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Cardioprotective effect of royal jelly on paclitaxel-induced cardio-toxicity in rats

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ABSTRACT

Objective(s): Paclitaxel is a potent chemotherapy agent with severe side effects, including allergic reactions, cardiovascular problems, complete hair loss, joint and muscle pain, which may limit its use and lower its efficiency. The cardioprotective effect of royal jelly was investigated on paclitaxel-induced damages.

Materials and Methods: Adult male Wistar rats were divided into control and test groups (n=8). The test group was assigned into five subgroups; 4 groups, along with paclitaxel administration (7.5 mg/kg BW, weekly), received various doses of royal jelly (50, 100, and 150 mg/kg BW) for 28 consecutive days. The last group received only royal jelly at 100 mg/kg. In addition to oxidative and nitrosative stress biomarkers, the creatine kinase (CK-BM) level was also determined. To show the cardioprotective effect of royal jelly on paclitaxel-induced damages, histopathological examinations were conducted.

Results: Royal jelly lowered the paclitaxel-elevated malondialdehyde and nitric oxide levels in the heart. Royal jelly could also remarkably reduce the paclitaxel-induced cardiac biomarker of creatine kinase (CK-BM) level and pathological injuries such as diffused edema, hemorrhage, congestion, hyaline exudates, and necrosis. Moreover, royal jelly administration in a dose-dependent manner resulted in a significant (P<0.05) increase in the paclitaxel-reduced total antioxidant capacity.

Conclusion: Our data suggest that the paclitaxel-induced histopathological and biochemical alterations could be protected by the royal jelly administration. The cardioprotective effect of royal jelly may be related to the suppression of oxidative and nitrosative stress.

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Introduction

Paclitaxel (Taxol ®, TXL), is a chemotherapy agent which is used for the treatment of breast, ovarian, lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers (1). It has also been used in Kaposi's sarcoma. Paclitaxel inhibits the growth of cancer cells and slows their spread in the body. The best-known mechanism of action of paclitaxel is provoking the tubulin polymerization to stable microtubules and also interacting directly with microtubules, stabilizing them against depolymerization. At the higher therapeutic concentrations, paclitaxel suppresses microtubule detachment from centrosomes, a process that is normally activated during mitosis (2).

Paclitaxel is an extremely potent chemotherapy agent, often producing a number of side effects in patients, including severe allergic reactions, cardiovascular problems, infections developing due to white blood cell deficiencies, hair loss, joint and muscle pain, irritation at the paclitaxel injection site, low red blood cell count, mouth or lip sore, stomach upset, and diarrhea (3). Some paclitaxel-induced side effects are so common and in some cases so severe that patients and their physicians may decide to reduce the dose or select another chemotherapy agent. While hitherto, medications designed to prevent or treat paclitaxel-induced nausea, vomiting and decreased white blood cell counts have been available, no treatments for other serious paclitaxel-

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induced nausea, vomiting and decreased white blood cell counts have been available, no treatments for other serious paclitaxel-induced side effects have been developed.

Royal jelly (RJ) is a honey bee secretion which is used for larvae and adult queens' nutrition. It is secreted from the glands in the hypopharynx of worker bees and fed to all larvae in the colony. It contains about 60% to 70% water, 12% to 15% proteins, 10% to 16% sugar, 3% to 6% fats, and 2% to 3% vitamins, salts, and amino acids (4). RJ exerts immunomodulatory and antibacterial effects as it contains biologically active chemicals such as 10-hydroxyl- 2-decenoic acid and antibacterial proteins (5). Its composition varies depending on geography and climate. There is evidence indicating that RJ increases energy, alleviates anxiety, sleeplessness, moodiness, and memory loss (6). One of the recently described side effects of paclitaxel is its cardiotoxicity (7). Hence, we in this investigation aimed to clarify the cardioprotective effect of RJ on paclitaxel-induced cardiotoxicity.

Materials and Methods

Chemicals

N-(1-naphthyl) ethylenediamine.2HCL and sulfanilamide were purchased from Sigma-Aldrich (Germany). 2, 4, 6-tri-2-pyridyl-1, 3, 5-triazin, thiobarbituric acid, phosphoric acid (85%), dimethyl sulfoxide (DMSO), and ethanol were obtained from Merck (Germany). N-butanol was obtained from Carl Roth, GmbH Co. (Germany). All other chemicals were commercial products of analytical grade. Royal jelly was collected from beehive no: 28 and 74, Sardrood, Hamedan province, Iran, during 2012 and kept at -20 ˚C until use. RJ was dissolved in distilled water and given orally.

