Progress in Nutrition 2022; Vol. 24, N. 1: e2022028 DOI 10.23751/pn.v24i1.12017 © Mattioli 1885

ORIGINAL ARTICLE

Does Ethanolic Propolis Extract Affect Blood Clotting Parameters?

Sevgi Kolayli[1], Huseyin Sahin[2], Meltem Malkoc[3], Hilal Ebru Cakir[1], Yakup Kara[1], Ozlen Bektas[4], Kaan Kaltalioglu[2]

[1]Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, Turkey; [2]Giresun University, Espiye Vocational School, Espiye, Giresun, Turkey; [3]Karadeniz Technical University, Vocational School of Health Sciences, Trabzon, Turkey; [4]Karadeniz Technical University, Faculty of Medicine, Department of Hematology, Trabzon, Turkey

Abstract. Ethanolic propolis extract (EPE) can be used as a food supplement since it is a rich source of phenolic compounds. However, it is also suspected that a high level of phenolic substances may interact with blood-thinning drugs, such as warfarin. This preliminary study investigated the effect of warfarin combined with EPE on blood coagulation in rats. Prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and fibrinogen were measured in all experimental groups to determine blood coagulation effects. In addition, the propolis extract was analyzed as *in vitro*. The total amounts of phenolic and flavonoid substances were 82.40±0.44 mg of gallic acid equivalents (GAE)/mL and 28.62±0.18 mg of quercetin equivalents (QUE)/mL, respectively. According to the HPLC analysis, rutin, caffeic acid phenyl ester (CAPE), and chrysin were identified as major components. Moreover, while the EPE [200 mg/kg, body weight (bw)] did not significantly affect coagulation parameters when used alone, it caused mild changes in combination with warfarin. When compared to the control group (G1), the levels of PT and fibrinogen of the combination group (G4) observed significant statistical difference ($p \leq 0.005$). However, the increases in all blood coagulation factors except fibrinogen were found to be higher in the warfarin group (G2) and these differences were also statistically confirmed ($p \leq 0.005$). In conclusion, the data obtained may be interpreted as evidence of a synergistic effect between the applied dose of EPE and warfarin, despite the expectation of a possible drug interaction.

Key words: bleeding, coagulation, flavonoids, propolis

Introduction

Propolis, one of the potential sources of natural dietary polyphenolic compounds, is produced by honeybees (*Apis mellifera*) (1). These collect propolis from the barks and leaves of various trees and plants to protect and isolate their hives from insect carcasses through mummification. Several studies in the literature have investigated propolis due to its significant phenolic compounds in terms of apitherapeutic applications (2). Dietary polyphenolic compounds are known to be capable of protecting cell constituents against oxidative damage by reducing the risk of numerous degenerative diseases related to oxidative stress (3).

Platelet function disorders that are closely correlated with oxidative stress are a group of bleeding disorders in which platelets exhibit abnormal functions. Platelet aggregation inhibitors perform important functions, such as blocking clot formation and preventing platelet adhesion by acting in different parts of the clotting cascade. Anti-platelet (anti-aggregant) drugs, such as aspirin, prevent clot formation due to platelet agglutination (4).

In addition to anti-platelet drugs, anti-coagulants, commonly known as blood-thinning drugs, prevent or reduce blood and slow down clotting time. Warfarin in particular, which is frequently employed in clinical applications, acts as a vitamin K antagonist, and is widely used for the primary and secondary prevention of arterial and venous thromboembolism in patients with common cardiovascular and peripheral vascular diseases (5,6).

In addition to studies investigating anti-coagulant and anti-platelet drugs, research has also been conducted into natural products that act as inhibitory agents due to their flavonoid capacities (7,8). It has also been strongly suggested that flavonoids extracted from herbs such as thyme and garlic may inhibit the synthesis of vitamin K in the intestinal system (9,10). CAPE, an important component of propolis, exhibits dose-dependent fibrinolytic activity in human whole blood clots (8,11). Two flavonoids, rutin and hesperetin, have been reported to exhibit *in vitro* anti-thrombotic effects for anticoagulant activity through coagulation tests including activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin time (TT) (12). Another study reported that extracts of the flavonoid-rich food *Rhizophora mucronata* Poir. affect coagulation factors (13).

When considered individually, these potent drugs and polyphenol-rich natural products exhibit positive effects. However, their use in combination can represent a potential risk owing to possible interactions or side-effects. Martina et al., (2018) and Rivera et al., (2012) both therefore recommended that individuals who use blood thinners should also pay close attention to their diets (4,14).

Despite a few negative indications, evidence concerning the interaction between warfarin and natural compounds in the literature is generally positive. For example, some vitamin K antagonists in natural products, including polyphenol-rich products, have been reported to affect coagulation mechanisms in warfarin users (10,15). It has also been suggested that when used in in high doses, some flavonoids, such as rutin, may be vitamin K antagonists and thus affect blood clotting (4,9,14). Moreover, there are also reports that some flavonoids in various fruits and teas can inhibit Cyt-P450 enzymes and the enzymes used to break down warfarin as a xenobiotic in the liver (14–16). Yuksel et al., (2010) suggested that flavonoids may be responsible for enzyme inhibition, since these interact with proteins and have a high affinity for their hydrophobic surfaces (17). One case report stated that the consumption of *Lycium barbarum* (Goji berry) juice together with warfarin affected blood coagulation (14).

