ORIGINAL ARTICLE

AL-ANBAR MEDICAL JOURNAL
Anb. Med. J. 21(2): 97–103, 2025

Role of Bee Propolis and Vitamin E in Attenuating Doxorubicin-induced Hepatic Toxicity in Rats

Hodhaifa Abdul Mohsin,[1] Mohannad E. Qazzaz,[1,*] and Mohanad Alfahad[2]

[1] *Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Mosul, Mosul, Iraq.*
[2] *Department of Pharmaceutics, College of Pharmacy, University of Mosul, Mosul, Iraq.*
(Received : 1 October 2024; Accepted : 19 January 2025; First published online: 11 March 2025)

ABSTRACT

Background: Doxorubicin (Dox) is a powerful chemotherapy medication in the anthracycline antibiotic group. However, due to its substantial toxic side effects in various non-target organs, its clinical application is restricted, which leads to hepatotoxicity and cardiotoxicity.
Objectives: To investigate the role of vitamin E and bee propolis as protective agents to ameliorate Dox-induced hepatic toxicity.
Materials and methods: A total of 50 adult male albino rats weighing 240±55g were randomly divided into seven groups. The time interval for the experiment was extended to 22 days. Liver function tests and histopathological inspection were performed according to documented protocols. At the end of the experiment, blood samples were collected, and serums were separated, numbered and used to assess the liver functions, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum albumin, and total serum bilirubin. Also, the liver was preserved and later examined microscopically with H & E stains.
Results: A severe decline in albumin levels was observed with 20 mg/kg of Dox, compared to the control, confirming the damaging effect of Dox on the hepatocyte-producing ability of albumin. The bee propolis or vitamin E showed significant protective effects on the liver by keeping the normal levels of total bilirubin, AST, ALT, and ALP compared to the control. Furthermore, the bee propolis or vitamin E in combination with Dox showed non-significant changes in the level of albumin compared to the Dox group. The histological investigation was in parallel with biochemical data in confirming the protective effects of bee propolis and/or vitamin E against Dox-induced hepatic toxicity.
Conclusion: The administration of specific drugs, like bee propolis and vitamin E, has demonstrated a protective effect against Dox-induced toxicity.
Keywords: Doxorubicin; Bee propolis; Vitamin E; Liver Function; Doxorubicin-induced hepatic toxicity.

DOI: 10.33091/amj.2025.154646.1968

INTRODUCTION

Doxorubicin (Dox) is effective in treating various tumours, including solid and haematological types such as breast cancer and paediatric leukaemia [1]. The liver serves as a key organ in the metabolism and detoxification of medications, the excretion of wastes in bile, and several other crucial functions [2]. The hepatotoxic mechanisms of Dox are primarily due to the creation of highly reactive chemicals formed from diatomic oxygen by the Dox throughout its hepatic breakdown. These highly reactive chemicals result in oxidative stress because of imbalanced redox potential and decreased antioxidant enzyme levels, inflammation, apoptosis, and mitochondrial dysfunction [3].

Bee propolis is a lipophilic substance that is naturally hard and brittle [4]. Bees extract propolis, a substance resembling resin, from the buds of poplar and cone-bearing trees [4]. It

* Corresponding author:E-mail:Mohannad.qazzaz@uomosul.edu.iq

may contain byproducts from bee colonies and is used by bees to construct hives. It appears that propolis aids in the defence against fungi, viruses, and bacteria. It may also help skin recover and have anti-inflammatory properties [4]. Typically, raw propolis contains between 50 and 70% resins, 30 to 50% waxes and essential oils, 5 to 10% pollen, and approximately 5% of various other chemical components [5].

Vitamin E refers to a group of fat-soluble chemicals with distinct antioxidant properties that Evans and Bishop discovered in 1922 and are vital to human health. Numerous diseases, including atherosclerosis, oxidative stress, cancer, cataracts, and Alzheimer's disease, are combated significantly by the antioxidative properties of vitamin E [6].