Experimental design

Forty-eight adult male Wistar rats (200–220 g) in good health were purchased from the animal resource of the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The animals were acclimatized for one week and had free access to food and water. The experimental protocols were approved by the ethical committee of Urmia University based on principles of laboratory animal care. Animals were assigned into control and test groups (n=8). Animals in the test group were subdivided to the following groups: TXL group (received TXL 7.5 mg/kg BW, IP, each 7 days); T1, T2 and T3 groups (in addition to TXL received RJ (50, 100 and 150 mg/kg BW, orally at 11:00 am, daily) and T4 group, which only received RJ (100 mg/kg BW, orally at 11:00 am, daily).

The control group received only saline (0.9%, 5 ml/kg) during the experiment. Other groups received RJ and TXL for 4 weeks. The RJ dose levels were selected based on previous reports (8) and our primary pilot experiments.

Serum preparation and tissue sample collection

On day 29, blood samples were obtained from the heart under light anesthesia, which was induced by using diethyl ether. The blood samples were centrifuged at 3000 × g for 10 min to obtain the serum. The serum samples were then stored at -20 ˚C until further analysis.

The anesthetized animals were euthanized by using CO_2 gas. The heart specimens were immediately removed and rinsed with chilled saline. The heart sample from each individual rat was divided into two equal parts, one part was snap frozen in liquid nitrogen and kept in -70°C until further biochemical analysis and the other part was fixed in formalin solution (10%) for histopathological examinations.

Assessment of total antioxidant capacity (TAC)

To determine the impact of TXL administration and any beneficial effect of RJ on TAC, the reducing capacity of serum was measured. The assessment was performed based on ferric reduction antioxidant power (FRAP) assay (9). Briefly, at low pH, which was achieved using acetate buffer (300 mM, pH 3.6), reduction of Fe^{III}-TPTZ (2, 4, 6-tri-2-pyridyl-1, 3, 5-triazin, Merck, Germany) complex to the ferrous form produces an intensive blue color that could be measured at 593 nm. The intensity of the complex following addition of the appropriate volume of the serum to the reducible solution of Fe^{III}-TPTZ is proportional to total reducing power of the electron donating antioxidant. An aqueous solution of Fe^{II} ($FeSO_4.7H_2O$) and appropriate concentration of freshly prepared ascorbic acid were used as blank and standard solutions, respectively.

Determination of creatine-kinase (CK-MB) activity in serum

The CK activity was measured by using a hybrid form of CK-MB (TS.M.91.17.4, Parsazmun, Tehran, Iran), which is specifically for detection of enzyme activity in the heart (10). The elevated CK-BM activity is detectable in serum. Measurement was performed based on the kit manufacturer's instructions.

Cardiac Malondialdehyde (MDA) analysis

To determine the lipid peroxidation rate, the MDA content of collected heart samples was measured using the thiobarbituric acid (TBA) reaction as described previously (11). In short, 0.3–0.4 g of the heart samples were homogenized in ice-cold KCl (150 mM), and then the mixture was centrifuged at 3000×g for 10 min. Thereafter, 0.5 ml of the supernatant was mixed with 3 ml phosphoric acid (1% v/v) and then following vortex mixing, 2 ml of 6.7 g l^{-1} TBA was added to the samples. The samples