Many investigation studies related to propolis as an anti-aggregant and/or anti-coagulant agent have originated from its containing a high percentage of polyphenols as well as flavonoids. However, it is unclear whether the consumption of EPEs rich in polyphenols has an effect on blood coagulation. It is also unclear whether individuals using blood-thinners such as warfarin should also use propolis. There has been only limited investigation concerning propolis extract administration and its interaction with warfarin, employed as a blood-thinning agent. One study reported that EPE interacted with warfarin in rats, although only international normalized ratio (INR) values were measured in that research (18). The present study therefore focused on investigating the effects of EPE on various blood clotting parameters, and on interaction with warfarin in terms of PT, aPTT, fibrinogen, and INR.

Materials and methods

Chemicals and propolis samples

Active warfarin was purchased from a pharmacy in the form of Coumadin tablets (5 mg, Zentiva, Turkey). Fifteen milligrams of Coumadin tablets were dissolved in 0.9% NaCI to obtain a stock solution (0.15 mg/mL). EPE was purchased from a manufacturer in Istanbul, Turkey. Bee&You (Bee'O®) (SBS Scientific Bio Solutions Inc, Istanbul, Turkey) brand Anatolian propolis samples were purchased in drop format containing 30% pure propolis in ethanol-water (70:30, v/v) extraction solvent. A combination solution of propolis and warfarin was then prepared. Warfarin (0.30 mg/mL) was first dissolved in 70% ethanol and then mixed (1:1) with a 400 mg/mL concentration of the propolis extract.

The INR measuring device (Coagu Chek® XS System) was purchased from Roche Diagnostic GmbH, Germany. PT, aPPT, and fibrinogen were measured using a Cobas Integra 800 biochemical automated BCS X P Coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) at the Karadeniz Technical University Medical Faculty Laboratory.

Experimental Groups and Treatment

Experimental procedures were approved by the Animal Experimentation Ethics Committee of the Karadeniz Technical University, Faculty of Medicine (Trabzon, Turkey) (protocol no. 2019/15). Twenty-four male Sprague-Dawley rats (250-300 g) were housed in cages under a 12h light/dark cycle at 20-25°C. Ad libitum access was allowed to standard rat chow and drinking water. The rats were randomly divided into four groups and all application procedures continued for 8 days. All samples were applied by oral gavage in the form of 1 mL. At the end of the experimental period, all rats were decapitated under xylazine-ketamine (10:50 mg/kg) anesthesia administered intraperitoneally. Blood was collected into plastic tubes containing trisodium citrate dihydrate, an anti-coagulant.

- **Group 1 (Control)**: Control rats were given distilled water via the oral route (1.0 mL, 0.9% saline).
- **Group 2 (Warfarin):** Rats in this group received 15 mg/kg, bw, 1.0 mL warfarin.
- **Group 3 (Propolis)**: Rats in this group received 200 mg/kg, bw, 1.0 mL propolis
- **Group 4 (Warfarin+Propolis):** Rats in this group received 0.30 mg/kg, bw, 0.50 mL warfarin and 400 mg/kg, bw, 0.50 mL propolis.

Ethanolic propolis extract (EPE) analyses
Determination of total phenolic and flavonoid contents

Total phenolic contents of the EPE were measured using the method described by Folin-Ciocalteu (19) and expressed as mg of gallic acid equivalents (GAE) per mL extract.

Total flavonoid contents of the EPE were determined by spectrophotometric assay using quercetin as standard (20). The results were expressed as mg of quercetin equivalents (QUE) per mL extract.

Determination of phenolic profiles

Before the chromatographic analysis of EPE, 19 phenolic standards were subjected to high-performance liquid chromatography (HPLC) (Elite LaChrom Hitachi, Japan). Separation was performed on a reverse phase C18 column (150 mm x 4.6 mm, 5 µm; Fortis), in gradient solvent systems including an A reservoir (2% acetic acid in water) and B reservoir (70:30, acetonitrile/water). The flow rate was kept constant at 1 mL/min using gradient programming, starting the flow of the mobile phase as B (5%) to 3 minutes, then gradually increasing (up to 15, 20, 25, 40 and 80% at 8, 10, 18, 25 and 35 min, respectively) and reducing this to 5% at 40 minutes, before being left for 10 minutes to equilibrate in the column. The standard phenolic substances chromatogram is given in Figure 1.

Statistical analyses

The study data were expressed as mean ± standard deviation (X±SD). Statistical analyses were performed using One-Way ANOVA followed by post-hoc Tukey. The unpaired *t* test was used for comparisons between groups. A minimum level of significance of $p \leq 0.05$ was adopted.

