Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.04.022

Propolis flavonoids liposome ameliorate doxorubicin-induced cardiotoxicity in rats

Ali M. Ghazi[1]*, Sijad M. Latif[2]
[1,2]Veterinary Medicine college / University of Al-Qadisiyah
***Corresponding author:** Ali M. Ghazi, Veterinary Medicine college / University of Al-Qadisiyah, Email: ali.Ghazi@qu.edu.iq

Submitted: 11 January 2023; Accepted: 14 February 2023; Published: 16 March 2023

ABSTRACT

The clinical applications of doxorubicin (DOX), a potent anthracycline antineoplastic drug, is often restricted by its deleterious cardiotoxic effects. Thus, for improving usage of DOX, the aim of this study was to assess the cardio-protective effects of nanopropolis in a rat model of DOX-induced acute cardiotoxicity. Through maceration method for 7 days in ethanol then 7 days in absolute ethyl acetate, flavonoidal propolis extract was obtained. twenty albino Rats were divided in to four equal groups: Control rats that received BPS for 10 days, DOX-treated rats that recived a single I.P injection of DOX (25 mg/kg BW) on day 7 from beginning of experiment. Rats received liposome orally for 10 consecutive days and single dose of doxorubicin (25 mg/kg BW IP) on day 7 from beginning of experiment. Rats groups received propolis liposome formulas orally for 10 consecutive days and single dose of doxorubicin (25 mg/kg BW IP) on day 7 from beginning of experiment. Sera were collected for screening of cardiac injury markers.The administration of DOX in single dose showed high cardiac biomarkers CK-MB, Troponin I, and LDH than in the negative group. Treatment with propolis nanoformulas shown ameliorated the DOX-induced cardiac tissue damage by decline the serum levels of CK-MB, Troponin I, and Lactate dehydrogenase.

The experiment parts endorsed conclusion that nanopropolis may exhibit remarkable protective effect in DOX-induced cardiac toxicity through improve a number of cardiac biomarkers linked with cardiac toxicity, and may be represent a potential agents for attenuation and prevention of the serious complications accompanied with DOX in clinical practice.

Keywords: *Propolis flavonoids liposome, Doxorubicin, cardiotoxicity*

INTRODUCTION

Anthracycline chemotherapy regimens play a central role in the treatment of many cancer types including breast cancer and lymphoma. Doxorubicin (derived from microbes), its precursor daunorubicin, are the most common anthracyclines (1). Cardiotoxicity is a frequent, fatal side-effect of the doxorubicin (DOX), that restrict its therapeutic usage for tumor therapy (2). The usage of DOX may result in pericarditis, left ventricular dysfunction, and arrhythmias, which ultimately result in heart failure (3). So the prevention of DOX-induced cardiotoxicity may be helpful to improve future DOX therapy (4) Despite the recent advances in the field of chemically synthetized pharmaceutical agents, the search of natural origin products is a valuable approach for the discovery and development of novel biologically active compounds possessing unique structures and mechanisms of action (1).

Propolis is a complex, natural, resinous substance which is collected by bees and mixed with bee saliva. It is used by bees as glue, a general-purpose sealer, and as draught-extruder for beehives. It has important pharmacological properties and it can be used for a wide range of purposes as anti-inflammatory and hypotensive agent, immune system stimulant, and bacteriostatic and bactericidal agent, among many other uses (5).

The potential of nanobiomedical area to create a promising nano-sized drug delivery system is considered as a tremendous pharmaceutical trend for encapsulation and release of various therapeutic substances (6). Targeted delivery of nutraceuticals is one of the main challenges in the treatment of the different diseases. Through the enhanced permeability and retention effect, which increases drug accumulation in affected area while decreasing it in normal cells, nanomaterials have demonstrated great promise for encapsulating and transporting drugs, penetrating cell membranes, and releasing drugs in affected organ (7). Under the light of these facts, The primary goal of this study will depend on the development of liposomal formalae including flavnoidal propolis as the active moiety for the intention of protection the heart from the harmful effects of doxorubicin in vivo. For this purpose, propolis liposomal formulation will be prepare and characterize and evaluate cardioprotective role in rats model. To achieve this aim, following objectives were drawn

1. Characterization of prepared propolis liposome complex

2. Establish the protective effect of propolis liposomal formulation against DOX-induced cardiotoxicity.

MATERIALS AND METHODS

Propolis collection and extraction

Raw native propolis samples were gathered between October-December of 2021 directly from bee keepers located in the Al-Diwaniya province, and manually cleaned from any undesirable materials. The propolis samples conserved through transportation in closed vessels at 4°and kept at -4°C until use (8).The extraction process was done according to the method reported by (9) with slight modifications. The propolis was cut into small pieces and frozen at -20°C for 24 hours for hardness. Then grinded to the fine powder by an electrical grinder, 50 grams of the powdered was mixed with 500 ml of 80% ethanol solution 1:10 w/v in an amber glass container. The mixture was kept at room temperature for 7 days and manually stirred once a day.

