

Effects of propolis on warfarin efficacy

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Abstract

Introduction: Warfarin is commonly used to avoid thromboembolism, predominantly for cardiovascular pathologies. However, the consumption of several herbal products is not permitted during its use due to the associated interactions. Propolis is a popular phytotherapy product made by honey bees. The use of propolis has been dramatically increasing in recent times.

Aim: To evaluate the possible interactions between propolis and warfarin in a mouse model with determination of the international normalized ratio (INR) values.

Material and methods: CD-1 mice were employed in the experimental model. The mice were warfarinized, and propolis was administered simultaneously. The INR values were obtained. All animals were sacrificed at the end of the study.

Results: The baseline INR value was 0.8 ± 0.1 . After 72 h, the INR value increased as expected. The INR value was 7.28 ± 1.08 in the control group and 5.8 ± 2.88 in the propolis group. At the end of the study, the INR value was 1.3 ± 0.37 . Propolis interacted with warfarin and caused a decrease in the INR value.

Conclusions: Propolis interactions, especially with warfarin, should be kept in mind and further studied. Healthcare specialists should be aware of this possible interaction between warfarin and propolis and inform patients about it.

Key words: propolis, warfarin, international normalized ratio, CD-1 mice, Coumadin.

Streszczenie

Wstęp: Warfaryna jest często używana w celu zapobiegania incydem zakrzepowo-zatorowym, głównie w schorzeniach sercowo-naczyniowych. W trakcie jej stosowania przyjmowanie części produktów ziołowych jest zabronione ze względu na interakcje lekowe. Propolis to popularny produkt ziołolecznicy wytwarzany przez pszczoły miodne. Jego użycie ostatnio zwiększa się gwałtownie.

Cel: Zidentyfikowanie możliwych interakcji pomiędzy propolisem a warfaryną w modelu mysim i ustalenie wartości międzynarodowego współczynnika znormalizowanego (INR).

Materiał i metody: W eksperymencie wykorzystano myszy CD-1. Mysiom podawano jednocześnie warfarynę i propolis, monitorując wartości INR. Wszystkie zwierzęta poddano eutanazji po zakończeniu badania.

Wyniki: Wyjściowa wartość INR wynosiła $0,8 \pm 0,1$. Po 72 godzinach, zgodnie z oczekiwaniami, nastąpił jej wzrost do $7,28 \pm 1,08$ w grupie kontrolnej i $5,8 \pm 2,88$ w grupie przyjmującej propolis. Na końcu badania wartość INR wynosiła $1,3 \pm 0,37$. Propolis wszedł w interakcję z warfaryną i spowodował zmniejszenie wartości INR.

Wnioski: Interakcje propolisu, zwłaszcza z warfaryną, są tematem wartym uwagi i dalszych badań. Specjaliści służby zdrowia powinni być świadomi możliwych interakcji pomiędzy warfaryną a propolisem i informować o nich pacjentów.

Słowa kluczowe: propolis, warfaryna, międzynarodowy współczynnik znormalizowany, myszy CD-1, Coumadin.

Introduction

Recently, the prevalence of heart failure (HF) has increased dramatically. The population is getting older; therefore, the incidence of HF has increased. At the same time, medical therapy for HF is progressing [1].

The HF is associated with a risk of thromboembolism, which often results in the patient's death. In order to prevent this, oral anticoagulants are administered. One very effective agent used for this purpose is the vitamin K antagonist warfarin (Coumadin, Turkey). Warfarin therapy is

recommended in order to thin the blood and minimize the risk of stroke [2]. Sometimes daily dietary habits affect the drug's metabolic pathway; therefore, it is crucial to pay attention to diet while using warfarin.

Propolis (bee glue) is a sticky, dark-colored material that honeybees collect from living plants, mix with wax, and use in the construction of their nests. Its resistance against micro-organisms is one of its essential characteristics, and it has been used by human beings since ancient times for its pharmaceutical properties [3].

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The chemical composition of propolis depends on the properties of the collection site. Bees collect it from different source plants in different ecosystems by choosing appropriate representatives of the local flora. The different plant choices of bees in different habitats complicate propolis standardization [4].

Aim

In this study, we aimed to test the effects of propolis on warfarin therapy in a mouse model and determine the international normalized ratio (INR) values.

Material and methods

Animals

Male CD-1 mice were used with the approval of the Animal Care and Use Committee of Hacettepe University (permit no. 2016/5-10); the tests were carried out in accordance with the National Guidelines for the Use and Care of Laboratory Animals. After twenty 30–40 g CD-1 mice were purchased from the Animal Laboratory Unit of Hacettepe University, they were allowed to get used to the conditions of the laboratory for 1 week. The animals were housed separately in polypropylene cages with a 12–12 h light-dark cycle.

Experimental design

The mice were randomly divided into four groups containing five animals per group. The first set of mice ($n = 5$) formed the control group used to determine the baseline INR. The second group received propolis, and the third group was administered Coumadin by oral gavage. The fourth group was treated with Coumadin, which after a while was followed with propolis by oral gavage. All administrations were done over eight days starting at 2:00 p.m. On the 8th day, all animals were sacrificed in accordance with the ethical rules.

