SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 7.
Issue: 11
Page N. 50-55
Year: 2023

Original Article www.wjahr.com

IN VITRO EVALUATION OF THE EFFECT OF YERBA MATE ON WARFARIN EFFICACY

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Received date: 14 September 2023 **Revised date:** 04 October 2023 **Accepted date:** 24 October 2023

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ABSTRACT

Yerba mate (YM) is a popular tea in Arab countries especially Syria, it is made from the infusion of the dried leaves of *Ilex paraguariensis*. Traditional and composed YM are a rich source of polyphenols. Warfarin is a widely prescribed anticoagulant drug with a narrow therapeutic index. Warfarin is known for its significant and extensive interaction with food and other drugs. This study aimed to investigate the effects of different concentrations of YM extract on the international normalized ratio (INR) of patients who receive Warfarin. We prepared many concentrations of each traditional and composed YM. The total polyphenolic content was determined using a spectrophotometric assay at 750 nm. The anticoagulant effect was evaluated by calculating the international normalized ratio INR depending on prothrombin time (PT). The results showed that the phenolic content of composed YM was (106.88 mg_{GAE}/g) which was higher than the one of the traditional YM (90.47 mg_{GAE}/g). *In-vitro* PT test showed that both traditional and composed YM could prolong prothrombin time and INR, in a concentration dependent manner. All results indicated that YM could interact with Warfarin and unfortunatly might cause bleeding.

KEYWORDS: *Ilex paraguariensis*, Yerba Mate, polyphenols, Ginger, Prothrombin time, Warfarin.

1. INTRODUCTION

Cardiovascular diseases (CVD) are the main causes of death worldwide. All patients who have artificial heart valves are taking antithrombotic drugs especially Warfarin, which is the racemic mixture of R and S Warfarin.[1,2] Warfarin's therapeutic dose is always challenging, the determination of it depends on the international normalized ratio (INR) and prothrombin time (PT).[3,4] INR is a ratio between the PT of the patient and the PT of a normal (control) sample.[5] PT is a global test to evaluate extrinsic coagulation, being sensitive to factors (II, V, VII, and X). This test is used for routine tests in the control of oral anticoagulants therapy (Warfarin).[5] INR of 2.0 to 3.0 is generally considered in the therapeutic range, and the risk of bleeding increases when the INR exceeds.[3,4,6]

Recently, herbal products have been extensively used around the world and patients who receive antithrombotic drugs are also receiving these herbs.[7] Several studies have confirmed the interactions between Warfarin, which has a narrow therapeutic index, and a variety of food and herbal products especially which have anticoagulant properties.[8] These interactions could be dangerous and fatal because herbal products may increase the risk of bleeding or potentially change the response to Warfarin treatment.[9,10,11] Also Many researchers suggested that polyphenol-rich extracts have anticoagulant effects.[12]

Yerba mate (YM) is a popular tea in southern Latin American countries and Arab countries, especially Syria.[13,14] It is made by infusion of the dried leaves of *Ilex paraguariensis*, a plant of the Aquifoliacae family.[15] Polyphenols are the major compound in YM, which are secondary metabolites widely disseminated in plants but not synthesized in animals.[16,17] Phenolic compounds have antioxidant,[18] anti-inflammatory,[19] antimicrobial,[20] anti-thrombotic activities.[21] and may reduce the risk of many cardiovascular diseases and cancer.[22]

Some consumers add herbal plants to YM to improve its bitter taste and in this case, it is called "Composed Yerba Mate". Many research showed that traditional and composed YM aren't similar in composition and properties.[23] Ginger is one of the most medicinal plants added to the YM[3]. Ginger's extract has a high antioxidant activity due to the presence of polyphenol compounds (6-gingerol and its derivatives).[24]

In this study, we aimed to investigate the effects of traditional and composed YM on Warfarin therapy *in-vitro*. For this purpose, PT and INR are used as an indicator.[12,25]

2. MATERIALS AND METHODS

2.1. Instruments and chemicals

Spectrophotometer (Jasco V-530 UV), PT analyzer (Coa DATA 4004), Centrifuge and Water bath ultrasonic (K & H Industries) were used in this study.

Folin-Ciocalteu reagent was purchased from Sigma-Aldrich, Switzerland. Gallic acid was purchased from Biotech LTD, India. Sodium Carbonate was obtained from BDH, England. Kit for the measurement of PT was purchased from Biorex, BT41 1QS, United Kingdom.