The mixture suspension of the extract was filtered by Whatman filter paper No. 1. With diameter 185 mm to remove waxes and insoluble constituents, The subsequent filtrate was then evaporated off using a rotary evaporator under reduced pressure at 60 °C. to remove all the ethanol content within the filtrate. The final step led to represent the balsam of propolis and is referred to as ethanolic extract of propolis (EEPs). In order to obtain a larger amount of and flavonoids compounds in extract a part of the ethanolic dry extracts (EEPs) were subsequently suspended in 500 mL of ethyl acetate and kept at room temperature for 7 days and manually stirred once a day. The suspension was then filtered through a Whatman No. 1 paper, the solvent was subsequently evaporated to dryness to obtain the ethyl acetate propolis extract (EAPE).

Liposome preparation

The liposomes were prepared with simple modification by dried thin lipid film technique as described previously by (10). L-α

phosphatidylcholine (PC) 0.25 gm and cholesterol 0.25 gm (w:w) were dissolved in 15 ml of chloroform: methanol (2:1) (v/v) mixing in the 25 ml glass test tube then vortexed for 30 minutes, 1500 rpm. The mixture was kept warm in a water bath 40°C for 15 minutes and transferred to a round bottom flask connected with a vacuumed rotary evaporator machine at; 80 rpm, 80° C attached to a vacuum pump. The round –bottom flask was immersed in a thermostatic water bath 40 °C for 2 hours to affirm the dryness of the thin lipid film deposited on the inner walls of the round bottom glass flask. The empty or liposome was formed and suspended by phosphate puffer.

Preparation stock solution of propolis

Propolis flavonoids extract stock were prepared by weighed 400 mg of extract and dissolved in the 2 ml ethyl acetate, vortexed for 30 seconds using a vortex mixer. The final concentration of each compound was 20gm /100 ml, 20%, and the solutions filtered through a 0.22 µm syringe filter and kept at 4°C until use.

Liposomal flavonoids propolis

The diluted ethanol 2 ml of 30%, with propolis 400 mg added to the 0.5 gm of empty liposome and vortexes 1500 rpm for 30 minutes and rejoined with the previous set of rotary evaporator under 80 rpm, 80° C for 30 minutes.

Standardization of liposomal propolis

Scanning electron microscope (SEM)

The liposomal flavonoids propolis were examined in scan electron microscope to illustrate the morphological characteristics of the prepared nanoparticles. scanning electron microscope with ultrahigh resolution field emission was used. To analyze the morphology and form of the particles, samples were mildly pressed into pellets at 0.5 ton-load to achieve the best picture under SEM (11).

Liposomal propolis lambda max determination

The spectral analysis of the empty liposome 2 mg and liposomal propolis 0.2 mg were done by dissolved them in 1 ml of PBS. The prepared solutions were then vortexed for 3 minutes and examined in a wavelength range of 200 to 800 nm, via a UV-visible spectrophotometer; the absorbance data were plotted with wavelength to drive the absorbance curve, and an estimate of the maximum absorbance for Nano form (12).

Encapsulation efficiency and loading efficiency

The encapsulation efficiency and loading efficiency were calculated on the spectrophotometer technique after determination of non-encapsulated propolis in the liposomal formulas. The load liposome was centrifuged at 5000 rpm for 20 minutes at 22° C to separate the supernatant containing liposomal vesicles from the sediments containing the unentrapped drug. The clear supernatant was collected and recentrifuged at 5000 rpm for 15 minutes.

The liposome was mixed in 10 ml of methanol (98%) and vortexed 1500 rpm for 10 minutes. The liposomes were dissolution to release the drug, to quantify the content of each propolis in supernatant and sediments of the samples and estimated by spectrophotometer then consequent hold in the calibration curve. The encapsulation efficiency of liposomal propolis and were calculated by the ratio of encapsulated drug to the initially added extract, The assay was done in triplicate. EE and LE calculations were done according to the following equations (13).

$$encapsulation\ efficiency\ \% = \frac{total\ propolis - free\ propolis}{total\ propolis} \times 100$$

Percent substance loading was calculated by the ratio of encapsulated drug to liposomal lipid amount according to the following equation.

$$loading\ efficiency = \frac{total\ prpoils - free\ propolis}{total\ liposome\ weight} \times 100$$

Experimental rats

A total of 20 healthy adult male wistar rats with initial body weights of 200-210 gm. were obtained from the animal house of the Veterinary Medicine College / Al-Qadisiyah University. The ages of rats used in this study ranged between 10 - 12 weeks, The animals were housed under standard laboratory conditions with a 12 h light dark cycle at a 24±3˚C, 40–60% relative air humidity and food and water provided ad libitum. After randomization into different experimental groups, they were allowed to acclimatized to the

laboratory conditions for two weeks before beginning the experiment.