The selected dose for the application of propolis was 100 mg/kg/day (20 μ l/day) [5]. In mice, the average daily warfarin dose for a body weight of 40 g should be 0.08 mg [6]. A Coumadin tablet contains 5 mg of warfarin. One tablet of Coumadin was dissolved in 20 ml of tap water, and this was used as a stock solution. Each mouse in the relevant groups (third and fourth) received 320 μ l of the stock solution.

Preparation of ethanol extract of propolis (EEP)

We used a local honey bee product from Düzce, Turkey. Frozen propolis was ground and dissolved in 70% ethanol (1 : 3). This mixture was kept in a refrigerator for 2 weeks in a tightly closed bottle. The supernatant was filtered twice with Whatman no. 4 and 1 filter papers (Whatman International Ltd, Maidstone, UK). The filtered solution was an ethanol extract of propolis (EEP), and the final supernatant was analyzed using gas chromatography-mass spectrometry (GC-MS) [7].

Determination of volatile compounds in propolis samples by gas chromatography and mass spectrometry (GC-MS)

The GC-MS analysis of the EEP samples was performed using a 6890N gas chromatograph from Agilent (Palo Alto, CA, USA) coupled with a mass detector (MS5973, Agilent) equipped with a DB-5 MS capillary column (30 m \times 0.25 mm; film thickness: 0.25 μ m). Helium was used as the carrier gas at a flow rate of 1 ml/min.

Organic compounds in propolis samples were identified in the Wiley/NIST Mass Spectral Library [8].

INR value measurements

Propolis and Coumadin were orally administered to the mice daily for eight days. Blood samples were collected carefully in accordance with the ethical permission from 72 h after the first administration of chemicals every day at 9:00 a.m. The INR was measured on the 4th, 6th, and 8th days with an INR device called the CouguChek XS System (Roche Diagnostics Japan, Tokyo). The reference values for mean INR values in CD-1 mice have been published elsewhere [9].

Statistical analysis

The results are presented as mean \pm SD. Statistical data analysis was conducted with R software [10]. One-way analysis of variance (ANOVA) was performed and followed by Tukey's test to determine statistical significance for multiple comparisons. A difference was considered statistically significant at $p < 0.05$.

Results

Chemical composition of propolis

The main components of propolis, rich in alcohol, aldehydes, and flavonoids, are summarized in Table 1.

Animal experiment

In this study, CD-1 mice were preferred due to the existence of previous research using the same species [9]. Oral administration of both warfarin and propolis solution to each mouse (except the control group) was performed by gavage. All mice were sacrificed on the 8th day of the experiment. No deaths occurred during the study.

Changes in INR values

The first blood samples were collected 72 h after the first administration, and INR values were measured. The mean INR value in the control group (no warfarin or propolis treatment) was determined as 0.8 ± 0.1 . The INR results in the propolis group were the same as in controls; no changes were observed. All data were calculated from five mice per group for separate days.

After the administration of Coumadin (0.40 mg/kg/day) for 3 days, the INR value increased to 7.28 ± 1.08 (4th day). On the 8th day the INR value for the Coumadin group became stable at 4.24 ± 2.33 . When Coumadin and propolis

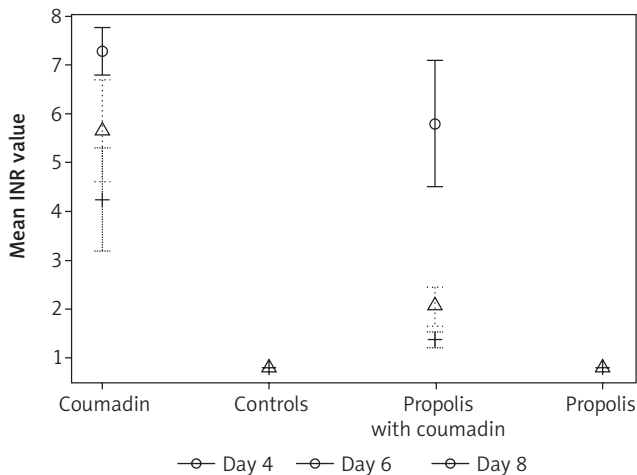


Fig. 1. All data were summarized with a plot of means

Circle: day 4, the first day of evaluating INR values; triangle: day 6, the second day of evaluating INR values; plus: day 8, the third day of evaluating INR values.

were administered concomitantly for 3 days, the INR value increased to 5.8 ± 2.88 (4th day). Then, on the last day of the experiment (8th day), the INR value gradually decreased to 1.38 ± 0.37 (Fig. 1).

These results demonstrate that propolis intake in combination with the Coumadin therapy dramatically reduced the anticoagulant effects of Coumadin in mice.