As far as we know, this work is novel in showing that taking vitamin E and bee propolis together can synergistically protect against Dox-induced hepatic toxicity. Therefore, this study aimed to ascertain the potential protective effect of vitamin E or bee propolis in acute Dox-induced hepatotoxicity and to investigate the synergistic protective effect of bee propolis and vitamin E as a combination therapy to decrease the toxicity of Dox on the liver.

MATERIALS AND METHODS

Materials

Dox injections were obtained from STADAPHARM GmbH, Germany and dissolved in saline to make an appropriate dose for administration [7]. Bee propolis 2000mg capsules from NATURAL ANSWERS, UK and vitamin E from PIONEER, Iraq was prepared in distilled water by mixing it at 25°C.

Study Approval

This experimental study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine at the University of Mosul in Iraq to conduct this study. The ethical approval reference number was UM.VET.2022.020. The study was conducted at the University of Mosul, College of Pharmacy. The current study was conducted from December 2023 to July 2024.

Animal Groups

The animals were divided as follows:

1. Group 1 (Control): Animals received distilled water orally for 22 days.
2. Group 2 (Dox): Animals received distilled water orally for 22 days and a single intraperitoneal dose (20 mg/kg) of Dox on day 21 [8, 9].
3. Group 3 (Bee propolis): Animals received 100 mg/kg of bee propolis, [10] Orally for 22 days.
4. Group 4 (Vitamin E): Animals received 100 mg/kg of vitamin E orally for 22 days [8, 11].
5. Group 5 (Dox and Bee propolis): Animals received 100 mg/kg of bee propolis orally for 22 days and a single intraperitoneal dose of Dox on day 21.
6. Group 6 (Dox and Vitamin E): Animals received 100 mg/kg of vitamin E orally for 22 days and a single intraperitoneal dose of Dox on day 21.
7. Group 7 (Dox, Bee propolis and Vitamin E): Animals received 100 mg/kg of bee propolis, and 100 mg/kg of vitamin E orally for 22 days and a single intraperitoneal dose of Dox on day 21.

Experiment Protocol (Figure 1).

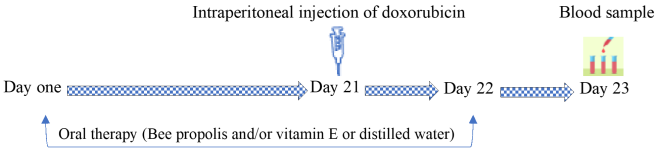

Figure 1. Experimental study protocol describing the administration schedule throughout the study period.

Biochemical and histopathological tests

In vitro test for quantitatively determining alkaline phosphatase (ALP) in rats' serum and plasma on Roche/Hitachi cobas c systems. Colourimetric assay by a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance [12]. ALP levels in rats normally range between 40-275 IU/L.

Alanine transaminase (ALT): This assay follows the recommendations of the IFCC but was optimized for performance and stability. ALT catalyses the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalysed by lactate dehydrogenase to form L-lactate and NAD+. The rate of NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance [12]. ALT levels in rats normally range between 15–70 IU/L.

Aspartate transaminase (AST): In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates on Roche/Hitachi basic systems. This assay follows the recommendations of the IFCC but was optimized for performance and stability. AST in the sample catalyses the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH) to form NAD+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance [12]. AST levels in rats normally range between 30-100 IU/L. Colorimetric diazo method for total bilirubin was used, in the presence of a suitable solubilizing agent, coupled with 3,5-dichloro phenyl diazonium in a strongly acidic medium. The color intensity of the redazo dye formed is directly proportional to the total bilirubin and can be determined photometrically [13]. Total bilirubin and albumin levels in rats normally range between 0.1–0.4 mg/dL and 3–5 g/dL respectively.

Tissue preparation and staining for histopathology have been done, according to AT Feldman and D Wolfe, 2014 [14].

Statistical analysis

The data was presented as means ± standard deviations (SD). A GraphPad Prism application (version 8.0.1) was used for statistical analysis (Graph Pad, San Diego, CA). An ordinary one-way ANOVA was used, followed by a Tukey's post hoc test against control values.

