Total phenolics content was assayed using Folin–Ciocalteu reagent. For this, 0.125 mL appropriately diluted ethanolic extract of was mixed with 0.5 mL of distilled water and 0.125 mL of dilluted Folin-Ciocalteu reagent. After 6 min of incubation, 1.25 mL of Na₂CO₃ (7%) was added and then adjusted with deionized water to 3 mL. After 90 min of incubation at 23°C, the absorbance was read at 760 nm (Dewanto *et al.*, 2002). Total phenolic content was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

Flavonoids content was measured according to Dewanto *et al.* (2002). A 250µL appropriately diluted ethanolic extract was mixed with 75µL NaNO₂ (5%). After 6 min, 150µL of 10% AlCl₃ and 500 µL of NaOH (1 M) were added. Using distilled water, the mixture was adjusted to 2.5mL and the absorbance was read at 510 nm. Total flavonoids content was expressed as (mg rutin eq /g) using rutin calibration curve.

DPPH assay

The electron donation ability of the obtained extracts and essential oils was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al.* (1988). 1 mL from different concentrations of EEP (200µg/mL, 100µg/mL, 50µg/mL and 25µg/mL) prepared in methanol was added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC₅₀ (µg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect} = [(A_0 - A_1)/A_0]100$$

where A₀ is the absorbance of the control at 30 min, and A₁ is the absorbance of the sample at 30 min. Ascorbic acid was used as a positive control. Tests were carried out in triplicate.

Animals treatment protocol

Female wistar rats (purchased from Pasteur institute, Algiers, Algeria) weighing 200-240 g were used in this study. Rats were subdivided into five main groups of 10 rats each. They had free access to water and food. The temperature was maintained to 22°C, relative humidity was 60% and photoperiod of 12H/24H was also applied. Group1, control rats: rats received, 1 mL of

0.9% saline solution in *po*. Group2, DOX -group: rats were treated with 1 mL of 0.9% NaCl solution followed by 1ml of doxorubicin (10mg/kg, *ip*). Group3, prophylactic group: rats were received daily 1mL of EEP (100mg /kg/ day, *po*) for 30 days followed by a single dose of DOX (10mg/kg, *ip*). After 10 days of injections, the rats were sacrificed their livers were rapidly excised, weighed and placed in ice-bath.

Isolation of liver mitochondria and Cytosolic fraction

Liver mitochondria were isolated via a differential centrifugation using the method of Rustin *et al.* (1994). Mitochondrial suspension was used for the measurement of superoxide anion production. Mitochondrial CAT, SOD and MDA were measured on the mitochondrial matrix.

Cytosolic fraction was extracted using the method of Sanmugapriya and Venkataraman (2006). Liver was cut into small pieces and homogenized with three volumes of phosphate buffer (0.15M; pH7.4) containing KCl (1.17%) using a manual poter, supernatant was centrifuged at 10,000 rpm (45 min at 4°C). The supernatant obtained called cytosolic fraction and used as a source for cytosolic parameters measurement.

Mitochondrial superoxide anion (O₂⁻) assay

Generation of superoxide anion was achieved as described by Zini *et al.* (1999). Reaction mixture contained mitochondria (0.2 mg/mL), CsA (1 µM), and NBT (100 µM) in 1.2 mL of respirant buffer (250 mM sucrose, 5mM KH₂PO₄, pH 7.2 at 25°C) supplemented with 2 µM rotenone at 25°C. The addition of (6 mM) succinate initiates the superoxide production and the NBT reduction to monoformazan was measured at 560nm.

Mitochondrial and cytosolic lipid peroxidation assessment

Lipid peroxidation was assessed according to Zini *et al.*, (1999) method. Mitochondrial MDA was assessed using an incubation mixture, containing 800µl of suspended mitochondria in 0.9% NaCl, 100µL of the oxidizing solution Fe²⁺/Fe³⁺ (50µM/ 50µM). After 30mn incubation at 37°C, the reaction was stopped (1mL of 10%). 1.5 mL of thiobarbituric acid (1% TBA) reagent was added and the mixture was heated at 100°C for 30 min. Tubes were recooled in ice for 5 min, then centrifuged (3000rpm, 15 min). The supernatant absorbance was read at 530 nm. The reaction without Fe²⁺/Fe³⁺ was carried out as blank.