Results

Blood clotting effects

Table 1 shows the values of three coagulation parameters measured in the experimental rats. PT(s) values increased significantly in the warfarin (G2) and the warfarin+propolis (G4) groups ($p \leq 0.05$), those which received the most the warfarin group (G2) (18.16±2.85 sec) compared to the control group (G1) (8.96±0.27 sec), but did not change in the propolis group (G3) (8.82±0.51 sec) ($p > 0.05$). Similarly to

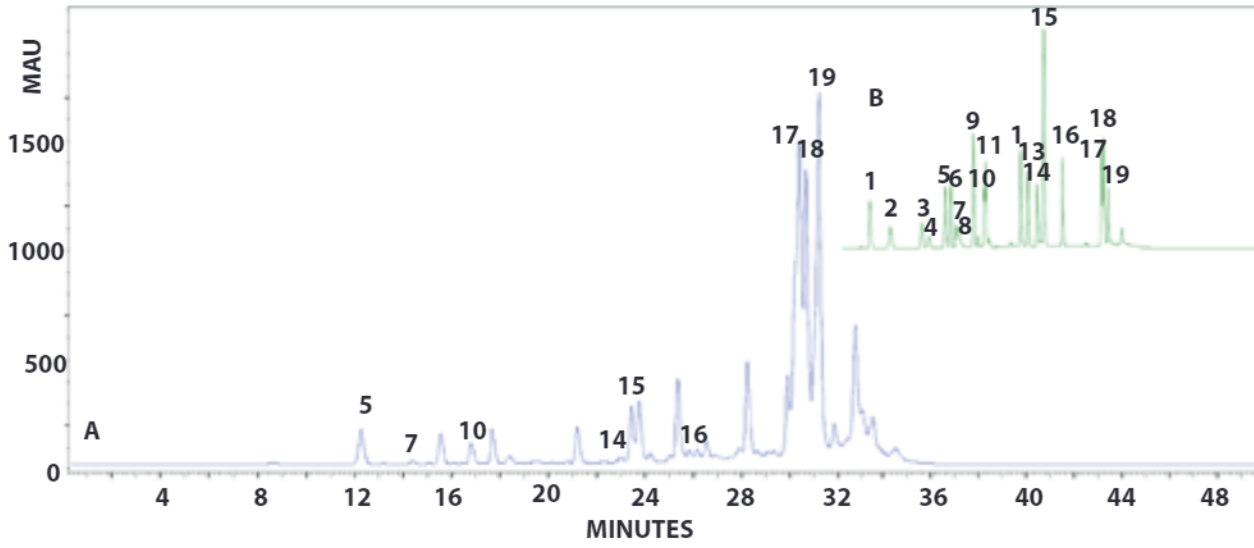


Figure 1. A: HPLC chromatogram of the ethanolic propolis sample (EPE), B: Chromatogram of 19 phenolic compounds at HPLC-UV; (1) gallic acid, (2) protocatechuic acid, (3) *p*-OH benzoic acid, (4) catechin, (5) caffeic acid, (6) syringic acid, (7) epicatechin, (8) *p*-coumaric acid, (9) ferulic acid, (10) rutin, (11) myricetin, (12) resveratrol, (13) daidzein, (14) luteolin, (15) *t*-cinnamic acid, (16) hesperetin, (17) chrysin, (18) pinocembrin, and (19) caffeic acid phenethyl ester (CAPE)

Table 1. The effect of ethanolic propolis extract (EPE) on blood coagulation parameters

	G1 Control (n=6)	G2 Warfarin (n=6)	G3 Propolis (n=6)	G4 Warfarin+Propolis (n=6)
PT (sec)	8.96±0.27[a]	18.16±2.85[c]	8.82±0.51[a]	13.03±1.60[b]
aPTT (sec)	13.69±0.74[a]	24.17±2.77[b]	14.64±0.13[a]	16.48±3.29[a]
Fibrinogen (mg/dL)	115.51±3.59[a]	146.06±9.15[b]	116.41±33.26[a]	189.61±25.16[c]

The same letters in each line for each parameter were not significantly different at $p \leq 0.05$

the PT(s) values, aPTT(s) values were almost twice as high in the warfarin group (G2) (24.17±2.77 sec) compared to the control group (G1) (13.69±0.74 sec) ($p \leq 0.05$), while a moderate increase was observed in the warfarin+propolis group (G4) (16.48±3.29 sec). However, no significant increase in aPPT values occurred in the propolis group (G3) ($p > 0.05$). The mean fibrinogen value in the healthy experimental animals (G1) was 115.51±3.59 mg/dL. Fibrinogen values increased in the warfarin group (G2) (146.06±9.15 mg/dL), the group receiving warfarin ($p \leq 0.05$), but the highest increase was in the warfarin+propolis group (G4) (189.61±25.16 mg/dL), the group given propolis extract combined with warfarin ($p \leq 0.05$).