Protection rate (%) = [(G2 - G5 or G6 or G7) / G2] 100.

http://doi.org/10.33091/amj.2025.154646.1968

When the P-value < 0.05, the difference is considered significant.

RESULTS

A. Biochemical data

1. AST

Compared to the control group, Dox increased the level of AST in the second group (G2) by 63%, and this is considered preliminary evidence of a disturbance in liver function as a result of the use of Dox (control, 60.70±1.7 vs. Dox, 99.23±14.52) (Figure 2, A). However, the bee propolis group (G3) and vitamin E group (G4) don't show a significant change in the level of AST compared to the control (G1, 60.70±1.7 vs. G3, 59.05±11.67 and G4, 59.58±10.04). The bee propolis + Dox (G5), vitamin E + Dox (G6) and bee propolis + vitamin E + Dox (G7) groups showed significant protection effects on the liver by keeping the normal level of AST compared to control (G1, 60.70±1.7 vs. G5, 67.9±15.19, G6, 72±6.13 and G7, 72.07±10.43) (Figure 2, A).

2. ALT

A dose of 20 mg/kg of Dox showed a significant increase (78%) in the level of ALT in G2 compared to G1 (control, 60.70±1.7 vs. G2, 108.1±8.26; Figure 2, D). The safety of bee propolis and vitamin E is confirmed by the insignificant effect on the ALT level in G3 and G4 compared to G1 (G1, 60.70±1.7 vs. G3, 59.04±11.68 and G4, 59.88±4.393). The protective effect of tested agents in treated groups is clearly shown by a significant decrease in ALT levels in G5, G6 and G7 compared to G2 and its approach to normal levels compared to the control group (G1, 60.70±1.7 vs. G5, 83.24±3.4, G6, 89.98±3.07 and G7, 70.36±6.93). Regarding potency, there is no significant difference in the level of ALT between G5 and G6. Interestingly, the combination therapy of vitamin E and bee propolis showed a potent synergistic protective effect compared to bee propolis and vitamin E separately (Figure 2, D).

3. ALP

The pathological effect of Dox on the liver appeared clearly in the second group through a sharp rise in the ALP level (50%) compared to the control group (G1, 261.2±9.535 and G2, 392.1±24.33; Figure 2, B). Still, both bee propolis and vitamin E showed safety features through the insignificant changes in the level of ALP compared to the control (G1, 261.2±9.535; G3, 248.3±11.5 and G4, 293.6±12.62). Both bee propolis and vitamin E administration in combination with Dox, G5 and G6, respectively, keep the level of ALP within normal range, representing the defensive ability of these therapies against the hepatotoxic induced by Dox (G5, 238.6±10.32 and G6, 241.2±20.19). As in ALT, combination therapy of 100 mg/kg of bee propolis and vitamin E demonstrated a remarkable synergistic effect in keeping the low level of ALP, as shown in G7 compared to G5 and G6. Furthermore, the level of ALP was significantly lower than that in the control group (G1, 261.2±9.535 vs G7, 173.6±12.37) (Figure 2, B).

4. Albumin

The serum level of albumin significantly decreased from 3.58±0.216 in the control group to 2.66±0.136 in G2, confirming the damaging effect of Dox on the hepatocytes-producing ability of albumin. Bee propolis and vitamin E showed no significant changes in the level of albumin as represented in the G3 and G4 compared to the control (G1, 3.58±0.216 vs G3, 3.75±0.191 and G4, 4.05±0.45, Figure 2, E). Furthermore, the bee propolis and vitamin E in combination with Dox, G5 and G6, respectively, showed a significant decrease in albumin levels compared to the control (G1, 3.58±0.216 vs G5, 2.95±0.258 and G6, 2.843±0.151), with non-significant changes in the same groups compared to G2 (G2, 2.66±0.136). However, the level of albumin is still showing insignificant changes in G7 in comparison to the control, which means the combination therapy of bee propolis and vitamin E synergistically works to overcome the hepatotoxic effect of Dox (G1, 3.58±0.216 vs G7, 3.15±0.1) (Figure 2, E).