Induction of cardiotoxicity

Acute cardiotoxicity was induced in male rats on day 7 from beginning of experiment by using single dose of doxorubicin antibiotic at 25 mg/kg BW Intraperitonially (14).

Animal grouping and treatment

Male rats were randomly divided into five equal groups; each one comprised of five male rats. Cardiotoxicity was induced as previously mention in the paragraph. The rats received respective treatments daily for 10 days. The dosed solutions were freshly prepared daily before each experimental series. The dosed amounts were administered orally in a 2 ml oral gavage. The Cardiotoxicity induced rats was grouped in 2 sets of dosing formulation, with dualistic control groups negative and positive, rats of all groups were treated as follows:

Negative control group

(NC) rats' treatment by vehicle (PBS) which are used for preparation of selected drug (n= 5 rats)

Doxorubicin group

(DOX) Receive single dose of doxorubicin (25 mg/kg BW IP) on day 7 from beginning of experiment (n= 5 rats)

Liposome group

(L) Receive liposome orally for 10 consecutive days and single dose of doxorubicin (25 mg/kg BW IP) on day 7 from beginning of experiment (n= 5rats)

Propolis flavonoids liposome treated group

(FPL) Receive propolis liposome formulas orally for 10 consecutive days and single dose of doxorubicin (25 mg/kg BW IP) on day 7 from beginning of experiment (n= 5 rats)

Rat scarification and samples collection

At the end of the study, the rats were fasted for overnight but were allowed free access to water. The blood samples were collected by heart puncture method under chloroform anesthesia. Blood was collected and centrifuged in a refrigerated centrifuge (4°C) at 3,000 rpm for 20 min, and the serum was stored at -20◦C until analysis done. The whole heart was isolated immediately after sacrificing the animal and washed with normal saline, rinsed, and weighted.

Relative heart weight

The total body weight and whole heart weight of grouped rats were estimated, and the relative heart weight was calculated as the ratio of heart weight to total body weight for each rat.

The relative heart weight = heart weight (gm) / total body weight (gm) × 100

Determination of serum CK-MB level

The levels of CK-MB (ng/mL) in the serum were estimated using an ELISA kit according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate ELISA (15).

Determination of serum high sensitivity Troponin I level

The levels of high sensitivity Troponin I (pg/mL) in the serum were determined using an ELISA kit according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate ELISA (15).

Determination of serum Lactate dehydrogenase level

The levels of Lactate dehydrogenase (ng/mL) in the serum were determined using an ELISA kit according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate ELISA (15).

Statistical analysis

Statistical evaluations were performed using One-way ANOVA test was used to find the differences between groups followed by least significant difference (LSD) multiple comparisons post hoc. Values of $P < 0.05$ were considered significant. All values are expressed as mean ±standard error. All statistical analysis were performed using SPSS program version 31

RESULTS

Propolis extract yield

The primary yield percentage of propolis ethanolic extraction (PEE) process (80%) for three patches was 37.25±0.62% while final yield percentage of ethyl acetate propolis extract was 23.35±0.28% as shown in table (1) the characteristics of final product of propolis after complete dryness was sticky in consistency and glossy brownish to dark brown in color with distinguish odor figure (1).

TABLE 1: yield of extraction and percentage yield of primary and final propolis extract.

Raw amount (gm)	Primary Yield recovery (gm)	Yield %	Final yield recovery (gm)	Final yield %
Propolis 100	37.25±0.62	37.25±0.62	23.35±0.28	23.35±0.62

The values are expressed as mean± SEM of the 3 patches for propolis samples

FIGURE 1: Propolis material (crude and ethyl acetate propolis extract)

liposomal propolis characterization

Absorbance curve, lambda max peak of liposomal propolis

The λ max peaks of the liposome, liposomal propolis were estimated in the Uv-visible spectrum band; at 37° C at 2 nm intervals, shard 332, and 288 nm respectively figure 2