Cytosolic MDA levels were assessed using (Ohkawa *et al.*, 1979) method. The reaction mixture containing 0.5 mL of cytosolic fraction 0.5 mL of trichloroacetic acid (TCA) (20%) and 1 mL of 0.67% thiobarbituric acid was placed in boiling water. moved to an ice-bath, tubes received 4ml of n-butanol and centrifuged (3000 rpm, 15 min). The supernatant absorbance was measured

at 532nm (UVmini1240UV-Vis spectrophotometer Shimadzu, China). MDA amounts are expressed as nM/g of liver using a standard curve of TEP (1,1,3,3-tetraethoxypropane).

Glutathione assessment

GSH amounts were measured using the method of Ellman (1959). Briefly, 50µL of cytosolic or mitochondrial fraction were diluted in 10 ml of phosphate buffer (0.1M, pH 8.0). 20µL of 0.01M (DTNB) were added to 3mL of the mixture dilution. After 15 min incubation at 25°C the absorbance was read at 412 nm. GSH levels were expressed in mM/g using a GSH standard curve prepared in the same conditions.

Catalase activity measurement

Cytosolic and mitochondrial CAT enzymatic activities assessment was performed by Clairborne (1985) method. Briefly, the reaction mixture contained 1 ml phosphate buffer (0.1M, pH 7), 950 µL hydrogen peroxide (0.019M) and 0.25 mL of cytosolic or mitochondrial fraction. The catalase activity was measured spectrophotometrically at 240 nm each minute for 2min. Enzymatic activity was expressed IU/g of protein.

SOD activity Measurement

Beauchamp and Fridovich (1971) method was adopted to assess SOD activity in both cytosol and mitochondria. 2ml of reaction medium (sodium cyanide 2.10^{-5} M, methionine solution 10^{-2} M, EDTA solution 6, 6.10^{-3} M, NBT solution 1, $6.7.10^{-4}$ M, riboflavin solution 2.10^{-6} M) were added to 5µL of cytosolic fraction or mitochondrial matrix. This solution was exposed for 15mn to a 15W lamp. The reduction of NBT to formazan by superoxide anion resulting from the photoreaction was then measured at 560 nm. SOD activity was expressed on IU/g of protein.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using the student t-test. $P < 0.05$ were considered significant, (ns) indicates a non significant effect ($p > 0.05$), (*) indicates a significant effect ($p < 0.05$), (**) indicates a very significant effect ($p < 0.01$).

RESULTS

Polyphenols and flavonoids content

The total phenolic content results indicate that EEP contain high concentrations of phenolic compounds, i.e. 410.25 (mg GAE/g) of extract, total Flavonoid concentration is equal to 320.71 mg RE/g. Our extract appears to be rich in flavonoids which could explain the strong free radical scavenging activity of EEP *in vitro*.

Scavenging activity of propolis

One of the more prominent properties of flavonoids is their excellent radical scavenging ability. Compared to vitamin E, EEP showed a high scavenging effect. Propolis extract reduced free radical DPPH in a concentration-dependent manner. At 200µg/mL, EEP reduce DPPH[°] with $91.22 \pm 0.5\%$ against $61.86 \pm 0.87\%$ observed with Vit. E (table 1). The IC₅₀ for antioxidant activity of EEP and Vit E are 31,54µg/mL and 60,65 µg/mL respectively.

Assessment of superoxide anion production by mitochondrial complex III

The measurement of mitochondrial superoxide anion production, especially by respiratory chain complex III, has a special importance in the study of oxidative stress because this phenomenon constitutes the primary event in the oxidative stress induction. The effect of Doxorubicin and propolis extract on mitochondrial superoxide anion production are shown in fig. 1. There was a height significant increase ($p < 0.01$) in superoxide anion production in DOX- treated rats (147%) compared to normal control (100%). EEP at the amount of (100 mg/kg) given by oral administration prior to the administration of doxorubicin 20mg/kg reduced significantly the mitochondrial production of superoxide anion ($p < 0.01$), the production percentage was 76% only.