2.2. Preparation of traditional and composed YM infusions

I.Paraguarensis and fresh ginger were obtained from a commercial shop in Tartous, Syria. Fresh ginger has been chopped and dried in a dark, well-ventilated place for two days, and then milled with a household grinder then stored in a dry place.

Four aqueous extracts were prepared according to the method described by Omar *et al* (2019) with minor modifications to simulate the popular use of YM in Syria. For the traditional YM, dried YM was prepared by the infusion (10, 16, 26, 32 g) per 300 ml of boiling water. First, 10g of YM were put with 30 ml of boiling water in a cup, waited for 5 min then filtered through 45 mm Millipore Filter. Then, the rest of YM was re-extracted with 30 ml of boiling water as prescribed before. This step was repeated again for eight times, and thus 300 ml was the final volume of the first extract. The extraction was repeated with larger amounts of YM (16, 26, 32 g). All resulted extracts were frozen at -8 ºC until usage.

For the composed YM, we followed the same steps but we added 0.5 g of dried ginger to each amount of YM (10, 16, 26, 32 g).

2.3. Determination of total phenolic content in yerba mate infusions

The total phenolic content in all aqueous extracts (AE) was determined by Folin-Ciocalteu Method described by Al Diab et al (2021).[26,27,28] 0.5 ml of diluted sample was transferred to Folin-Ciocalteu reagent (0.1 ml). Then, the solution was vigorously mixed and neutralized with sodium carbonate (0.1ml, 1%). After 30 minutes of incubation in the absence of light at room temperature, the absorbance was recorded at 750 nm. Distilled water was used instead of the sample as a blank. Phenolic contents were expressed as milligrams of Gallic acid equivalents (GAE) per gram of AE. The method was performed in triplicate for each extract.

2.4. Blood samples collection

Ten volunteers, who receive Warfarin in a daily dose of (2.5 mg), with a mean age of 55 years (40-70 years), were instructed not to drink YM during the previous day of blood sample collection and to fast for 12 h. The blood sample was drawn from the antecubital vein by venipuncture into citrate tubes. Platelet-poor Plasma was isolated by centrifugation at 4000 rpm for 10 min and used immediately in a PT test.

2.5. *In-vitro* determination of PT and INR in the presence of YM extracts

50 µl of platelet-poor plasma were incubated with 50 µl of mate extract at 37ºC for 2 min. Afterward, 100 µl of Biorex kit was added and PT time was recorded. To remove water's effect on PT, a result (PT blank – PT control) was deleted from PT after adding extract.

According to the World Health Organization (WHO), INR is obtained by the following formula to calculate it:
INR = Patient PT / Control PT.[29,5]

2.6. Ethical Approval

All procedures were approved by the Bioethics Committee of Tishreen University and the ethical approval has been to this article.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation. PT was statistically compared using one-way ANOVA and Student's paired t-test[5]. Differences were considered to be significant when p-value < 0.05. All statistical analyses were performed using Microsoft Excel 2016 Software.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content (TPC) in YM extracts

TPC in all aqueous extracts of traditional YM are present in Table 1. The average TPC in YM was (90.47mg $_{GAE}$/g).

The highest concentration was found in the fourth extract (15.42 g_{GAE}/l), which was prepared with the highest amount of *I. Paraguarensis* (32.203 g). De Mejia *et al* (2010)[15] reported also that TPC in YM ranged (90-176 mg_{GAE}/g).

The phenolic content in our study was higher than that reported by Cheminet *et al* (2021) where the TPC was (15-45 mg_{GAE}/g)[23] However, it was lower than that obtained by Silva *et al* (2020) of the Brazilian study which was (187.36 mg_{GAE}/g).[30]

The difference in our results and those of the researchers can be attributed to many factors like the different raw materials, the analytical methods, and the types of solvent used.[31,32]

Table 1: Total phenolic content in the Traditional YM extracts.

A mount of dry YM (g)	Concentrations of phenolic compounds in aqueous extracts (g/l)	TPC in 1g of YM (mg_{GAE}/g)
10	3.278 ± 0.02	82.9 ± 0.05
16	6.407 ± 0.05	94.85 ± 0.07
26	9.104 ±0.07	87.87 ± 0.06
32	15.42 ±0.03	96.28 ± 0.08

Results are expressed as mean ± SD, n=3

Table 2: Total phenolic content in the composed YM extracts.