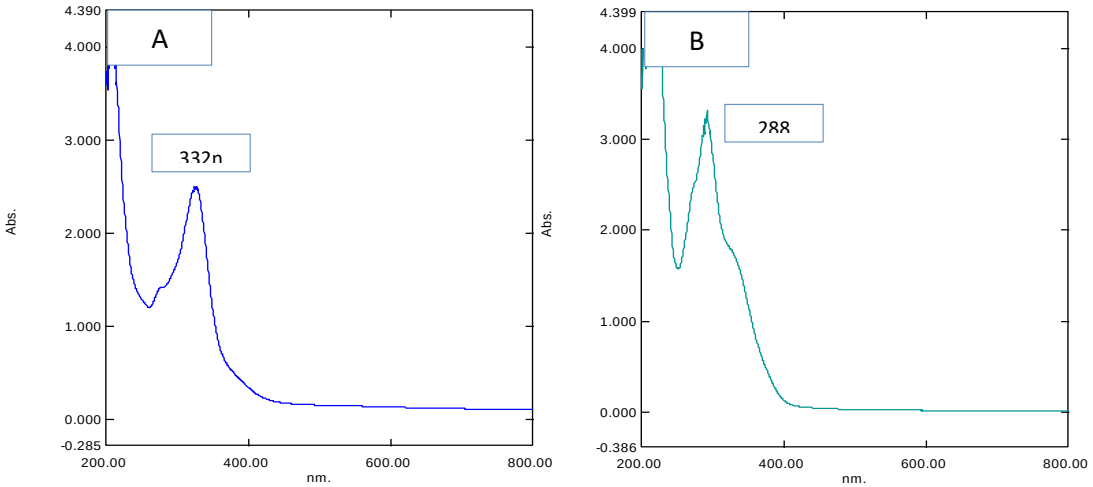

FIGURE 2: Nano-formulas lambda max.(a) liposome only (b) liposomal propolis formulas.

J Popul Ther Clin Pharmacol Vol 30(4):e230–e240; 16 March 2023.

Scanning electron microscope

The type and morphology of liposomal formulas scanning electron microscope. A scan depiction of the liposomal propolis was shown fine spherical separated vesicular assemblies. The particle size of the Nano-metric scale was valued at 93.5±0.87 nm as shown in the figure 3

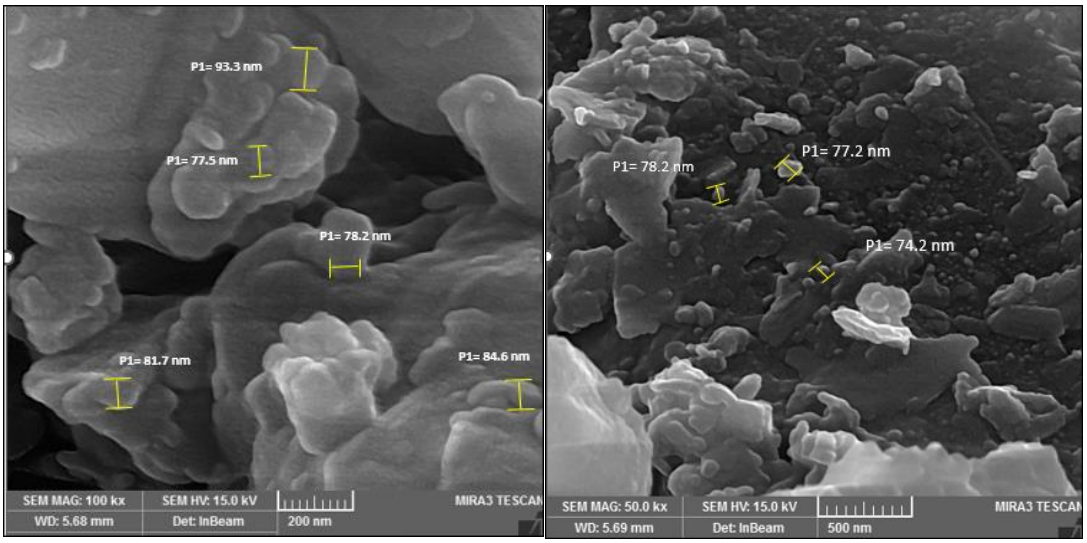

FIGURE 3: Scanning electron microscope image of liposomal propolis formulas

Liposome entrapment efficiency and loading

As illustrated in the table 2 The encapsulation efficiency and loading efficiency of propolis flavonoids liposome was recorded for 5 patches. The percentage of propolis incorporated in the liposome nanoparticles relative to the initial amounts of propolis in the mixture was 82.19±2.76% whereas the percentage of loading efficiency of propolis incorporated in the liposome nanoparticles relative to the content of the total lipid used was 65.75±2.51%.