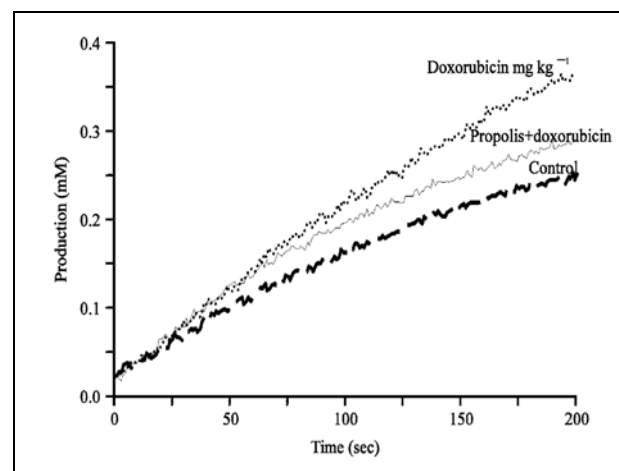


Fig. 1: Changes in mitochondrial superoxide anion production *in vivo* after a single dose of doxorubicin (10 mg/kg) alone or combined with EEP (100 mg/kg).

Propolis extract inhibits mitochondrial and cellular lipid peroxidation

Propolis flavonoids inhibit the lipid peroxidation of liver mitochondrial and cytosolic membranes. Data in fig. 2 shows highly significant increase ($p < 0.001$) in cytosolic (46.73 ± 8.42) nmol/g and mitochondrial (31.14 ± 1.1) nmol/g MDA levels after DOX-treatment compared to the control (34 ± 1.4) and (25 ± 6.8) nmol/g. The pre-treatment with EEP at the amount of (100 mg/kg) significantly reduces MDA rates in both cytosol (18.26 ± 8.42) nmol/g and mitochondria (14.72 ± 1.17) nmol/g (fig. 2).

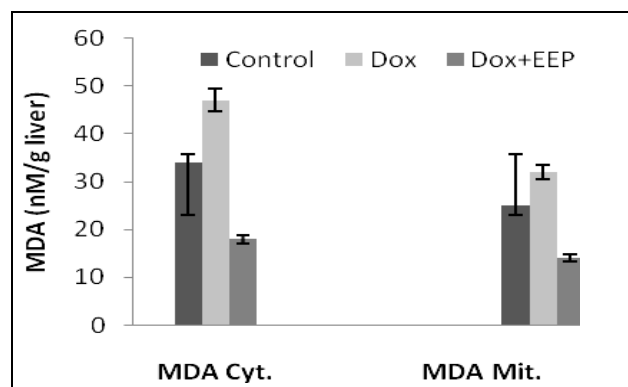


Fig. 2: Effect of a single dose of doxorubicin (10 mg/kg) alone or combined with EEP (100 mg/kg) on mitochondrial and cytosolic MDA.

Propolis extract restored mitochondrial and cellular glutathione GSH content

As shown in fig. 3, administration of DOX caused a significant depletion ($p < 0.001$) in both cellular and mitochondrial GSH levels (0.73 ± 0.21) and (0.34 ± 0.05) mM/g respectively when compared to the control group (2.85 ± 0.31) and (1.05 ± 0.2) mM/g. The pretreatment of animals with EEP (100mg/kg) before DOX treatment normalized GSH content.

Propolis extract (EEP) restored SOD and CAT activities in both cytosol and mitochondria

Mitochondrial and cytosolic CAT and SOD activities in the three treatment groups are shown in table 2 and table 3. DOX (10mg/kg) decrease significantly ($p < 0.001$) CAT and Mn-SOD and Cu/Zn SOD antioxidant enzymes activities in cytosol and mitochondria. In EEP-treated rats the activities of these enzymes in both cytosol and mitochondria is not varying significantly when compared to those measured in control groups (table 2, table 3).