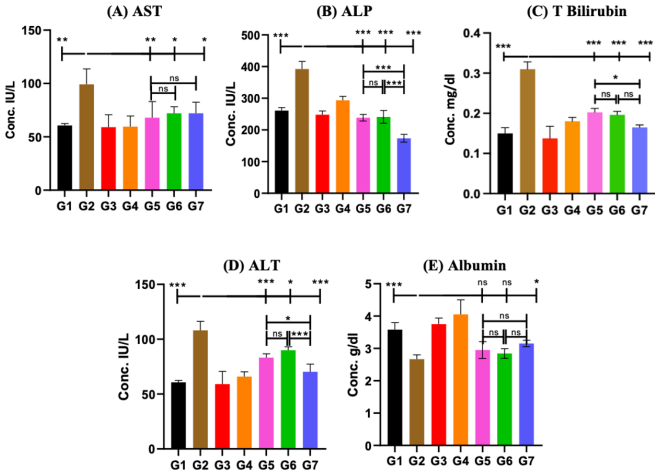

Figure 2. The bee propolis and vitamin E effect against Doxorubicin (Dox) effects on serum parameters. Serum levels are assessed following a single dose of Dox (20 mg/kg) or placebo, as well as with or without bee propolis (100 mg/kg orally daily) and/or vitamin E (100 mg/kg orally daily). The data are presented as mean ± standard deviation. * Indicates P-value < 0.05, ** indicates P-value < 0.01 and *** indicates P-value < 0.001. An ordinary one-way ANOVA was used, followed by Tukey's post hoc test against control values. (G1: Control, G2: Doxorubicin, G3: Bee Propolis, G4: Vitamin E, G5: Doxorubicin + Bee Propolis G6: Doxorubicin + Vitamin E, G7: Doxorubicin + Vitamin E + Bee Propolis).

5. Total Bilirubin

The serum level of bilirubin in G2 was significantly increased by 106% compared to the control, Figure 2, C, (G1, 0.15±0.014 vs G2, 0.31±0.018). Both bee propolis and vitamin E didn't conduct significant changes in levels of bilirubin in G3 and G4 (G1, 0.15±0.014 vs G3, 0.137±0.029 and G4, 0.18±0.01). In G5 and G6, bee propolis and vitamin E showed a significant decrease in the level of bilirubin compared to G2, which confirms the protective effects of these agents when used simultaneously with Dox (G2, 0.31±0.018 vs G5, 0.202±0.009 and G6, 0.196±0.008). However, there is no significant variation concerning potency between bee propolis and vitamin E as shown in the G5 and G6 comparison. Combination therapy of bee propolis and vitamin E, in group 7, kept the level of bilirubin close to normal level compared to the control (G1, 0.15±0.014 vs G7, 0.165±0.005).

Furthermore, combination therapy of bee propolis and vitamin E showed statistically significant potency in the comparison of bilirubin levels between G5 and G7 (G5, 0.202±0.009, and G7, 0.165±0.005) with nonsignificant reduction potency in the comparison of bilirubin level between G6 and G7 (G6, 0.196±0.008 and G7, 0.165±0.005) (Figure 2, C).

The rate of protection of tested agents against Dox-induced hepatotoxicity

The combination treatment of bee propolis with vitamin E achieved high protective effectiveness compared to the single treatments. However, an exception was observed with AST, in which it was shown that the effectiveness of bee propolis gave a better protective effect than vitamin E and the combination treatment of vitamin E and bee propolis. Although the differences between them were small (bee propolis, 31.57%; vitamin E, 27.44% and combination therapy, 27.37%) as shown in Table 1.

B. Histological data

Table 2 shows the histological changes in the liver according to 7 groups.

DISCUSSION

Despite being widely used in clinical oncology treatment because of its excellent therapeutic efficiency, Dox is thought to be the most damaging anthracycline on body tissues. Moreover, no drug has yet been licensed to protect against Dox's organ toxicities [15]. As a result, a lot of studies have been carried out to understand the Dox toxicity mechanisms and develop treatments, including antioxidants, that minimize this adverse effect. It has been documented that Dox seriously harmed the liver [16]. Dox-induced hepatic toxicity is primarily caused by increased oxidative stress, which results in the accumulation of free radicals in these tissues and the depletion of endogenous antioxidants [11, 17].