TABLE 2: The entrapment efficiency and loading of flavonoid propolis liposome

material	Total propolisa mount (mg)	Liposome amount (mg)	Free drug amount (mg)	Encapsulated efficiency (5)	Loaded efficiency (%)
Propolis	400	500	71.21±13.18	82.19±2.76	65.75±2.51

Total body weight and relative heart weight

As illustrated in the table 3 there was no significant (P>0.05) differences in the total body weight among all experimental groups' rats. In regarding to the relative heart weight recorded significant (P<0.05) decline in both DOX group and Liposome group compared with other groups. Propolis flavonoids liposome group recorded significant difference (P<0.05) as compared with negative control group.

J Popul Ther Clin Pharmacol Vol 30(4):e230–e240; 16 March 2023.

TABLE 3: Effect of FPL on the total body weight, heart weight and relative heart weight in DOX-induced cardiotoxicity rats' model

Groups	Symbol	Total body weight (gm)	Heart weight (gm)	Relative heart weight (%)
Negative control(G1)	NC	220.6±3.37A	0.804±0.006A	0.364±0.004A
Doxorubicin (G2)	DOX	223.2±2.88A	0.652±0.019C	0.292±0.008C
Liposome(G3)	L	221±4.84A	0.655±0.019A	0.296±0.009C
Propolis flavonoids liposome (G4)	FPL	226.4±1.56A	0.710±0.005B	0.313±0.003B
LSD (P<0.05)		9.87	0.033	0.014

Data presented as mean± SEM. The different superscript letters denoted to significant differences p<0.05, n=5.

Serum CK-BM level

The table (3) showed the serum level of the Ck-BM in the treated and control groups. There was marked significant (P<0.05) elevation in the serum CK-MB in the doxorubicin given rats 94.23±1.45 ng/ml compared with negative control rats 58.16±1.56 ng/ml. In the same time, Propolis flavonoids liposome administrated rats exhibited a significant (P<0.05) reduction in CK-MB serum levels 61.16±1.14 ng/ml as compared with DOX group. However, no significant (P>0.05) difference in the CK-BM levels in both DOX and Liposome administered rats.

TABLE 4: Effect of FPL on serum CK-MB level in DOX-treated rats

Groups	Symbol	CK-MB (ng/ml)
Negative control(G1)	NC	58.16±1.56B
Doxorubicin (G2)	DOX	94.23±1.45A
Liposome(G3)	L	91.58±2.06A
Propolis flavonoids liposome (G4)	FPL	61.16±1.14B
LSD (P<0.05)		5.15

Data presented as mean ± SEM. The different superscript letters denoted to significant differences p<0.05, n=5, PEE: Propolis ethanolic extract, FPL: Propolis flavonoids liposome

Serum cardiac Troponin I level

As shown in the table (5) the serum level of the cardiac troponin I was significantly (P<0.05) elevated in the doxorubicin given rats 981.4±3.94 pg/ml as compared with negative control rats 426.12±1.08 pg/ml. On the other hand, Propolis flavonoids liposome rats showed a significant (P<0.05) reduction in troponin I levels 628.52±3.78 pg/ml as compared with DOX group. Conversely, there was no significant (p>0.05) alterations in the Troponin I value between liposome group and DOX group.

TABLE 5: Effect of FPL on serum cardiac troponin I level in DOX-treated rats

Groups	Symbol	Troponin I (pg/ml)
Negative control(G1)	NC	426.12±1.08D
Doxorubicin (G2)	DOX	981.4±3.94A
Liposome(G3)	L	987.11±6.14A
Propolis flavonoids liposome (G4)	FPL	628.52±3.78C
LSD (P<0.05)		18.86

Data presented as mean ± SEM. The different superscript letters denoted to significant differences p<0.05, n=5, PEE: Propolis ethanolic extract, FPL: Propolis flavonoids liposome

Serum cardiac lactate dehydrogenase level

The results in table (6) revealed the lactate dehydrogenase levels in the serum, The lactate dehydrogenase values in the group of DOX rats 47.62±1.02 ng/ml was significantly ($p<0.05$) higher than basic values of negative control 21.15±0.36 ng/ml and no significant alteration with liposome group 46.18±0.68 ng/ml. Conversely, there was significant ($p<0.05$) reduction in the serum cardiac lactate dehydrogenase values in liposomal formulas 27.08±0.55 ng/ml as compared with the both DOX and Liposome treated groups.