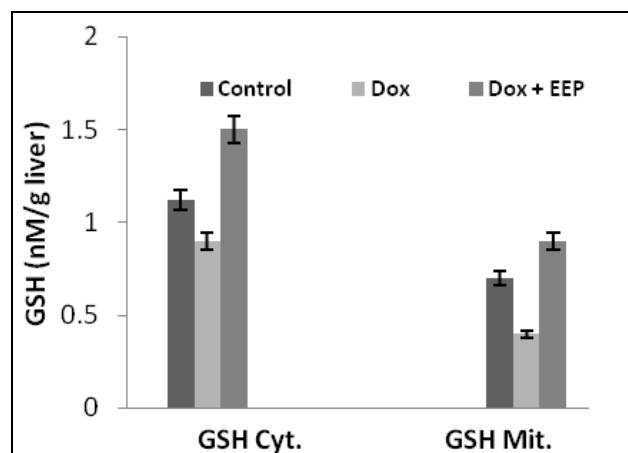


Fig. 3: Effect of a single dose of doxorubicin (10mg/kg) alone or combined with EEP (100mg/kg) on mitochondrial and cytosolic glutathione GSH.

DISCUSSION

Doxorubicin is considered the most toxic anthracycline on hepatocyte (Andersson *et al.*, 1999). Despite this toxicity, DOX is widely used in clinical oncology practice due to its good therapeutic efficacy. Considerable effort has been expended to understand the mechanisms of doxorubicin toxicity and to identify therapies that reduce this adverse response such as antioxidants (Della *et al.*, 1999).

Algerian propolis is known to have antioxidant and hepatoprotective properties. Our results indicate that EEP contains high concentrations of phenolic compounds 410.25 mg GAE/g, total flavonoids concentration is equal to 320.71 mg RE/g. Propolis appears to be rich in flavonoids which could explain the strong free radical scavenging activity of EEP *in vitro*. The redox property of propolis is measured by DPPH, widely used method to evaluate the free radical scavenging ability in various plant species in short period (Ebrahimzadeh *et al.*, 2009a). It showed an excellent scavenging property which was more than that of α -Tocopherol, given in table 1. There are number of reports that proved that phenols have scavenging ability due to the presence of hydroxyl group. It was also reported they are effective hydrogen donors and the position and degree of hydroxylation of phenolic compounds especially in the Bring play a major role make them excellent antioxidants (Fukumoto and Mazza, 2000).

Free radicals play an important role in doxorubicin toxicity and are in part generated by its redox-cycling (Nohl, 1987). In the current study, the pro-oxidant effect of DOX in liver was investigated using mitochondrial superoxide anion O_2^- production assessment. Superoxide can generate more dangerous species, like singlet oxygen and hydroxyl radicals ($OH^\bullet$), which may induce cellular lesions, potentially leading to cell death by necrosis or apoptosis (Halliwell and Gutteridge, 1999). In DOX-treated animals superoxide anion O_2^- production by mitochondrial complex III was significantly increased, this rate was significantly reduced in EEP-treated animals. During liver DOX metabolism, a semiquinone radical is formed suggesting that propolis mechanism of action is similar in part to trans-resveratrol (De Matteis, 1994). Propolis polyphenols and trans-resveratrol inhibit O_2^- production in cardiac and nervous cells respectively. This effect is due to complex III partial inhibition (Delgi Esposti, 1998; Zini *et al.*, 1990). The interaction of propolis polyphenols with the Q cycle inhibits radical ubisemiquinone formation; they can capture the first electron yielded by the cytochrome to the ubiquinol or can add an electron to ubisemiquinone radical. The reduction of O_2^- production in liver mitochondria can also be explained by the strong scavenger effect of EEP. In fact, our study shows that EEP has a dose dependant scavenger effect *in vitro* against DPPH $^\circ$ free radicals which is better than that of

Table 1: Scavenging effect and IC₅₀ of the ethanolic extract of propolis (EEP) and Vit. E against DPPH° free radicals.

	200µg/mL	100µg/mL	50µg/mL	25µg/mL	IC ₅₀ µg/mL
EEP	91,22±0,5	86,16±1,32	71,46±1,35	40,68±1,9	31,54
Vit E	61,86±0,87	53,06±0,6	37,18±3,89	24,66±2,01	60,65

Table 2: Effect of a single dose of doxorubicin (10 mg/kg) alone or combined with EEP (100 mg/kg) on liver mitochondrial catalase (CAT) and superoxide dismutase (Mn-SOD).