A mount of dry YM (g)	Amount of dry ginger (g)	Concentrations of phenolic compounds in aqueous extracts (g/l)	TPC in 1g of YM (mg_{GAE}/g)
10	0.5	4.506 ± 0.02	106.84 ± 0.04
16	0.5	8.752 ±0.04	109.97 ± 0.01
26	0.5	12.607 ±0.03	106.92 ± 0.01
32	0.5	17.074 ±0.04	104.44 ± 0.07

TPC in the aqueous extracts of composed YM are present in Table 2. The TPC was higher in composed than traditional YM (106.88mg_{GAE}/g versus 90.47mg_{GAE}/g). The highest concentration was also found in the fourth extract (17.074g_{GAE}/l) that was obtained from 32 g YM. Interestingly, determination of the phenolic content of mixture of ginger and YM was not reported in the literature, however, De Mejia *et al* (2009) estimated that TPC in YM with other flavoring ingredients was (40-113 mg_{GAE}/g).[15]

Results are expressed as mean ± SD, n=3

3.2. Effect of traditional and composed YM on PT and INR

Based on the obtained results, all the concentrations of Traditional YM demonstrated an effect on the coagulation cascade by prolonging PT (23.9 sec) and INR (2.46) significantly (Table 3), especially the fourth extract obtained from 32g YM (15.42 g_{GAE}/l) which resulted an INR of (6.13) ($P<0.05$).

The effect of composed YM on PT and INR is present in Table 4. All concentrations of the phenolic extracts resulted an INR value significantly higher than the basal INR (2.46) ($P<0.05$). The fourth extract, which obtained from 32 g YM with 0.5g ginger (17.074 g_{GAE}/l), also resulted the highest effect on PT (50.5 sec) and INR (6.53).

Table 3: Effect traditional YM on PT and INR.

A mount of dry YM (g)	PT control (sec)	PT blank (sec)	PT after adding extract (sec)	INR after adding extract
10	23.9 ± 2.95	24.9 ± 2.92	26.5 ± 2.98	2.80 ± 0.42
16	23.9 ± 2.95	24.9 ± 2.92	28.9 ± 3.13	3.14 ± 0.45
26	23.9 ± 2.95	24.9 ± 2.92	38.8 ± 3.18	4.68 ± 0.51
32	23.9 ± 2.95	24.9 ± 2.92	48.3 ± 2.82	6.13 ± 0.48

Results are expressed as mean ± SD, n=3

Table 4: Effect composed YM on PT and INR.

Amount of dry YM with ginger (g)	PT control (sec)	PT blank (sec)	PT after adding extract (sec)	INR after adding extract
10.5	23.9 ± 2.95	24.9 ± 2.92	28.3 ± 3.02	3.05 ± 0.42
16.5	23.9 ± 2.95	24.9 ± 2.92	31.0 ± 3.29	3.45 ± 0.48
26.5	23.9 ± 2.95	24.9 ± 2.92	41.7 ± 3.07	5.08 ± 0.51
32.5	23.9 ± 2.95	24.9 ± 2.92	50.5 ± 2.83	6.53 ± 0.41

Results are expressed as mean ± SD, n=3

All extracts of traditional YM resulted INR higher than the therapeutic range [2-3] and this effect depends on

concentration as shown in Fig. 1. Also all extracts of composed YM prolonged INR more than [2-3]. Fig. 2 presents the effect of composed YM on INR.

It was clearly noticed that the aqueous extract of YM either alone or with ginger had an anticoagulation effect as they increased INR values significantly relative to the control. It is known that ginger may lead to bleeding when consumed with Warfarin,[33] this property might be enhanced if it is taken with YM because of its phenolic content. Many *in-vitro* studies have confirmed that polyphenols rich -plant extracts might interact with Warfarin. The interaction between phenolic extracts of YM and warfarin was not reported in the literature despite the its wide consumption in our country -even so in many other countries- and despite the importance of this interaction which could be fatal.