TABLE 6: Effect of FPL on serum Lactate dehydrogenase level in DOX-treated rats

Groups	Symbol	Lactate dehydrogenase (ng/ml)
Negative control(G1)	NC	21.15±0.36C
Doxorubicin (G2)	DOX	47.62±1.02A
Liposome(G3)	L	46.18±0.68A
Propolis flavonoids liposome (G4)	FPL	27.08±0.55B
LSD ($P<0.05$)		1.55

Data presented as mean ± SEM. The different superscript letters denoted to significant differences $p<0.05$, n=5,

DISCUSSION

In general, propolis and its nano-formulas reported to have anti-inflammatory and anti-oxidant properties that can be contribute to decrease toxicity of different drug materials (16). The limitation of the extract uses was related to large dosed amount, non-specified targeted effects, and inadequacy of efficacious effects. For this reason, the current experimental datum resolved short come of uses synthetic, and non-specified oriented nutraceutical extracts via preparation and characterization of the Nano-propolis medication via loading of propolis in liposome nanoparticles and manged exploration the relative efficacy of orally dosed of formulas outcomes as a potential candidate for ameliorate toxic actions of the doxorubicin antibiotic on the heart tissue.

DOX-induced cardiomyotoxicity has been well documented in various animal species (17). Doxorubicin (DOX) is a highly active antineoplastic agent and widely used to treat various solid tumors, however, its clinical use is greatly limited by its dose-dependent toxicity, particularly severe cardiac and hepatic toxicity (18). Therefore, it is important to work for develop effective natural products could be attribute to metigate the adverse reactions of this chemotherapeutic agent and other related drugs. In the present work, the rats that administrated single high dose of doxorubicin (25 mg/ kg BW IP) resulted cardiomyopathy associated markedly alterations in the a selected heart markers (Serum CK-MD level, serum high sensitivity cardiac troponin I level, serum Lactate dehydeogenase). These biomarker enzymes aspecially CK-MB and LDH are extensively used in the clinical practice as markers for the diagnosis of myocardial necrosis and toxicity which reflects the extend of damage in its musculature (19). These results were in accordance with previous findings of direct toxic effects induced by DOX on rats myocardial tissue (20; 17 and 21). In the cardic tissue, Its converted into its semiquinone form, which is a toxic, short-lived metabolite that can interacts with molecular oxygen initiating a cascade of reaction leading to ROS generation (22) beside that, its play pivotal role in the formation of an anthracycline-iron (Fe^{2+}) free radical complex which reacts with hydrogen peroxide to produce hydroxyl ($OH^{\bullet}$) radical and this events lead to react ROS with lipids, protein and other cellular constituents causing damage to mitochondria and cell membranes of the heart muscle cells (23; 24). Studies have reported that the acute cardiotoxicity caused by DOX is observed within 2-3 days of its single-dose administration related with excessive production of free radicals induced lipid peroxidation that ultimately caused injury to the membrane intergrity (14).

In our study, single large dose of DOX has significantly ($P<0.05$) elevated CK-MB, LDH, and Troponin I. Raised levels of CK-MB, LDH, and troponin I in serum are considered as vital indicators of myocardial injury (25) DOX

induced these biochemical changes that were brought back to close to normal in rats group treated with nanopropolis. The significant ($P<0.05$) increase in serum CK-MB, LDH, and troponin I in DOX treatment group correlates with the previous reports which suggest that DOX-induced oxidative stress can lead to lipid peroxidation that is accompanied by the release of these cardiac indicators into serum and these outcomes were in agreement with the previous works (26). Since DOX has markable antitumor effectiveness, novel methods to reduce or prevent its detrimental side effects are expected to increase its activity as anticancer agent. Propolis extract and its nanoformulas has also been demonstrated to exhibit notable antitumor effects (27; 28). Therefore, it was hypothesized that propolis may have synergistic antitumor effects with DOX, as well as ameliorate the Dox induced systemic toxicity.

The present study demonstrated that nanopropolis formulas, markedly minimized the cardiomyotoxicity induced by high dose DOX administration. Pretreatment with nanopropolis ameliorated the DOX-induced cardiac injury via lowering the serum levels of CK-MB and Troponin I, and LDH as well as the However, the mechanism underlying the effects of propolis formulas is not completely clear, The protective effects of propolis pre-treatment could be due to increase in the antioxidant enzyme activities result in suppression of the oxidative stress damage of DOX in cardiomyocytes (29).

The outcome of experiment treatment maneuvers of induced DOX rats by nutraceutical and Nano nutraceutical formulas for 10 days showed markedly inhibit The superior healthiness recovery indices of the heart in Nano formulated nutraceutical was may be attributable to the incorporation of these drugs in a suitable vesicle carrier for their efficient dissemination in the body by increasing their concentrations at targeted cardiac cells and decreasing the accumulations of drugs to the non-targeted areas. This mechanism can aid in increasing solubility, stability, toxicity protection, pharmacological activity, and sustained delivery (30). Due to drug accumulation, ideally at the targeted location, the nano size carrier also improved the drug's pharmacokinetic, pharmacodynamic, and in vitro stability. This improves the biodistribution and retention effect of the drug (31). Several compounds with antioxidant properties have proven to be cardioprotective in in vitro and in vivo trials (32).The particle size in nanopropolis is very small, so active ingredient remaining in nanopropolis can log into the network with ease, propolis which was active against cardiotoxicity is caused by the presence of the active components in propolis prevent harmful effects of DOX.