In addition to these three coagulation parameters (PT, aPTT, and fibrinogen), INR values were measured from tail blood collected on the 3rd and 8th days. Figure 2 shows the INR values on both days. The INR value in the control group rats (G1) was 0.8, which increased to 0.9 in the group receiving propolis (G3). INR values increased significantly in the warfarin with propolis group (G3) (3.2 on the 3rd day and 4.6 on the 8th day) compared to the control group (G1).

In vitro analyses of EPE

The *in vitro* properties of EPE summarized in terms of total phenolic content, total flavonoid

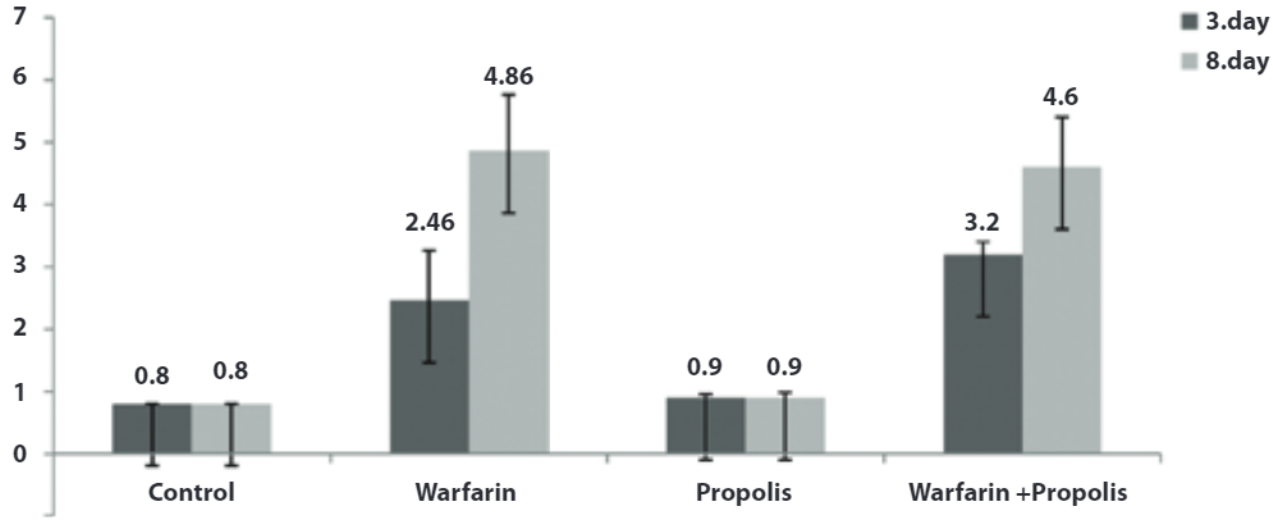


Figure 2. INR values of the control, warfarin and ethanolic propolis extract groups on days 3 and 8

content, and phenolic profile analysis are shown in Table 2. Total phenolic and total flavonoid contents of the EPE were calculated at 82.40±0.44 mg GAE/mL and 28.62±0.18 mg QUE/mL, respectively. HPLC chromatogram of the phenolic composition of EPE was obtained by investigating 19 phenolic standards; this is given in Figure 1. Accordingly, CAPE (1851.412 µg/mL), rutin (1260.851 µg/mL), and chrysin (1132.587 µg/mL) were the major compounds of the extract sample. More detailed examination of the HPLC chromatographic analysis revealed a number of variations among the sub-polyphenolic compound classes. The current table was therefore prepared by dividing the phenolic compounds into six different sub-polyphenolic compound classes such as catechins, flavonols, flavanones, hydroxy benzoic acids, hydroxy cinnamic acids, and hydroxy stilbenes.

Some hydroxybenzoic acids, such as gallic acid, *p*-OH benzoic acid, syringic acid, and protocatechuic acid were not detected in the sample, while some hydroxycinnamic acid members such as caffeic acid, *p*-coumaric acid, *t*-cinnamic acid, and CAPE were detected in differing amounts. Especially CAPE was identified as the dominant compound with the value of 1851.412 µg/mL. Although the catechin standards used (catechin and epicatechin) were not identified, pinocembrin, a type of flavanone, was detected at the moderate level of 880.571 µg/mL.

Discussion

Evaluation of the anticoagulant activity of EPE

As a supplementary natural product, EPEs are frequently used for a wide range of purposes in apitherapy. Since propolis extracts are rich in phenolic acids and flavonoids, they exhibit significant antioxidant, anti-inflammatory, and antimicrobial activities. However, high-concentration propolis extract is also thought to cause numerous side-effects in some individuals who use it together with blood thinners, such as warfarin and aspirin (4,8). Some studies have also reported that propolis may exhibit antiplatelet effects (4,8). Although several previous studies have investigated the effects of different plant extracts on blood coagulation, research using propolis is more limited.