This study aims to evaluate the potential effectiveness of propolis or vitamin E, separately or in combination, to protect the liver from the toxic effects associated with the use of doxorubicin and to evaluate the synergistic preventive effectiveness resulting from these two substances. Propolis has received a lot of interest lately as a possible medicinal and preventive agent because of its many advantages, safety, and affordability. To the best of our knowledge, this work is the first to identify propolis and vitamin E's synergistic protective potential against Dox-induced liver toxicity. The elevated activities of serum ALT, AST, and ALP in the Dox group compared to the control one further supported our study's conclusion that Dox-induced liver damage occurred. Since hepatocytes are the primary target of reactive oxygen species attack, an increase in apoptotic processes in liver tissue may be the cause of the rise in these liver function indices [18]. Due to the abundance of AST and ALT enzymes in the liver, small elevations in these enzyme levels have been linked to myocardial infarctions as well as liver damage. Consistent with these findings, M. Sahlan et al. showed that Dox-induced increased AST and ALT activities could be linked to hepatocyte cell death brought on by oxidative stress, which would cause the hepatic cytosolic components to leak out, just like in the case of the heart [4].

In contrast, compared to the Dox group, propolis or vitamin E pre-treatment dramatically reduced serum levels of ALT, AST, and ALP. This could be because, as previously mentioned [19], propolis contains flavonoids with anti-inflammatory and antioxidant properties that help capture and neutralize free radicals before they cause liver damage. According to the research, propolis's IC_{50} value falls into the category of extremely potent antioxidants; in comparison, it differs only slightly from ascorbic acid's IC_{50} value. According to Jun et al., the classification of antioxidant strength levels is based on the following: Strong (IC_{50} < 50 ppm), strong enough (IC_{50} 50–100 ppm), moderate (IC_{50} 101–250 ppm), weak (IC_{50} 250–500 ppm), and extremely weak (IC_{50} > 500 ppm) [20]. This suggests that propolis at a dose of 100 mg/kg may have a hepato-protective effect, as the flavonoid components in it function as antioxidants to lower levels of hepatic enzymes. Furthermore, it is thought that propolis' antioxidants shield hepatocytes from Dox-induced lipid peroxidation [21].

Vitamin E exhibit potent free radical scavenging action, and it inhibits lipid peroxidation [22]. Additionally, it appears that vitamin E can prevent Dox-induced lipid peroxidation through an enzyme-based mechanism [23]. Our findings, which aligns with previous data, no discernible difference between the groups receiving Dox with vitamin E and the controls [16]. This shows that Dox's hepatotoxic and overall toxic effects may be inhibited by vitamin E. Furthermore, the study found that the combination of bee propolis and vitamin E enhanced the protective effect of the former, and that Dox-induced hepatotoxicity could be prevented by utilizing this combination therapy. This finding may pave the way for future research into this synergistic effect and the development of novel formulations that offer dual protective effects.

Further serum measurements were performed to assess the degree of hepatic toxicity and tissue damage. The results showed that the Dox-treated group's level of bilirubin was much higher than that of the control animals, indicating liver dysfunction [24]. Although bilirubin levels were significantly greater in the Dox-treated rats (G2) than in the control group, they were significantly lower in the propolis-treated rats (G5) and vitamin E-treated rats (G6) compared to G2. As new in this study, the combination treatment of vitamin E and bee propolis (G7) provided additional protection for the liver by maintaining the bilirubin level as close to the normal level as possible compared to the control group.

The exact mechanism of the synergistic protective effect between propolis and vitamin E, which was shown in this study,

Table 1. The protection rate of tested agents against Doxorubicin (Dox)-induced hepatotoxicity. The negative sign (–) indicates a decrease in the treated groups compared to the Dox group. Except for albumin, there is an increase in the treated groups compared to the Dox group. * Indicates P-value < 0.05, ** indicates P-value < 0.01 and *** indicates P-value < 0.001.
Protection rate (%) = [(G2 - G5 or G6 or G7) / G2] × 100.