Discussion

Cardiovascular diseases are often associated with a high risk of recurrence and stroke. Today the most common cardiac dysrhythmia is atrial fibrillation. The standard treatment for this condition is oral anticoagulant therapy. For nearly a century, warfarin has been widely used for its anticoagulant effects exerted by inhibiting the activity of vitamin K epoxide reductase. Warfarin dosage is routinely checked with INR measurements. In recent years, the anticoagulant effects of warfarin have been affected by the intake of herbal products in concurrence with warfarin therapy, resulting in unstable INR values [11]. This has become a significant source of concern for health care providers because of the increasing concomitance of warfarin and herbal supplement use [12].

Propolis (bee glue) is a complex product combined with many different compounds (phenolic compounds, enzymes, and organic substances), which is used worldwide as a folk medicine [13, 14]. A great number of experiments have reported protective effects of propolis, but current reports on drug interactions with propolis are lacking [15]. The only thing that is known about propolis is that it might increase the risk of bleeding by slowing down the blood clotting process. Skalli *et al.* reported that alkaloids contained in propolis could lead to adverse cardiovascular events [16].

Flavonoids are polyphenolic compounds with a wide distribution including plant foods. Several *in vitro* studies have shown that polyphenols such as resveratrol and the flavonoids quercetin and catechin inhibit platelet aggregation.

Tab. I. The main components of propolis from Düzce, Turkey

Component	Percentage
Alcohols:	
2-Naphthalenemethanol, decahydro-alpha.,alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	1.47
α -Bisabolol	0.23
2-Propen-1-ol,3-phenyl-	2.05
2-Methoxy-4-vinylphenol	2.05
Benzyl alcohol	0.34
Phenylethyl alcohol	0.32
2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-, (4bS-trans)-	0.77
Aldehydes:	
Vanillin	0.41
Aliphatic acids and their esters:	
2-Propenoic acid, 3-phenyl-, phenylmethyl ester	0.29
6-Octadecenoic acid	1.24
9-Octadecenoic acid, ethyl ester	1.35
Tetradecanoic acid, ethyl ester	0.25
Hexadecanoic acid, ethyl ester	0.39
2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-	1.61
Dodecanoic acid, ethyl ester	0.41
n-Hexadecanoic acid	0.31
Carboxylic acids and their esters:	
1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	0.42
Benzoic acid	1.22
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.50
Cinnamic acids and their esters:	
Benzyl benzoate	0.49
p-Hydroxycinnamic acid, ethyl ester	0.31
Trans-cinnamic acid	0.74
2,4-Dimethoxycinnamic acid	0.86
Flavonoids:	
2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-	4.82
4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl(Pinocebrin)	16.08
4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl	1.26
4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-phenyl	2.54
4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxy	1.28
Chrysin	6.94
Others:	
Naphthalene, hexahydro-1,6-dimethyl-4-(1-methylethyl)-, [1S-(1.alpha.)]-	0.34
2-Naphthalenecarboxylic acid, 3,4-dihydro	2.51

Results obtained by incubating human or animal platelets with isolated flavonoids suggest that the antiplatelet properties may be attributed to the inhibition of TxA₂ formation, thromboxane receptor antagonism, protein kinase C activation, and phosphoinositide synthesis [17]. In the investigated propolis sample, we determined the ratio of flavonoids at 32.92%. Although flavonoids have anticoagulant effects, the propolis sample applied in our research (obtained from Düzce, Turkey) suggested an opposite effect.

Furthermore, the compound “1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)ester” was the major component of the used propolis; its antiviral, antimicrobial, antioxidant, and anti-inflammatory properties were discussed by Govindappa *et al.* [18]. The platelet effect of the propolis samples can be attributed to this compound, or it may be a synergistic effect of the other minor compounds. To analyze this effect, further research should be conducted by observing the effects of all the possible coagulant compounds after isolation.

To date, only two reports have been published about interactions between warfarin and bee products [19, 20]. However, no papers about interactions between propolis and warfarin have yet been published. In this study, we aimed to clarify the possible interactions of propolis and warfarin. For this purpose we used a mouse model. We examined whether propolis administration after warfarin decreased the anticoagulant effects of warfarin in mice using INR as an indicator. After the concomitant application of propolis and warfarin, a decline in the INR values was observed in the mice. Based on our data (INR values obtained on successive days), we can underline that phytotherapy may have a negative effect on warfarin treatment. By reducing the effects of warfarin, it can lead to increased thromboembolic risk.

Conclusions

Although there are many studies on the protective and therapeutic effects of bee products, the use of these products without a detailed analysis of their contents could lead to serious health problems. More information is required about the possible interactions between these products and other agents. Further experiments are needed to better understand the precise mechanism through which propolis affects INR values. The fact that the chemical composition of propolis varies depending on the botanical source makes it difficult to standardize its use. Determining the effect of propolis compounds on INR values will provide more explanatory results; this requires more trials with different propolis samples. Another important issue is that physicians should ask their patients about propolis intake before starting warfarin therapy.

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Disclosure

Authors report no conflict of interest.

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