This study investigated the effect of EPE on blood coagulation parameters in rats exposed to warfarin. Four blood coagulation parameters were measured - INR, PT, aPTT, and fibrinogen. These coagulation values in the experimental animals were compared with those of the control group. In addition to the control application, single-dose propolis, single-dose warfarin, and a combined propolis and warfarin dose were administered to the relevant groups, in the form of a single dose administered by oral gavage. Except for INR, the other three coagulation parameters were measured on day 8. Ideally, the coagulation parameters should

Table 2. *In vitro* properties of the ethanolic propolis extract (EPE) used

Antioxidant characterization	
Total Phenolic Contents (mg GAE/mL)	82.40±0.44
Total Flavonoid Contents (mg QUE/mL)	28.62±0.18
Phenolic profiles (μg/mL)	
Catechins	
Catechin	N.D.*
Epicatechin	N.D.*
Flavonols	
Rutin	1260.851
Myricetin	N.D.*
Daidzein	N.D.*
Luteolin	21.691
Hesperetin	26.298
Chrysin	1132.587
Flavanones	
Pinocembrin	880.571
Hydroxy benzoic acids	
Gallic acid	N.D.*
p-OH Benzoic acid	N.D.*
Syringic acid	N.D.*
Protocatechuic acid	N.D.*
Hydroxy cinnamic acids	
Caffeic acid	236.232
Ferulic acid	N.D.
p-coumaric acid	111.930
t-cinnamic acid	85.697
Caffeic acid phenethyl ester (CAPE)	1851.412
Hydroxy stilbenes	
Resveratrol	N.D.*

*N.D. Not Detected

be monitored daily, but the amount of tail blood collected was not sufficient to allow this. INR values were therefore monitored on days 3 and 8. INR is the principal monitoring parameter used to measure the therapeutic index and safety of anticoagulation therapy in humans. It is employed for the purpose of comparison against other bleeding time parameters. INR values in healthy individuals range between 2.0 and 3.0. These values are generally considered to be in the therapeutic range. However, the risk of bleeding increases when INR exceeds 4.0. The use of warfarin may be seen as a reason for a significant increase in INR values due to the prolongation of bleeding clotting time. A mouse study similar to the present research reported an INR value of 0.8 in the control group, while bleeding time increased significantly in animals given Coumadin (warfarin) (18). Significant increases in INR were observed in the other two groups, warfarin (G2) and warfarin+propolis (G4), compared to the control group (G1), although not with propolis alone (G3). The main reason for the increase in INR in the combination group was the presence of warfarin. This was confirmed through the minimal and variable contribution of propolis shown in Figure 2. This contribution was more apparent in the form of a 0.74 increase on the 3rd day and a 0.26 decrease on the 8th day. The numbers of studies investigating the antiplatelet efficacy of bee products based on bleeding time are limited.

One case report described a significant increase in INR values in in humans using bee pollen (1). That report also noted that the probable interaction between bee pollen and warfarin was correlated with the long-term use of bee pollen. The anti-platelet activity of bee pollen may have been due to the variety of flavonoids. Some flavonoids such as chrysin, baicalein, apigenin, luteolin, kaempferol, quercetin, and rutin, found in bee pollen, have been described as a competing inhibitor with Cytochrome P-450 (CYP) isoenzymes (21). In contrast, another study reported that bee pollen did not exhibit antiplatelet effects since the difference between the bee pollen and placebo groups was not significant. In the same preclinical study, although a negative effect was observed in terms of bee pollen, the propolis yielded a positive effect in terms of average bleeding times in mice. These were reported as 102 sec for placebo, 442 sec for aspirin, and 310 sec for propolis, thus confirming the antiplatelet effect of propolis administration (4). This result, which was not compatible with our data, may be due to the active substances in the propolis used, the range of extract dose, and/or the type of propolis.

PT, aPTT, and fibrinogen are three important coagulation parameters for determining coagulation

effects (22). PT and aPTT are common screening anticoagulant tests used for exogenous and endogenous coagulation systems, respectively (23). Table 1 shows the values for these parameters in the study groups. The mean baseline PT value in the control group was 8.96 sec, and a significant increase was observed in the warfarin group. No change was observed following the administration of propolis. Although the PT value of the propolis + warfarin group was significantly lower than that of the warfarin group, no change occurred in the propolis group. Similarly to the present research, safflower extract treatment combined with warfarin has been observed to affect blood coagulation parameters, and to prolong PT and aPTT (24). Hydroxy safflower yellow A (HSYA), a chalcone derivative of flavonoids, is the main component of safflower extract, and this inhibition mechanism is also based on the inhibition of the Cyt-P450 enzymes (CYP1A2 and CYP2C11) in rats (24,25). We observed a significant increase in aPTT in rats receiving warfarin, while no significant increase was observed in the propolis group. A combination of prolonged PT and aPTT values was also reported in a similar rat study involving the administration of 6,7-Dihydroxy-3-phenyl coumarin with warfarin, and the measurement of coagulation parameters (22).