Groups	Mit. CAT (UI/g Pr.)	Mit. SOD (UI/g Pr.)
Control	2583.51±123	1544±49
Dox. 10mg/kg	1024.45±329**	601.34±42.1**
Dox. 10mg/kg+EEP (100mg/kg)	2657.67±143 ns	1434.4±68.9 ns

Table 3: Effect of a single dose of doxorubicin (10 mg/kg) alone or combined with EEP (100 mg/kg) on cytosolic catalase (CAT) and superoxide dismutase (Cu/Zn-SOD) in liver rats.

Goups	Cyt. CAT (UI/g Pr.)	Cyt. SOD (UI/g Pr.)
Control	3543.43±67.56	2644±209.01
Dox. 10mg/kg	2287.70±98.65**	1257.34±152.83**
Dox. 10mg/kg+EEP (100mg/kg)	3698.74±213.6 ns	2534.83±138.91 ns

Vit.E. This result confirmed the *in vivo* effect of propolis obtained in this study.

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids. Its occurrence in biological membranes causes impaired membrane function, impaired structural integrity, decrease in fluidity, and inactivation of a number of membrane bound enzymes and protein receptors (Alyane *et al.*, 2008). In this study, a significant increase in MDA levels in both liver cells and mitochondria was observed in DOX-treated animals. The increased lipid peroxidation was attributed to the overproduction of superoxide anion production in liver mitochondria observed in current study. Increased lipid peroxidation could be attributed also to the reduction in detoxifying hyperperoxides. Our study shows that EEP inhibit DOX-induced mitochondrial and cellular lipid peroxidation. This protective effect can be due to scavenging MDA molecules by propolis active ingredients or to inhibition of mitochondrial and cytosoliclipoperoxydation chain reactions. Propolis extracts give comparable effect with that of quercetin. This lets us think that these extracts have the same mechanism as that of quercetin. Indeed, quercetin presents an antilipoperoxydative activity; by its scavenging effect of peroxy radicals, by ions chelating capacity and its capacity to reduce Fe⁺² and thus inhibiting fenton reaction and lipid peroxydation (Vasilyeva *et al.*, 2000). Recent studies show also that quercetin and rutin are able to stop the radicalizing reactions by inhibition of superoxide anion, hydroxyl radical and peroxy radical's production (Elicoh-Meddleton *et al.*, 2010). Our results are similar to those obtained by several other authors (Bridger, 1998; Lopez-Lazaro *et al.*, 2000). To protect themselves against free

radicals, cells have developed antioxidant defences which include enzymes like superoxide dismutase (SOD), ctalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSHPx) and glutathione-S-transferase, as well as non enzymatic molecules such as GSH and vitamin E (Venukumar and Latha, 2002).

Glutathione (GSH) is a non –enzymatic anti-oxidant defense. It plays a critical role in cellular functions, which includes the maintenance of thiol status of proteins, the destruction of H₂O₂, lipid peroxides, free radicals and drug biotransformation and detoxification. In our study, results revealed a significant depletion of mitochondrial and cytosolic of hepatic GSH in DOX -treated rats. Decrease in GSH level might be due to its increased utilization by the hepatocytes in scavenging doxorubicin-metabolites. It has been reported that most covalent binding of toxicant to hepatic protein occurs only after depletion of GSH, and the severity of hepatic necrosis is related to the degree of covalent binding (Jollow *et al.*, 1973). On the other hand, GSH levels were increased in propolis-treated rats. flavonoids found in our propolis are known to increase GSH production. The effects of propolis in maintaining the mitochondrial and cellular GSH might be due to direct neutralising free-radicals, or increasing of GSH synthesis by propolisbioactive compounds. Indeed, recent study showed that propolis at 100 mg/kg prevents doxorubicin cardiotoxicity by the improvement of intracellular and serum GSH rates (Alyane *et al.*, 2008). In this study we have also found that doxorubicin (10mg/kg) decrease significantly Mn-SOD, Cu/Zn SOD and CAT antioxidant enzymes activities in cytosol and mitochondria. SOD plays an important role in the elimination of ROS derived from xenobiotics metabolism in liver tissues. This

decrease could be do to mitochondrial overproduction of superoxide anion following DOX administration. The increase in this enzymatic activities in pretreated rats with propolis can be the consequence of an induction SOD and CAT genes and protein expression synthesis. During ischemia-reperfusion, it is found that quercetin can either increase or preserves MnSOD activity (Paul *et al.*, 2002).