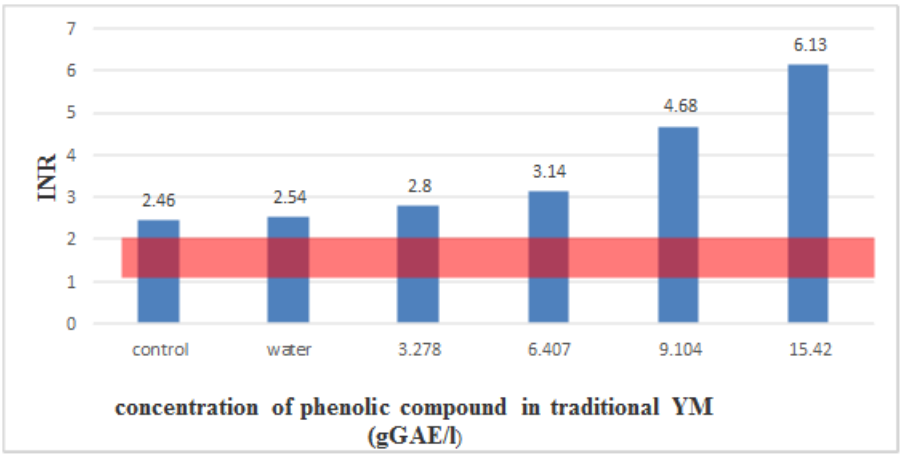

Figure 1: effect of traditional yerba mate on the INR time. P<0.05.

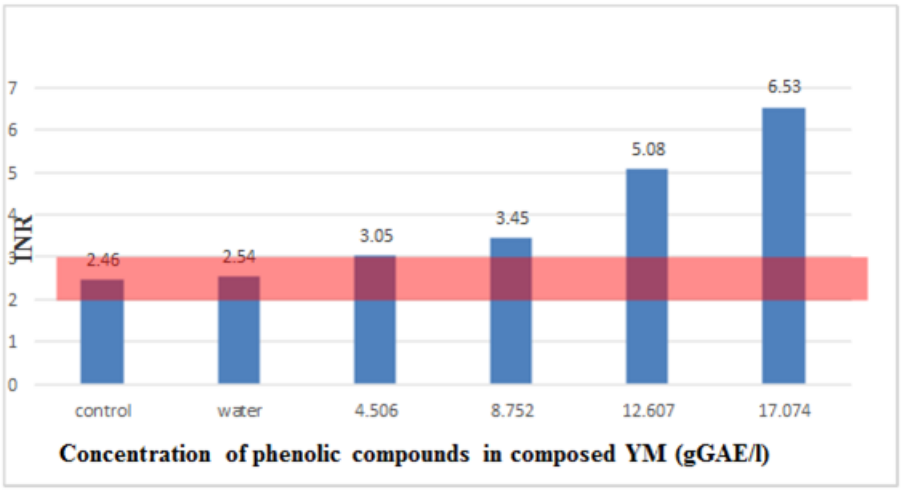

Figure 2: effect of composed yerba mate on the INR time. P<0.05.

Ahmad *et al* (2023) suggested that a phenols-rich extract of *Rosa damascene* has interacted with Warfarin *in-vitro* by prolonging PT and INR.[21] Also, Yu *et al* (2021) confirmed that *Cranberry* which contains a variety of polyphenols could influence Warfarin therapy and prolong bleeding time.[34] Many *in-vivo* studies suggested that polyphenols have an inhibitory effect on factor X activity. In this case, polyphenols affect the coagulation cascade and PT.[35]

The potential mechanism of the anticoagulant effect of polyphenols is mediated through the inhibition of the activity of several factors like thrombin.[35,36,37] Also polyphenols could inhibit cytochrome P450, the main metabolizing enzyme for Warfarin, and enhance the anticoagulant effect of Warfarin.[34]

Pharmacists and other healthcare professionals should stay aware of the potential interaction between drugs-both prescribed and over the counter- and traditional herbs and teas. Therefore, they should ask patients about the use of alternative therapies.[33,38]

More clinical research is needed taking into consideration the limitations of *in-vitro* and *in-vivo* studies because Herb-Warfarin interaction has considerable clinical significance, so it is necessary to determine the herbs that interact with Warfarin.

These results also suggest that further research on the action of these plants could be of real clinical value in identifying potential alternative anticoagulant therapies.[37]

CONCLUSION

This study highlighted the importance of herb-drug interaction, the case of traditional and composed YM. Unlike what the public usually expects, herbs are not always safe even if they are natural. Especially for drugs with narrow therapeutic index like Warfarin.

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