Fibrinogen (coagulation factor I) is a major component of blood coagulation. Although soluble fibrinogen in the bloodstream is not a pro-inflammatory agent, activation of the coagulation cascade results in the formation of fibrin (26). While fibrinogen levels did not change in the propolis groups in the present study, the propolis plus warfarin group exhibited a significant increase. CAPE, one of the active ingredients of propolis, was shown to exhibit dose-dependent fibrinolytic activity in one *in vitro* study (11). The main reason for the dissimilarity to our results may be that the present study was dose-independent because of the single-dose application of propolis. Fibrinogen is also an acute-phase protein (27), suggesting that propolis combined with warfarin can increase this acute-phase reactant. In addition, warfarin combined with propolis may cause a synergistic effect in rats. Inconsistent findings have been reported on this subject in the previous literature. Consistent with our findings, warfarin has been reported to raise fibrinogen levels in rats (28). However, another study reported that fibrinogen remained unchanged in warfarin-treated rats (22). The majority of studies have involved other natural extracts, and unfortunately there has been no direct research into propolis. A previous study involving CAPE, an important component of propolis, reported prolonged PT, aPPT, and TT in rats, but decreased fibrinogen levels (29). However, in that study, CAPE was used as a pure compound, and the matrix effect due to polyphenols in propolis was not taken into consideration.

In vitro properties of EPE

Propolis is an important apitherapeutic agent used for various different purposes in recent years. This bee product consists of two main components, balsam and wax. The balsamic part, which includes various polyphenolic compounds, is proportionally greater than the wax part. Plant phenolic compounds, known as secondary metabolites, are responsible for numerous biological functions such as anti-oxidant, anti-microbial, anti-inflammatory, and anti-tumor activities (5). In addition to these bio-properties, specific members of phenolic compounds undertake some specific tasks in the metabolism. For example, some flavonoids, which represent the largest molecular subclass of the family of phenolic compounds, inhibit various Cyt-P450 enzymes and affect the coagulation cascade, as emphasized in the current study (11,14). Propolis is the best source of phenolic acid and flavonoid contents among the various bee products. This has been tested and correlated using numerous *in vitro* methodologies, such as spectrophotometric or chromatographic analysis (30). In the current study, both methodologies were used to evaluate the total phenolic content, total flavonoid content, and phenolic composition of EPE. In contrast to the present study in which the relevant unit was expressed per mL of extract, unit data for both total phenolic content and total flavonoid content have generally have been expressed in terms of dry sample in the literature. Therefore, although our findings appear compatible with previous propolis studies, full similarity has not been ensured. In one study, ethanol extracts of 20 propolis samples were compared with those of Australian, Brazilian, and Chinese samples. The total phenolic contents of all analyzed and compared propolis samples ranged from 49 to 239

mg GAE/g ethanolic extract of propolis (EEP), while total flavonoid contents ranged from 21 to 53 mg QE/g EEP (31). Fikri et al., (2019) analyzed the ethanolic extract and water extract of propolis from Indonesia in terms of total phenolic and total flavonoid contents and reported values ranging from 10.00 to 28.65 mg/g GAE and 0.76 to 3.39 mg/g QE, respectively. The values for total phenolic and total flavonoid content were compatible with these previous studies (32).

The HPLC results from the present research and the data from previous studies appear broadly similar. According to our results, CAPE was the dominant compound among the hydroxycinnamic acid derivatives, followed by caffeic acid and coumaric acid. According to the literature, some studies were focused on CAPE known as one of the major phenolic compounds in propolis samples, especially in poplar type propolis (33, 34). Substantially, this important compound isolated from natural compounds including also propolis is responsible for some bioactivity processes associated with anti-oxidant, anti-inflammatory, anti-microbial, anti-diabetic, etc. (33, 34). The propolis extract was found to be rich in flavonoids, particularly rutin and chrysin for flavonols, and pinocembrin for flavanols (Table 2, Figure 1). We also examined some previous studies in order to correlate our HPLC results. Ristivojević et al. (2015) suggested that CAPE was a major constituent of propolis and was responsible for a wide spectrum of its biological activity (35). Another report described chrysin and *p*-coumaric acid as dominant in nine propolis samples collected from different regions of Poland (36).

Comparing our HPLC results with previously reported data, we speculated that flavonoids and phenolic acids were responsible for the potential anticoagulant activity in synergy with other specific propolis components. Various data from the literature appear to support this thesis. For example, Helal Ashour (2014) revealed that CAPE, which was quite dominant in our sample, was capable of counteracting hematological and blood coagulation disturbances, oxidative stress, and hepatorenal damage in Cd intoxication (37). The flavonoid rutin has been shown to exhibit a platelet anticoagulant effect by reducing the racemic effect of warfarin in rats (38). Another study involving vitamin P compounds, which also include the flavonoid rutin, reported that these counteracted the activity of the oral anticoagulant in rats by reducing PT (39). *In vitro* evidence suggests that chrysin may exhibit interactions with warfarin, meaning that it affects the cytochrome P450 metabolism as a 1A2 inhibitor (40). When our results were assessed in terms of flavonoids, with their known anticoagulant effects, the propolis sample used in the present research exhibited no anticoagulant effect, despite its high flavonoid content. Akbay et al, (2017) observed a very similar situation to that in the present study, and also reported that 1,2-benzene dicarboxylic acid, mono(2-ethylhexyl)ester and/or other minor compounds in propolis may be responsible for the synergistic platelet effect of the propolis samples (18).