CONCLUSION

From these results it is concluded that Algerian propolis presents a strong antioxidant activity and can reverse doxorubicin toxicity by protecting mitochondria and reinforcement of enzymatic and non enzymatic antioxidant defenses. Polyphenolic fraction of Algerian propolis might be able to solve the problem of the anticancer drug toxicity. It can open perspectives in the treatment of a wide variety of human malignancies.

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REFERENCES

- Alyane M, Benguedouar L, Kessa W, Boussenane HN, Rouibah H and Lahouel M (2008). Cardioprotective effects and mechanism of action of Polyphenols extracted from Propolis against Doxorubicin toxicity. *Pak. J. Pharm. Sci.*, **21**(3): 201-209.
- Andersson B, Eksborg S, Vidal R, Sundberg M and Carlberg M (1999). Anthraquinone-induced cell injury: Acute toxicity of carmi-nomycin, epirubicin, idarubicin and mitoxantrone in isolated cardiomyocytes. *Toxicology*, **135**: 11-20.
- Angélica MW, Ehara KA, Marla BJ, Conti JM and Sforcin A (2011). Cytotoxic constituents of propolis inducing anticancer effects. *J. of Pharmacy and Pharmacology*, **63**(11): 1378-1386.
- Bachiega TF, Orsatti AC, Pagliarone JM and Sforcin A (2012). The Effects of Propolis and its Isolated Compounds on Cytokine Production by Murine Macrophages. *J. Nutr.*, **111**:150-170.
- Beauchamp C and Fridovich I (1971). Assay of superoxide dismutase. *Anal. Biochem.*, **44**: 276-287.
- Boutabet K, Kessa W, Alyane M and Lahouel M (2011). Polyphenolic fraction of Algerian propolis protects rat kidney against acute oxidative stress induced by doxorubicin. *Indian J. of Nephrol.*, **21**(2): 101-106.
- Bridger S (1998). Deaths from low dose paracetamol poisoning. *Br. Med. J.*, **316**: 1724-1725.
- Bruneton J (1993). Pharmacognosie, Phytochimie, Plantes médicinales. *Paris Tec & Doc Lavoisier*, p. **26**.
- Clairborne A (1985). Catalase activity. In: Greenwald RA, editor. *CRC Handbook of Methods for Oxygen Radical Research*. Boca. Raton. FL. CRC. Press, pp: 283-284.
- De Matteis F (1994). The role of cytochrome P450 in drug metabolism and toxicity. *CRC press*, New York, 81.
- Delgi-Esposti M (1998). Inhibitors of NADH-ubiquinone reductase: an overview. *Biochim. Biophys. Acta.*, 364-222.
- Della Torre P, Imondi AR, Bernardi C, Podestà A, Moneta D, Riflettuto M and Mazué G (1994). Cardioprotection by dexrazoxane in rats treated with doxorubicin and paclitaxel. *Cancer Chemother. Pharmacol.*, **44**: 138-142.
- Dewanto V, Wu X, Adom K and Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.*, **50**: 3010-3014.
- Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR (2008). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechnol.*, **7**(18): 3188-3192.
- Elicoh-Middleton Jr, Chithan K and Theoharis C (2010). Effect of plant flavonoids on mammalian cells: implications for inflammation, heart diseases and cancer. *Pharmacology and Experimental therapeutics*. **4**(52): 673-751.
- Ellman GL (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, **82**: 70-77.
- Farooqui T and Farooqui AA (2012). Beneficial effects of propolis on human health and neurological diseases. *Front Bioscience (Elite Ed.)*, **1**(4):779-793.
- Fukumoto LR, Mazza G (2000). Assessing antioxidant and prooxidant activity of phenolic compounds. *J. Agri. Food Chem.*, **48**(8): 3597-604.
- Gardjeva PA, Dimitrova SZ, Kostadinov ID, Murdjeva MA, Peyche LP, Lukanov LK, Stanimirova IV and Alexandrov AS (2007). A study of chemical composition and antimicrobial activity of Bulgarian propolis. *Folia. Med.*, **49**: 63-69.
- Gebara EC, Lima LA and Mayer MP (2002). Propolis antimicrobial activity against periodontopathic bacteria. *Brazilian J. of Microbiol.*, **33**: 365-369.
- Gutteridge JM and Halliwell E(1988). Oxygen radicals and tissue injury. *FASEB J.*, **13**: 9-19.
- Halliwell Band Chirico S (1999). Lipid peroxidation: its mechanism, measurement and significance. *Am. J. Clin. Nut.*, **57**: 715-725.
- Hanato T, Kagawa H, Yasuhara T and Okuda T (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. *Chem. Pharm. Bull.*, **36**: 1090-1097.
- Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR and Brodie BB (1973). cetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J. Pharmacol. Exp. Ther.*, **187**: 195-202.