REFERENCES

1. Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Pinzaru, I. (2021). Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy. Molecules, 26(4), 1109-1139.
2. Lipshultz SE, Franco VI, and Miller TL.(2018). Cardiovascular disease in adult survivors of childhood cancer. Annu Rev Med; 66:161–176.
3. Force T, Wang Y. (2013). Mechanism-based engineering against anthracycline cardiotoxicity. Circulation. 128: 98–100.
4. Fadillioglu, E., Oztas, E., Erdogan, H., Yagmurca, M., Sogut, S., Ucar, M., & Irmak, M. K. (2004). Protective effects of caffeic acid phenethyl ester on doxorubicin-induced cardiotoxicity in rats. Journal of Applied Toxicology, 24(1), 47– 52.
5. Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., Dash, C. K. (2018). Composition and functional properties of propolis (bee glue): A review. Saudi Journal of Biological Sciences.
6. Zidan, Omar Helmi. (2020). PEGylated chitosan / doxorubicin nanoparticles and conjugated with monoclonal antibodies for breast cancer therapy. American University in Cairo, Master's Thesis. AUC Knowledge Fountain.
7. Yuan, Y., Cai, T., Xia, X., Zhang, R., Chiba, P., & Cai, Y. (2016). Nanoparticle delivery of anticancer drugs overcomes multidrug resistance in breast cancer. Drug Delivery, 23(9), 3350–3357.
8. Al-mohana, Al-Mohana, A.(2004). A study of activity of the local alcoholic propolis extract in the treatment of the external wounds that experimentally infected with some pathogenic bacteria and fungi in mice.Master thesis. college of veterinary medicine at the university of Baghdad.
9. Boufadi, Y., Soubhye, J., Riazi, A., Rousseau, A., Vanhaeverbeek, M., Nève, J., Van Antwerpen, P. (2014). Characterization and Antioxidant Properties of Six Algerian Propolis Extracts: Ethyl Acetate Extracts Inhibit Myeloperoxidase Activity. International Journal of Molecular Sciences, 15(2), 2327–2345.
10. Ramana, M.V. Chaudhari, A.D. Himaja, M. Satyanarayana, D. and Dua, K. (2017). An