Parameters	Bee propolis	Vitamin E	Bee propolis + vitamin E
AST	–31.57 %**	–27.44 %*	–27.37 %*
ALT	–22.99 %***	–16.76 %*	–34.91 %***
ALP	–39.14 %***	–38.48 %***	–55.72 %***
Albumin	10.90 %	6.87 %	18.42 %*
Total Bilirubin	–34.83 %***	–36.77 %***	–46.77 %***

http://doi.org/10.33091/amj.2025.154646.1968

Table 2. Comparison between groups in terms of severity of changes and therapeutic effects occurring in liver tissue. (–) indicates no change, (+) indicates mild change, and (++) indicates moderate.

Parameters	Groups						
	G1	G2	G3	G4	G5	G6	G7
Hepatic portal pattern	–	+	–	–	–	–	–
Hepatic sinusoids dilation	–	+	–	–	+	+	–
Fatty changes	–	–	–	–	–	–	–
Vacuolar degeneration and cell swelling	–	++	–	–	–	+	–
Pyknosis of nucleus	–	+	–	–	–	+	–
Necrosis	–	++	–	–	–	+	–
Apoptosis	–	+	–	+	+	+	–
Infiltration of inflammatory cells	–	++	–	–	–	+	–
Hemorrhage	–	–	–	–	–	–	–
Congestions	–	++	+	+	+	+	+
Hyperplasia of bile ducts	–	++	–	–	–	–	–
Hypertrophy of hepatocytes	–	+	–	–	–	–	–

is still unknown and needs more clinical and molecular study. However, in one research, comparisons were made in tissue concentrations of vitamin C, E, and lipid hydroperoxides in the presence of bee propolis [25]. This study found that the use of bee propolis may lead to an increase in the level of vitamin E in the plasma. Although this increase was not significant, it could be one of the mechanisms that contribute to the synergistic effect between bee propolis and vitamin E. The biochemical results agreed with our histology observations.

The histological characteristics of the livers in the Dox group of animals were consistent with earlier findings [26]. In alignment with biochemical data, propolis and vitamin E mitigate the majority of the histological alterations in the liver, leading to the restoration of the nearly normal architecture of the hepatic tissue in groups G5 and G6. Furthermore, the combination therapy of these agents demonstrated dramatic preservative effects on the hepatic tissues, suggesting the possible synergistic effects of using this combination therapy against Dox-induced hepatotoxicity. These histopathological results indicated that the endogenous antioxidant defence system and the histological structure were protected against Dox-induced liver injury by bee propolis and vitamin E. The histology evidence in this study is consistent with the results of Kaya et al. [27], who discovered that propolis protects rats against oxidative stress and hepatotoxicity caused by furans. This is also supported by Tanvir et al. [28]. Who found that the phenolic chemicals in propolis protected against tetracycline-induced hepatic and kidney damage. Additionally, an aqueous extract of propolis could reduce the damage and toxicity effects of octylphenol on liver cells, according to another study, owing to its antioxidant qualities [29]. Also, a microscopic examination in another study concluded that vitamin E significantly decreased Dox-induced hepatotoxicity in rats, which align with our finding [30]. Furthermore, Amirhosain et al. reported the same data, showing that vitamin E therapy could reduce Dox-induced hepatic lesions and protect the liver against vascular damage [31].

Two limitations of this study could be recognised. First, the study's small sample size, could limit how broadly the results can be applied. A limited sample size might not accurately reflect the general population; larger-scale research is needed to validate these results. The second limitation is the species specificity. Although rats are frequently employed in toxicity studies, their reactions to some treatments (such as vitamin E and bee propolis) may differ from those of humans. Therefore, human study is important to confirm these promising results.

CONCLUSION

The imbalance in the liver parameters observed in this study demonstrated how Dox use adversely affects the liver's vital functions. What makes this study novel? is the finding that the combine use of vitamin E and bee propolis together provides a synergistic protective effect, preventing Dox-induced damage.