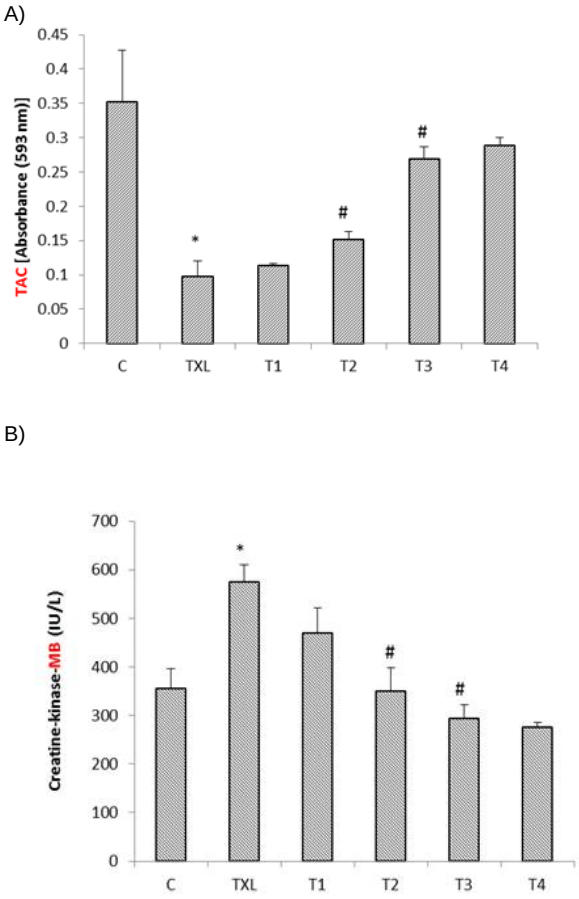

Figure 1. Effect of royal jelly on: (A) TXL-reduced TAC and (B) CK-BM activity in serum; data is given as mean±SD (n=8). * indicates a significant difference (P<0.05) between the control and non-treated TXL-received groups, and # represents significant differences between treated and non-treated TXL-received groups

were heated at 100 ˚C for 45 min and chilled on ice. Finally, N-butanol (3 ml) was added and the samples were further centrifuged at 3000 x g for 10 min again. The absorbance of the supernatant was measured spectrophotometrically at 532 nm and the MDA concentration was calculated according to the simultaneously prepared calibration curves using MDA standards. The amount of MDA was expressed as nmol per mg protein of the samples. The protein content of the samples was measured according to the Lowry method (12).

Nitric oxide measurement

The total nitrate/nitrite content of cardiac tissues was measured according to the Griess reaction (13). In Griess reaction, nitric oxide is converted into more stable nitrite, which in acidic environment is converted to HNO_2. In reaction with sulfanilamide, HNO_2 forms a diazonium salt, which reacts with N-(1-naphthyl) ethylenediamine.2HCL to form an azo dye that can be detected by absorbing at 540 nm wavelength. The NO content of the heart was expressed as nmol per mg of protein in samples.

Histopathological Examinations

Previously fixed heart samples in formalin solution (10%) were subjected to histological examinations. The paraffin-embedded sections (5-6 µm) were stained with hematoxylin and eosin and were analyzed under a light microscope using multiple magnifications.

Statistical analysis

For all numerical results mean and standard deviation of the measured parameters were calculated. The results were analyzed using the Graph Pad Prism software (version 2.01. Graph Pad software Inc. San Diego, California, USA). The comparisons among groups were made by analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. A *P*-value less than 0.05 was considered significant.

Results

RJ augmented TAC and attenuated the CK-BM activity of TXL-received animals

Total antioxidant power was measured in serum and the results revealed that the TXL administration resulted in a significant (P<0.05) reduction of TAC. While, RJ in a dose-dependent fashion protected from the TAC reduction (Figure 1-A). Although RJ alone also reduced the TAC but it was found statistically non-significant (P>0.05). To evaluate any possible myocardial disturbances due to the TXL-administration, CK-BM activity was measured in serum. Results showed a remarkable elevation of CK-BM activity in the TXL-treated animals, whereas RJ-received groups demonstrated a dose-dependent reduction of CK-BM activity (Figure 1-B). We failed to find any significant differences between the control group and those animals that received only RJ.

RJ lowered the TXL-elevated lipid peroxidation and nitric oxide concentration in the heart

To evaluate the effect of RJ administration on the TXL-induced lipid peroxidation, the concentration of lipid peroxidation end product (MDA) was measured in the heart. Results showed that the MDA content of the heart was significantly (P<0.05) elevated in the TXL-received (IP) group (8.18±0.91 *vs* the control group 3.75±0.35 nmol/mg of protein), while RJ at 100 and 150 mg/kg dose levels could lower significantly the lipid peroxidation rate (5.35±0.95 nmol/mg of protein in T3 group). Administration of RJ alone (100 mg/kg) also increased the MDA level compared to the control group (Figure 2-A). The NO content of the heart in the TXL-received animals was remarkably elevated (14.76±0.36 *vs* 10.0±0.56 nmol/mg of protein in the control group). All three given doses of RJ could reduce the TXL-induced NO concentration. RJ alone at 100 mg/kg dose level