approach to minimize Pseudomembranous colitis caused by clindamycin through liposomal formulation. Indian Journal of Pharmaceutical Sciences 69: 384-389.
11. Barsola, B and Kumari, P. (2022). Green synthesis of nano-propolis and nanoparticles (Se and Ag) from ethanolic extract of propolis, their biochemical characterization: A review" Green Processing and Synthesis, vol. 11, no. 1, pp. 659-673.
12. Miyataka, H. Nishlkl, M. Matsumoto, H. FujImoto, T. Matsuka, M. and Satoh, T. (1997). Evaluation of Brazilian and Chinese Propolis by Enzymatic and Physico-Chemical Methods. Biological & Pharmaceutical Bulletin. 20: 496-501.
13. Izgü F, Bayram G, Tosun K, İzgü D. (2017). Stratum corneum lipid liposome-encapsulated panomycocin: preparation, characterization, and the determination of antimycotic efficacy against Candida spp. isolated from patients with vulvovaginitis in an in vitro human vaginal epithelium tissue model. Int J Nanomedicine.
14. Ahmed AZ, Satyam SM, Shetty P, D'Souza MR. (2021). Methyl Gallate Attenuates Doxorubicin-Induced Cardiotoxicity in Rats by Suppressing Oxidative Stress. Scientifica (Cairo). 2021 Jan 13;2021:6694340.
15. Al-Rubii, A.K. (2018). Effect of Tribulus terrestris L. extract on prostatic and testicular functions of castrated rabbits. M.Sc thesis. College of science. Baghdad University. Baghdad. Iraq.
16. Kong, A.S.-Y.; Lai, K.S.; Hee, C.-W.; Loh, J.Y.; Lim, S.H.E.; Sathiya, M. (2022). Oxidative Stress Parameters as Biomarkers of Cardiovascular Disease towards the Development and Progression. Antioxidants, 11, 1175.
17. Abdel-Daim, M. M., kilany, O. E., Khalifa, H. A., & Ahmed, A. A. M. (2017). Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemotherapy and Pharmacology, 80(4), 745–753. doi:10.1007/s00280-017-3413-7.
18. Zulhendri, F. Perera,O. Chandrasekaran,K. Ghosh, A. Tandean,S. Abdulah,R Herman,H Lesmana,R. (2022). Propolis of stingless bees for the development of novel functional food and nutraceutical ingredients: A systematic scoping review of the experimental evidence, Journal of Functional Foods, Volume 88
19. Rawat, P. S., Jaiswal, A., Khurana, A., Bhatti, J. S., & Navik, U. (2021). Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. Biomedicine & Pharmacotherapy, 139, 111708.
20. Chen, X., Zhang, Y., Zhu, Z., Liu, H., Gou, H., Xiong, C., Su, S. (2016). Protective effect of berberine on doxorubicin-induced acute hepatorenal toxicity in rats. Molecular Medicine Reports, 13(5), 3953–3960.
21. Danese, E., and Montagnana, M. (2016). An historical approach to the diagnostic biomarkers of acute coronary syndrome. Annals of Translational Medicine, 4(10), 194–194.
22. Radeva-Ilieva, M. P., Georgiev, K. D., Hvarchanova, N. R., Stoeva, S. S., Slavov, I. J., Dzhenkov, D. L., & Georgieva, M. P. (2020). Protective Effect of Methylxanthine Fractions Isolated from Bancha Tea Leaves against Doxorubicin-Induced Cardio- and Nephrotoxicities in Rats. BioMed Research International, 2020, 1–9.
23. Podyacheva EY, Kushnareva EA, Karpov AA and Toropova YG. (2021). Analysis of Models of Doxorubicin-Induced Cardiomyopathy in Rats and Mice. A Modern View From the Perspective of the Pathophysiologist and the Clinician. Front. Pharmacol. 12:670479.
24. Abd Elbaky NA, A.A. Ali and Ahmed R.A, (2010). Cardioprotective Effect Of Simvastatin On Doxorubicininduced Oxidative Cardiotoxicity In Rats. Journal of Basic and Applied Sciences, 6, (1): 29-38.
25. Singal, P. Li, T. Kumar, D. Danelisen, I. and Iliskovic, N.(2000). Adriamycin-induced heart failure: mechanisms and modulation," Molecular and Cellular Biochemistry, vol. 207, no. 1-2, pp. 77–86.
26. Billingham, M.E. J.W. Mason, M.R. Bristow and J.R. Daniels, (1998). Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat. Rep., 62:865-872.
27. Swain, S. Whaley, F. Ewer, M. (2003). Congestive heart failure in patients treated with doxorubicin," Cancer, vol. 97, no. 11, pp. 2869–2879.
28. Kong, A.S.-Y.; Lai, K.S.; Hee, C.-W.; Loh, J.Y.; Lim, S.H.E.; Sathiya, M. (2022). Oxidative Stress Parameters as Biomarkers of Cardiovascular Disease towards the Development and Progression. Antioxidants, 11, 1175.
29. Mouse, H. A., Tilaoui, M., Jaafari, A., M'barek, L. A., Aboufatima, R., Chait, A., & Zyad, A. (2012). Evaluation of the in vitro and in vivo anticancer properties of Moroccan propolis extracts. Revista Brasileira de Farmacognosia, 22(3), 558–567.
30. Forma E. and Bryś,M. (2021). Anticancer activity of propolis and its compounds," Nutrients, vol. 13, no. 8, p. 2594.
31. Chopra, S., Pillai, K. K., Husain, S. Z., & Girl, D. K. (1998). Propolis Protects against Doxorubicin-Induced Myocardiopathy in Rats. Experimental and Molecular Pathology, 62(3), 190–198.
32. Singh, A. Ray, A. Mishra, R. Biswal, P. Yadar, R. and Ghatuary, K. (2020). Phyto-Phospholipid Complexes: Innovative Approach to Enhance the

Bioavailability and Therapeutic Efficacy of Herbal Extract. Pharmaceutical and Biosciences Journal 8: 01-09.
33. Babazadeh, A. Zeinali, M. and Hamishehkar, H. (2018). NanoPhytosome: A developing platform for herbal anti-cancer agents in cancer therapy. Current Drug Targets, 19: 170-180.
34. Nathalie Chahine, Jean Hanna, Hassane Makhlouf, Laurent Duca, Laurent Martiny & Ramez Chahine (2013). Protective effect of saffron extract against doxorubicin cardiotoxicity in isolated rabbit heart, Pharmaceutical Biology, 51:12, 1564-1571.

J Popul Ther Clin Pharmacol Vol 30(4):e230–e240; 16 March 2023.