ETHICAL DECLARATIONS

Acknowledgments

Our research group acknowledges the University of Mosul and the College of Pharmacy for assistance, guidance, and support.

Ethics Approval and Consent to Participate

This experimental study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine at the University of Mosul in Iraq to conduct this study. The ethical approval reference number was UM.VET.2022.020. Informed consent was not required for such study.

Consent for Publication

Not applicable.

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

No funding.

Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented to it being published.

REFERENCES

[1] P. Vejpongsa and E. T. H. Yeh. Prevention of anthracycline-induced cardiotoxicity: Challenges and opportunities. *J. Am. Coll. Cardiol.*, 64(9):938–945, 2014.

[2] L. P. Bechmann, R. A. Hannivoort, G. Gerken, G. S. Hotamisligil, M. Trauner, and A. Canbay. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J. Hepatol.*, 56(4):952–964, 2012.

[3] P. Prša *et al.* The potential use of natural products to negate hepatic, renal and neuronal toxicity induced by cancer therapeutics. *Biochem. Pharmacol.*, 173:113551, 2020.

[4] M. Sahlan *et al.* Potential hepatoprotective effects of flavonoids contained in propolis from South Sulawesi against chemotherapy agents. *Saudi Journal of Biological Sciences*, 28(10):5461–5468, 2021.

[5] T. Ozdal, F. D. Ceylan, Na. Eroglu, M. Kaplan, E. O. Olgun, and E. Capanoglu. Investigation of antioxidant capacity, bioaccessibility and LC-MS/MS phenolic profile of Turkish propolis. *Food Research International*, 122:528–536, 2019.

[6] N. A. Md Amin, S. H. Sheikh Abdul Kadir, A. H. Arshad, N. Abdul Aziz, N. A. Abdul Nasir, and N. Ab Latip. Are vitamin e supplementation beneficial for female gynaecology health and diseases? *Molecules*, 27(6):1896, 2022.

[7] T. Afsar, S. Razak, and A. Almajwal. Effect of Acacia hydaspica R. Parker extract on lipid peroxidation, antioxidant status, liver function test and histopathology in doxorubicin treated rats. *Lipids in Health and Disease*, 18(1):1–12, 2019.

[8] N. Hadi, N. G. Yousif, F. G. Al-Amran, N. K. Huntei, B. I. Mohammad, and S. J. Ali. Vitamin E and telmisartan attenuates doxorubicin induced cardiac injury in rat through down regulation of inflammatory response. *BMC Cardiovascular Disorders*, 12:1–7, 2012.

[9] P. L. Prasanna, K. Renu, and A. V. Gopalakrishnan. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. *Life sciences*, 250:117599, 2020.

[10] E. K. Mohamed, A. A. Osman, A. M. Moghazy, and A. A. S. Abdel Rahman. Propolis protective effects against doxorubicin-induced multi-organ toxicity via suppression of oxidative stress, inflammation, apoptosis, and histopathological alterations in female albino rats. *Biointerface Research in Applied Chemistry*, 12(2):1762–1777, 2022.

[11] T. Donia, S. Eldaly, and E. M. M. Ali. Ameliorating oxidative stress and inflammation by Hesperidin and vitamin E in doxorubicin induced cardiomyopathy. *Turkish Journal of Biochemistry*, 44(2):207–217, 2018.

[12] P. R. N. Kind and E. King. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *Journal of clinical Pathology*, 7(4):322, 1954.

[13] S. P. Roche and R. Kobos. Jaundice in the adult patient. *American family physician*, 69(2):299–304, 2004.

[14] A. T. Feldman and D. Wolfe. Tissue processing and hematoxylin and eosin staining. *Histopathology: methods and protocols*, pages 31–43, 2014.

[15] A. Sikandar, K. Farhat, A. Afzal, K. Ajmal, M. Laeeq, and A. Khokhar. Protective effects of trimetazidine against doxorubicin-induced cardiotoxicity and hepatotoxicity in mice. *Journal of Ayub Medical College Abbottabad*, 32(3):304–309, 2020.