- Kosalec I, Bakmaz M, Pepeljnjak S and Vladimir-Knezevic S (2004). Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta. Pharm.*, **54**(1): 65-72.
- Lahouel M, Boulkour S, Segueni N and Fillastre JP (2004). The flavonoids effect against vinblastine, cyclophosphamid and paracetamol toxicity by inhibition of lipid-peroxidation and increasing liver glutathione concentration. *Pathol. Biol.*, **52**: 314-322.
- Lopez-Lazaro M, Martin-Cordero C and Ayuso MJ (2000). Two new flavonol glycosides as DNA topoisomerase I poison. *Z. Naturforsch.*, **55**(11-12): 898-902.
- Lowry OH, Rosegrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.
- Miguel MG and Antunes MD (2011). Is propolis safe as an alternative medicine. *J. Pharm. Bioallied Sci.*, **3**(4): 479-495.
- Nohl H (1987). Demonstration of the existence of an organo-specific NADH dehydrogenase in heart mitochondria. *Eur. J. Biochem.*, **169**: 585-591.
- Ohkawa H, Ohishi N and Yag K (1979). Assay of lipid peroxides in animal tissue by thiobarbituric reaction. *Anal. Biochem.*, **95**: 351-358.
- Paul SB, Stanley BD, Dale QP, and Victor DU (2002). Mitochondrial function in response to cardiac ischemia-reperfusion after oral treatment with quercetine. *Free Rad. Biol. Med.*, **32**: 1220-1228.
- Rustin P, Chre'tien D, Bourgeron T, Ge'ard B, Rotig A and Munnich A (1994). Biochemical and molecular investigation in respiratory chain deficiencies. *Clin. Chem. Acta.*, **228**: 35-51.
- Sanmugapriya E and Venkataraman S (2006). Studies of hepatoprotective and antioxidant actions of *Strychnos potatorum* Linn. Seeds on CCl₄ induced acute hepatic injury in experimental rats. *J. Ethnopharmacol.*, **105**: 154-160.
- Singal PK and Iliskovic N (1998). Doxorubicin-induced cardiomyopathy. *N. Engl. J. Med.*, **339**: 900-905.
- Suh JH, Shevni SV, Dixon BM and Liu RM (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversed with lipoic acid. *Proc. Nat. Acad. Sci.*, **101**: 3381-3386.
- Takemura G and Fujiwara H (2007). Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Prog. Cardiovasc. Dis.* (Please include vol and page number)
- Vasilyeva OV, Lyubitsky OI and Klebanov GI (2000). Effect of the combined action of flavonoids, ascorbate and alpha-tocopherol on peroxidation of phospholipid liposomes induced by Fe²⁺ ions. *Membr. Cell. Biol.*, **14**: 47-56.
- Velikova M, Bankova V, Sorkun K, Houcine S, Tsetkova I and Kujemgiev A (2000). Propolis from the Mediterranean region: Chemical composition and antimicrobial activity. *Z. Naturforsch.*, **55**(9-10): 790-803.
- Venukumar MR and Latha MS (2002). Antioxidant activity of *Curculigo orchoides* in carbon tetrachloride induced hepatopathy in rats. *Indian J. Clin. Biochem.*, **17**: 80-87.
- Weiss RB (1992). The anthracyclines: will we ever find a better doxorubicin. *Semin. Oncol.*, **19**: 670-686.
- Wickens AP (2001). Aging and the free radical theory. *Resp. Physiol.*, **128**: 379-391.
- Zini R, Morin C, Bertelli CC and Tillement JP (1999). Effects of resveratrol on the rat brain respiratory chain. *Drugs Exp. Clin. Res.*, **25**: 87-97.