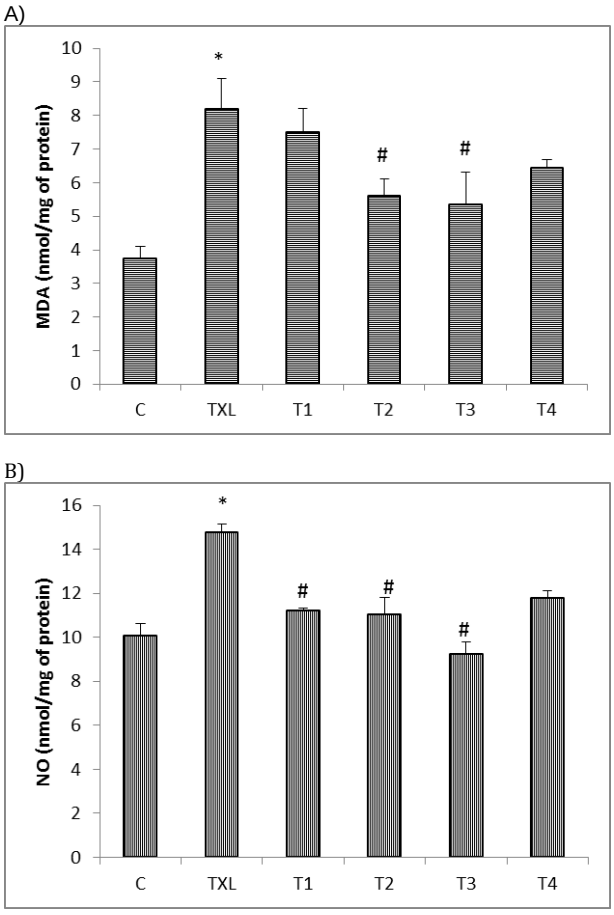

Figure 2: Effect of royal jelly on TXL-increased levels of: (A) MDA and (B) NO in the heart; data is given as mean±SD (n=8). * indicates a significant difference (P<0.05) between the control and non-treated TXL-received groups and # represents significant differences between treated and non-treated TXL-received groups

resulted in a slight but non-significant (P>0.05) elevation of NO content in the heart (Figure 2-B).

RJ improved the TXL-induced histopathological damages in the heart

Unlike the normal feature of cardiac muscles in the control animals (Figure 3-A), diffused edema, hemorrhage and congestion, hyaline exudates, and necrosis were found in the TXL-received rats (Figure 3-B). Although RJ administration relatively improved the cardiac damages, even at the high dose level of RJ (T3), there are still scattered edema and hyaline exudates (Figure 3-C, D, and E). There was no remarkable difference between the control and the RJ-alone-received animals (Figure 3-F).

Discussion

This study showed that during 4 consecutive weeks TXL-administration resulted in a remarkable augmentation of lipid peroxidation along with NO level elevation in the heart. Moreover, TXL-received animals showed a significant increase in serum levels of CK-MB. Histopathological examinations, on the other hand, revealed severe congestion and necrosis in the heart of TXL-treated animals. In animals that received RJ along with TXL, all indices that are used to monitor the TXL-induced impacts on the heart, show a dose-dependent cardioprotective effect of RJ.

Paclitaxel has been described as one of the most commonly used chemotherapeutic agents, whose cytotoxic mechanism still remains a controversial scientific debate. Its antitumor effect may result from interference with the normal function of microtubules and inhibition of cell cycle from rogression in late G2-M phases (14). We in our *in vivo* study tried to show TXL-induced impacts in the heart tissue, as previously cardiac arrhythmia has been reported one of the common side effects of TXL, which limits its administrations (7). Recently, it has been shown that ROS and reactive nitrogen species are involved in taxol-induced apoptosis (15).