Conclusion

Scientific research suggests that warfarin interacts with various drugs and natural compounds, and potential drug interactions, therefore, require very careful scrutiny. All phenolic agents, including flavonoids, may be responsible for these interactions. Although numerous studies have been conducted with pure chemicals to confirm this idea, the numbers of natural compound studies are more limited. Essentially, the investigation on the natural products is extremely substantial because the different numbers of bioactive components of these products as a whole may show different characteristic properties rather than individual behaviors of each ones in *in vivo*. This is called the synergistic effect. As supporting this claim from a different perspective, the present study was designed with propolis known as a valuable natural compound to reveal its anticoagulant effect and its possible interaction degree exhibited with warfarin. The study results suggested that the ethanolic propolis extract applied in this research had no significant effect on blood clotting factors and did not exhibit any interaction with warfarin. It should not be forgotten that propolis contains numerous different bio-compounds in addition to phenolic agents some of which were dominant such as CAPE and rutin in this current study, and these may also exhibit a synergistic effect with warfarin. Further studies involving a wide range of dose-dependent *in vivo* investigations with ethanolic extract of propolis are now needed to shed further light on the drug interaction potentials.

Acknowledgments: The authors are grateful to Dr. Sait Al, the animal laboratory veterinary surgeon, for his assistance, and to Bee&You (Bee'O®) (SBS Scientific Bio Solutions Inc., Istanbul, Turkey) for supplying ethanolic propolis extract without any financial expectation.

Conflict of Interest: The authors declare that there is no conflict interest related to this work.

References

1. Hurren KM, Lewis CL. Probable interaction between warfarin and bee pollen. *Am J Heal Pharm*, 2010; 67 (23): 2034–7.
2. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, propolis, and royal Jelly: A comprehensive review of their biological actions and health benefits. *Oxid Med Cell Longev* 2017; 2017 (1259510): 1–21.
3. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary Polyphenols and the Prevention of Diseases. *Crit Rev Food Sci Nutr* 2005; 45 (4): 287–306.
4. Martina SJ, Luthfi M, Govindan P, Wahyuni AS. Effectivity comparison between aspirin, propolis, and bee pollen as an antiplatelet based on bleeding time taken on mice. *MATEC Web Conf* 2018; 197: 1–7.
5. Zhang Z, Ge B, Zhou L, Lam TN, Zuo Z. Induction of liver cytochrome P450s by Danshen-Gegen formula is the leading cause for its pharmacokinetic interactions with warfarin. *J Ethnopharmacol* 2014; 154 (3): 672–86.
6. Li Z, Seeram NP, Carpenter Cl, et al. Does Not Affect Prothrombin Time in Male Subjects on Warfarin. *J Am Diet Assoc* 2006; 106 (12): 2057–61.
7. Wu YP, Huisman A, Weeterings C, et al. Caffeic acid phenyl ester, a component of the Chinese herb propolis, inhibits platelet aggregation via competition with fibrinogen for binding to aIIbb3. *Blood* 2008; 112 (11): 5367.
8. Chen T-G, Lee J-J, Lin K-H, Shen C-H, Chou D-S, Sheu J-R. Antiplatelet activity of caffeic acid phenethyl ester is mediated through a cyclic GMP-dependent pathway in human platelets. *Chin J Physiol* 2007; 50 (3): 121–6.
9. Plungian MB, Munch JC, Wolffe JB. Effect of rutin on coagulation time of rat blood. *J Pharmacol Exp Ther* 1948; 93 (3) 383–7.
10. Rahimi S, Zadeh ZT, Torshizi MAK, Omidbaigi R, Rokni H. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. *J Agric Sci Technol* 2011; 13: 527–39.
11. Elnager A, Hassan R, Idris Z, et al. Fibrinolytic activity and dose-dependent effect of incubating human blood clots in caffeic acid phenethyl ester: in vitro assays. *Biomed Res Int* 2015; 2015: 627471.
12. Kuntic V, Filipovic I, Vujic Z. Effects of rutin and hesperidin and their Al(III) and Cu(II) complexes on in vitro plasma coagulation assays. *Molecules* 2011; 16 (2): 1378–88.
13. Hari R, Vadivu R, Radha R. Effect of leaf extract from mangrove species rhizophora mucronata poir on homocysteine induced coagulation factors. *Res J Pharm Technol* 2019; 12 (10): 4807–11.
14. Rivera CA, Ferro CL, Bursua AJ, Gerber BS. Probable interaction between Lycium barbarum (Goji) and warfarin. *Pharmacotherapy* 2012; 32 (3): e50–e53.
15. Bhamra SK, Slater A, Howard C, Heinrich M, Johnson MRD. Health care professionals' personal and professional views of herbal medicines in the United Kingdom. *Phyther Res* 2019; 33 (9): 2360–8.
16. Li H, Wu F, Tan J, et al. Caffeic acid phenethyl ester exhibiting distinctive binding interaction with human serum albumin implies the pharmacokinetic basis of propolis bioactive components. *J Pharm Biomed Anal* 2016; 122: 21–8.
17. Yuksel Z, Avci E, Erdem YK. Characterization of binding interactions between green tea flavanoids and milk proteins. *Food Chem* 2010; 121 (2): 450–6.
18. Akbay E, Özenirler Ç, Çelemli ÖG, Durukan AB, Onur MA, Sorkun K. Effects of propolis on warfarin efficacy. *Kardiochirurgia i Torakochirurgia Pol* 2017; 14 (1): 43–6.
19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965; 16 (3) :144–58.
20. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem* 2000; 48 (8): 3597–604.
21. Korobkova EA, Effect of natural polyphenols on CYP metabolism: Implications for diseases, *Chem Res Toxicol*, 2015; 28 (7) 1359–1390.
22. Tunali T, Yarat A, Bulut M, Emekli N. 6,7-Dihydroxy-3-phenylcoumarin inhibits thromboplastin induced disseminated intravascular coagulation. *Br J Haematol* 2004; 126 (2): 226–30.
23. Liu YJ, Pan BS. Inhibition of fish gill lipoxygenase and blood thinning effects of green tea extract. *J Agric Food Chem* 2004; 52 (15): 4860–4.
24. Liu Y, Liu S, Shi Y, Qin M, Sun Z, Liu G. Effects of safflower injection on the pharmacodynamics and pharmacokinetics of warfarin in rats. *Xenobiotica*, 2018; 48 (8): 818–23.
25. Xu RA, Xu ZS, Ge RS. Effects of hydroxysafflor yellow A on the activity and mRNA expression of four CYP isozymes in rats. *J Ethnopharmacol*, 2014; 151 (3): 1141–6.
26. Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. *Semin Immunopathol* 2012; 34 (1): 43–62.
27. Tsiakalos A, Karatzaferis A, Ziakas P, Hatzis G. Acute-phase proteins as indicators of bacterial infection in patients with cirrhosis. *Liver Int* 2009; 29 (10): 1538–42.
28. El-Gendy AA, Abbas AM. Effect of omega-3 fatty acids on haemostatic functions in urocortin-treated obese rats. *J Physiol Biochem* 2014; 70 (3): 809–20.
29. Korish AA. Effect of caffeic acid phenethyl ester on the hemostatic alterations associated with toxic-induced acute liver failure. *Blood Coagul Fibrinolysis* 2010; 21 (2): 158–63.
30. El Adaouia Taleb R, Djebli N, Chenini H, Sahin H, Kolayli S. *In vivo* and *in vitro* anti-diabetic activity of ethanolic propolis extract. *J Food Biochem* 2020; 44 (7): e13267.