[16] A. Gokcimen *et al.* Protective effect of N-acetylcysteine, caffeic acid and vitamin E on doxorubicin hepatotoxicity. *Human & experimental toxicology*, 26(6):519–525, 2007.

[17] Y. Octavia, C. G. Tocchetti, K. L. Gabrielson, S. Janssens, H. J. Crijns, and A. L. Moens. Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. *Journal of Molecular and Cellular Cardiology*, 52(6):1213–1225, 2012.

[18] M. O. T. Badr *et al.* Biochemical and antioxidant effect of Doxorubicin hydrochloride and Propolis on N-methyl-N-nitrosourea (MNU) Induced Adenocarcinoma in Rats. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine*, 72(2), 2015.

[19] R. Miyata, M. Sahlan, Y. Ishikawa, H. Hashimoto, S. Honda, and S. Kumazawa. Propolis components from stingless bees collected on South Sulawesi, Indonesia, and their xanthine oxidase inhibitory activity. *Journal of natural products*, 82(2):205–210, 2019.

[20] M. Jun, H. Fu, J. Hong, X. Wan, C. S. Yang, and C. Ho. Comparison of antioxidant activities of isoflavones from kudzu root (Pueraria lobata Ohwi). *Journal of food science*, 68(6):2117–2122, 2003.

[21] S. I. Anjum *et al.* Composition and functional properties of propolis (bee glue): A review. *Saudi journal of biological sciences*, 26(7):1695–1703, 2019.

[22] Y. Al-Tonbary, M. Al-Haggar, R. El-Ashry, S. El-Dakroory, H. Azzam, and A. Fouda. Vitamin e and N-acetylcysteine as antioxidant adjuvant therapy in children with acute lymphoblastic leukemia. *Advances in Hematology*, 2009(1):689639, 2009.

[23] A. Pace *et al.* Neuroprotective effect of vitamin E supplementation in patients treated with cisplatin chemotherapy. *Journal of Clinical Oncology*, 21(5):927–931, 2003.

[24] S. Singla, N. R. Kumar, and J. Kaur. In vivo studies on the protective effect of propolis on doxorubicin-induced toxicity in liver of male rats. *Toxicology international*, 21(2):191, 2014.

[25] F. Sun *et al.* In vivo antioxidative activity of propolis evaluated by the interaction with vitamins C and E and the level of lipid hydroperoxides in rats. *Journal of Agricultural and Food Chemistry*, 48(5):1462–1465, 2000.

 http://doi.org/10.33091/amj.2025.154646.1968

[26] E. Mansouri, A. Jangaran, and A. Ashtari. Protective effect of pravastatin on doxorubicin-induced hepatotoxicity. *Bratislavske lekarske listy*, 118(5):273–277, 2017.

[27] E. Kaya, S. Yilmaz, and S. Ceribasi. Protective role of propolis on low and high dose furan-induced hepatotoxicity and oxidative stress in rats. *Journal of Veterinary Research (Poland)*, 63(3):423–431, 2019.

[28] E. M. Tanvir *et al.* Ameliorative effects of ethanolic constituents of Bangladeshi propolis against tetracycline-induced hepatic and renal toxicity in rats. *Journal of food biochemistry*, 43(8):e12958, 2019.

[29] E. M. Saleh. Antioxidant effect of aqueous extract of propolis on hepatotoxicity induced by octylphenol in male rats. *Acta Toxicol. Argent*, 20(2):68–81, 2012.

[30] N. S. Al-Sowayan and U. Kishore. Prophylactic efficacy of a combination of proanthocyanidin and vitamin E on hepatotoxicity induced by doxorubicin in rats. *Int. Res. J. Pharm*, 2:161–169, 2012.

[31] A. Lotfi, S. M. Rafie, S. Hesaraki, and K. Amini. Protective Effects of Coadministration of the Vitamin E and Omega-3 on Doxorubicin-Induced Hepatotoxicity in Mice. *International Journal of Cancer Management*, 15(10), 2022.