Moreover, using several human cancer cell lines, it has been concluded that the cellular TAC is a critical determinant of cellular sensitivity to paclitaxel (16). Our results indeed confirmed the previous reports indicating the crucial role of oxidative and nitrosative stress in TXL-induced damages and extended it to the heart tissue, which may partly contribute in the mechanism of TXL-induced cardiotoxicity. We showed a significant elevation of MDA in the heart of animals that received TXL, suggesting an increase of lipid peroxidation. Cell membranes contain a variety of polyunsaturated fatty acids, in which an attack of one reactive free radical can convert multiple fatty acid side chains into lipid peroxides, making the membrane leaky and eventually causing breakdown of the membrane (17). As the second goal of the current study, we investigated the cardioprotective effect of RJ and the obtained results showed that RJ in a dose-dependent fashion was able to reduce the TXL-induced lipid peroxidation rate, suggesting its anti-lipid peroxidation property. The antioxidative effect of RJ on cisplatin-induced damages in the testis and paracetamol-induced hepatic injuries have been previously reported (8, 18). Moreover, our results are supported by the previous findings, which showed the ameliorative effects of the RJ supplementation in rats against gamma irradiation-induced injuries in the heart tissue (19).

An interesting finding of this experiment was a significant elevation of MDA in the animals that were only treated with RJ. To explain this finding, one should note that RJ acts as a known antioxidant and there are two basic mechanisms for the action of antioxidants: mechanism of removal of ROS initiators and a chain breaking mechanism. Removal of ROS initiators is mainly based on the inhibition of the enzymes involved in the generation of ROS such as xanthine oxidase and lipoxygenase (20). On the other hand, in the chain breaking mechanism, antioxidants

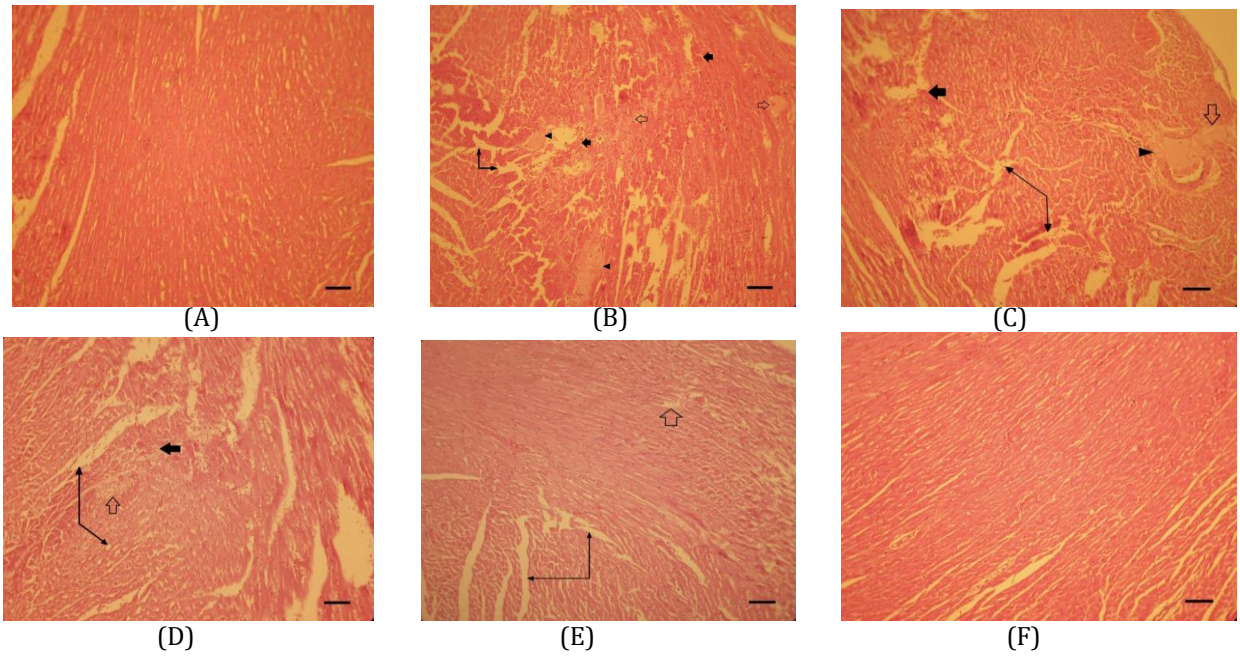

Figure 3. Photomicrograph of the rat heart; (A) normal appearances of cardiac structure, (B) represent the cardiac injuries due to the TXL administration and diffused edema (thin arrows), hemorrhage and congestion (bold arrows), hyaline exudates (hollow arrows), and necrosis (arrowheads) are observed. (C, D and E) are showing the cardioprotective effect of various dose levels of royal jelly. There are scattered edema and hyaline exudates. (F) no remarkable difference in the cardiac structure between the control and the royal jelly-alone-received animals are seen (H& E staining and scale bar: 100 µm)

scavenge free radicals by donating an electron to neutralize them. Our results from lipid peroxidation experiment indicate that RJ most likely acts via the chain breaking mechanism and due to extra electron donation (when only RJ was administered for a long time); we witnessed the extra ROS generation and consequently more lipid peroxidation. There is evidence indicating that vitamin E and vitamin C as endogenous and exogenous antioxidants are the main vitamins in the composition of RJ and work mainly through the chain breaking mechanism, which prevents the peroxidation of membrane phospholipids (21, 22).