31. Wang X, Sankarapandian K, Cheng Y, et al. Relationship between total phenolic contents and biological properties of propolis from 20 different regions in South Korea. *BMC Complement Altern Med* 2016; 16: 65.
32. Fikri AM, Sulaeman A, Marliyati SA, Fahrudin M. Antioxidant activity and total phenolic content of stingless bee propolis from Indonesia. *J Apic Sci* 2019; 63 (1): 139–47.
33. Šuran J, Cepanec I, Mašek T, et al. Propolis extract and its bioactive compounds—from traditional to modern extraction technologies. Molecules 2021; 26(10): 2930.
34. Yordanov Y. Caffeic acid phenethyl ester (CAPE): pharmacodynamics and potential for therapeutic application. *Pharmacia* 2019; 66(3): 107-114.
35. Ristivojević P, Trifković J, Andrić F, Milojković-Opsenica D. Poplar-type propolis: Chemical composition, botanical origin and biological activity. *Nat Prod Commun* 2015; 10 (11): 1869–76.
36. Socha R, Gałkowska D, Bugaj M, Juszczak L. Phenolic composition and antioxidant activity of propolis from various regions of Poland. *Nat Prod Res* 2015; 29 (5): 416–22.
37. Helal Ashour T. Preventative effects of caffeic acid phenyl ester on cadmium intoxication induced hematological and blood coagulation disturbances and hepatorenal damage in rats. *ISRN Hematol*, 2014; 2014 (764754): 1–7.
38. Chan E, Hegde A, Chen X. Effect of rutin on warfarin anticoagulation and pharmacokinetics of warfarin enantiomers in rats. *J Pharm Pharmacol*, 2009; 61 (4): 451–8.
39. Martin GJ, Swayne V. Effectiveness of vitamin P compounds in counteracting anticoagulant action of dicoumarol. *Science* 1949; 109(2826): 201–2.
40. Nutescu EA, Shapiro NL, Ibrahim S, West P. Warfarin and its interactions with foods, herbs and other dietary supplements. *Expert Opin Drug Saf* 2006; 5 (3): 433–51.

Correspondence:
Dr. Sevgi Kolayli
Karadeniz Technical University, Faculty of Science, Department of Chemistry, 61080
Trabzon, Turkey
E-mail: skolayli61@yahoo.com
Tel: +90 462 377 24 87
ORCID: 0000-0003-0437-6139