Determination of nitric oxide concentration in the heart samples was the second biomarker that was used to evaluate the TXL-induced impact on the heart tissue and consequently the possible cardioprotective effect of RJ. Results indicated that the TXL administration significantly elevated the NO level while RJ was able to protect from the aforementioned increase of NO. It is clear that NO plays a crucial role in various autoimmune and inflammatory diseases. Increased level of NO in the heart tissue following the TXL administration could be explained by the pathological findings such as diffused edema, hemorrhage and congestion, hyaline exudates and necrosis, suggesting an inflammation state in the TXL-received animals. During the inflammatory response due to the release of an excessive amount of $NO^{\bullet}$ and O_2 from activated macrophages and neutrophils, a potent and destructive metabolite of peroxynitrite ($ONOO^-$) is produced, which in turn results in more tissue damages. Our results at the same time showed that RJ both protected from the TXL-induced pathological injuries and declined significantly the NO content of the heart tissue, suggesting its cardioprotective effect at least partly via anti-nitrosative capacity. Anti-nitrosative effect of RJ on excessive production of NO in ram sperms stored at 4°C and in the testis tissue of rats that were treated with TXL has been recently reported (23, 24).

During recent decades, so many researchers used many markers to evaluate the oxidative stress-related damages in various tissues, including measurement of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin, and metallothioneins (25). Miller *et al*, (1993) introduced a new method, which measures the total antioxidant status, with the major advantage of measuring the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound (26). We –in this study– used the TAC measurement to show the TXL-induced oxidative stress and equally antioxidant potency of RJ in the reduction of TXL-induced oxidative stress. Involvement of oxidative stress in TXL-induced apoptosis of lymphoma cells has previously been

reported (27). At the same time, antioxidant effect of RJ on cisplatin-induced damages in the testis and on paracetamol-induced liver damages has been documented (8, 18).

Another finding of the current study is a dose-dependent protective effect of RJ on TXL-elevated serum level of CK-MB. Serum level of CK-MB is extensively used in daily practice as a known biomarker in the diagnosis of cardiac necrosis and toxicity. The TXL-elevated level of CK-MB and TXL-induced myocardial necrosis has been previously shown in the rodents (28). Moreover, we showed that RJ administration remarkably could protect from TXL-induced myocardial necrosis and other pathological damages along with a significant reduction of CK-MB levels; these two findings support the cardioprotective effect of RJ on TXL-induced injuries. In agreement with our findings, there are studies indicating that doxorubicin-induced cardiotoxicity was prevented through suppression of oxidative and nitrosative stress by using phytochemicals with high antioxidant capacity (29).

Conclusion

We in this study, for the first time, showed the protective effect of RJ on TXL-induced cardiotoxicity, which is characterized by biochemical changes such as elevation of cardiac lipid peroxidation rate and NO content, serum level of CK-BM, reduction of TAC, and histopathological injuries including myocardial necrosis, diffused edema and congestion. Our findings may help to develop new RJ-based therapeutic agents for patients that essentially have to use chemotherapy agents such as paclitaxel.

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References

1. Rowinsky EK, Donehower RC .The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmacol Ther 1991; 52:35-84.
2. Needleman DJ, Ojeda-Lopez MA, Raviv U, Ewert K, Miller HP, Wilson L, *et al.* Radial compression of microtubules and the mechanism of action of taxol and associated proteins. Biophys J 2005; 89:3410–3423.
3. Onetto N, Canetta R, Winograd B, Catane R, Dougan M, Grechko J, *et al.* Overview of Taxol safety. J Natl Cancer Inst Monogr 1993; 15:131–139.
4. Nagai T, Inoue R. Preparation and functional properties of water extract and alkaline extract of royal jelly. Food Chem 2004; 84:181–186.
5. Caparica-Santos C, Marcucci MC. Quantitative determination of trans-10- hydroxy-2-decenoic acid (10-HDA) in Brazilian royal jelly and commercial products containing royal jelly. J Apicultural Res 2007; 46:149–153.
6. Matsui T, Doi YA, Sugimoto S, Yamada H, Matsumoto HK. Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. J Nutr Biochem 2002; 13:80–86.
7. Zhang K, Heidrich FM, De Gray B, Boehmerle W, Ehrlich BE. Paclitaxel accelerates spontaneous calcium oscillations in cardiomyocytes by interacting with NCS-1 and the InsP3R. J Mol Cell Cardiol 2010; 49:829–835.
8. Silici S, Ekmekcioglu O, Ersalan G, Demirtas S. Antioxidative effect of royal jelly in cisplatin-induced testes damage. J Urol 2009; 74:545-551.
9. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth Enzymol 1999; 299:15-27.
10. Apple FS, Murakami MM. Cardiac troponin and creatine kinase MB monitoring during in-hospital myocardial reinfarction. Clin Chem 2005; 51:460-463.
11. Niehaus WG, Samuelsson JRB. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6:126–130.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.
13. Green LC, Wagne, AD, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982; 12:131-138.
14. Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem: Anticancer Agents 2002; 2:1-17.
15. Lin HL, Liu TU, Chau GY, Lui WY, Chi CW. Comparison of 2-methoxyestradiol-induced, docetaxel-induced, and paclitaxel-induced apoptosis in hepatoma cells and its correlation with reactive oxygen species. Cancer 2000; 89:983-994.
16. Ramanathan B, Jan K, Chen C, Hour T, Yu H, Pu Y. Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res 2005; 65:8455-8460.
17. Schafer FQ, Qian SY, Buettner GR. Iron and free radical oxidations in cell membranes. Cell Mol Biol 2000; 46:657–662.
18. Kanbur M, Erasian G, Beyaz L, Silici S, Liman BC, Altinordulu S, *et al.* The effects of royal jelly on liver damage induced by paracetamol in mice. Exp Toxicol Pathol 2009; 61:123-132.
19. Azab KS, Bashandy M, Salem M, Ahmed O, Tawfik Z, Helal H. Royal jelly modulates oxidative stress and tissue injury in gamma irradiated male Wister Albino rats. N Am J Med Sci 2011; 3: 268–276.
20. Borges F, Fernandes E, Roleira F. Progress Towards the discovery of xanthine oxidase inhibitors. Curr Med Chem 2002; 9:195–217.
21. Maguire JJ, Wilson DS, Packer L. Mitochondrial electron transport-linked tocopheroxyl radical reduction. J Biol Chem 1989; 264:21462–21465.
22. Mellio E, Chinou I. Chemistry and bioactivity of royal jelly from Greece. J Agric Food Chem 2005; 53:8987-8992.
23. Moradi AR, Malekinejad H, Farrokhi-Ardabili F, Bernousi I. Royal Jelly improves the sperm parameters of

ram semen during liquid storage and serves as an antioxidant source. Small Rumin Res 2013; 113:346-352.
24. Delkhoshe-Kasmaie F, Malekinejad H, Khoramjouy M, Rezaei-Golmisheh A, Janbaze-Acyabar H. Royal jelly protects from taxol-induced testicular damages via improvement of antioxidant status and up-regulation of E2f1. Syst Biol Reprod Med 2014; 60:80-88.
25. Ferrari CKB. Oxidative stress pathophysiology: Searching for an effective antioxidant protection. Int Med J 2001; 8:175-184.
26. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 1993; 84:407-412.
27. Wang YF, Chen CY, Chung SF, Chiou YH, Lo HR. Involvement of oxidative stress and caspase activation in paclitaxel-induced apoptosis of primary effusion lymphoma cells. Cancer Chemother Pharmacol 2004; 54:322-330.
28. Sherif Y, Saad Tawfeeg A, Najjar O, Alashari M. Cardiotoxicity of doxorubicin/paclitaxel combination in rats: effect of sequence and timing of administration. J Biochem Mol Toxicol 2004; 18:78-86.
29. Andreadou I, Sigala F, Iliodromitis EK, Papaefthimiou M, Sigalas C, Aligiannis N, *et al.* Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. J Mol Cell Cardiol 2007; 42:549–558.