Research Article

Pharmacodynamic and Pharmacokinetic Interaction of Warfarin in the Presence of Some Commonly Used Complementary and Alternative Medicines (CAMs) in Rat Plasma by Using HPLC

Farah Al-Mamoori[1*], Kenza Mansoor[1], Eyad Mallah [1], Feras Elhajji[2]

[1]Department of Pharmaceutical medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman-Jordan.

[2] Department of Pharmacy and Medical Sciences, Applied Science University, Amman-Jordan.

***Corresponding author's E-mail:** farahalmaamori@yahoo.com

Received: 28-09-2018; **Revised:** 30-10-2018; **Accepted:** 10-11-2018.

ABSTRACT

Complementary and alternative medicines (CAM), specifically herbal medicine, has been used since antiquity for the treatment of different ailments. However, a large number of studies on minor and significant drug–herb interactions have been recently reported since many people believe that "herbal products are harmless". The study was conducted regarding the pharmacodynamic (PD) and pharmacokinetic (PK) interaction between a warfarin and some commonly used CAMs (turmeric, hawthorn and cinnamon). To address this aim, Prothrombin Time (PT) and International Normalized Ratio (INR) were monitored. In vivo studies were conducted on Wistar laboratory rats and were divided into multiple and single dosing groups. Besides, they were analyzed by using High performance liquid chromatography (HPLC). It was developed and validated over concentration range100-4000 ng/ml of warfarin. The mobile phase was (40% acetonitrile, 60% water with triethylamine 1 ml/1L), BDS herpasil C18colum (150mm x4.6 mm, 5µm), 1.0 ml/min flow rate, 20µl auto-sampler injection volume and metronidazole as an internal standard. The pharmacodynamic results showed that the three CAMs contributed to a significant ($p<0.05$) increase in PT and INR of warfarin. The pharmacokinetic results showed that no significant difference ($p>0.05$) in mean plasma concentration of warfarin and pharmacokinetic parameters (C max, AUC, t0.5, Kel) when combined with CAMs. The type of interaction between warfarin in combination with turmeric, hawthorn and cinnamon is a pharmacodynamic interaction. Therefore, patients using warfarin as an anti-coagulant have to use these herbal medicines cautiously.

Keywords: Warfarin, Complementary and alternative medicine (CAM), Turmeric, Hawthorn, Cinnamon, Drug-herb interaction

INTRODUCTION

Complementary and Alternative Medicines (CAMs) is a highly various group of approaches to health care and are based on philosophies towards health and illness that are essentially different from the approach of conventional, biomedicine and pharmacy[1]. Herbal medicine (HM) is described as "holistic" system of healing together with a distinct tactic to the diagnosis and treatment of a wide range of complaints [1].

Drug-Herb Interaction (DHI) is an important issue affecting the efficacy and clinical safety of therapeutic treatment. There have been an increasing number of reports on significant DHI worldwide. Due to the popularity of using herbal products, and using multiple medicines, this has significantly increased the risk of potential interactions, especially in the elderly or specific groups of consumers[2]. The risk of interaction is increased if HMs are used concurrently with drugs which have a narrow therapeutic index like warfarin[3].

HMs are gaining popularity these days. Turmeric, hawthorn and cinnamon. Turmeric (*Curcuma longa)* is extensively used as a spice, food preservative and coloring material for thousands of years, it has been reported that it exerts significant effect on arthritis, alzheimer's disease, cancer therapy, and antimicrobial activity[4-7].

Hawthorn (*Crataegus spp.*) has been as a medicinal material and food for hundreds of years. It is used for cardiovascular conditions such as: heart failure, hypertension, angina and arrhythmias. Furthermore, it is used for sexual dysfunction[8-10].

Cinnamon (*Cinnamomum spp.)* is known and widely used in the ancient world, as a popular cooking spice and traditional medicine. It is used in Type 2 Diabetes, improves the insulin resistance in women with poly cystic ovary syndrome (PCOS), in addition to its antimicrobial activity [11-13].

Warfarin is one of the most widely used anticoagulants worldwide for prophylaxis and treatment of venous thrombosis and its extensions, reduction in the risk of death, recurrent myocardial infarction, and thromboembolic events such as stroke[14]. This study was aimed to investigate the pharmacodynamic and pharmacokinetic interaction between warfarin and some commonly used CAMs (turmeric, hawthorn and cinnamon). Also, to develop and validate chromatographic methods for the simultaneous estimation of warfarin and some commonly used CAMs in rat plasma.

MATERIALS AND METHODS

Samples and reagents

Warfarin sodium 5mg tablet (ORION PHARMA B#1624628, Finland) was purchased from a local pharmacy. Turmeric; Curcu-Truw 81mg hard capsule (TRUW B#4009694) (Truw, *Gütersloh*, Germany), whereas hawthorn; Crataegutt novo 450mg tablet (SCHWABE B#5961214) was obtained from (Schwabe, Karlsruhe, Germany). Cinnamon; Diabetruw 112mg hard capsule (TRUW B#306827) was obtained from (TRUW company, *Gütersloh*, Germany). For prothrombin test, Neoplastine® C1 Plus (lyophilized fresh rabbit brain thromboplastin with a specific heparin inhibitor hydrated with a solvent containing calcium with stabilizers, polybrene, buffer and preservatives).

Preparations of warfarin oral solution

Warfarin (10mg) dissolved in 100ml of distilled water to obtain (100 µg/ml) stock solution of warfarin.

Preparations of CAMs oral suspension

Turmeric (4mg), hawthorn (6mg), and cinnamon (5mg) were suspended in 1 ml of distilled water.

Pharmacodynamic Interaction Study

Study design

Study was performed on male and female Wister laboratory rats (weight range 170–200 g) supplied by the animal house of Applied Science University. All animal experiments were performed in compliance with Federation of European Laboratory Animal Science Association (FELASA) guidelines. The rats were divided into multiple dosing groups. Groups of rats in the multiple dosing study were divided into 3 groups (n=8); A, B, and C in each experiment.

A syringe which has a specific needle was used for oral administration of both warfarin solution and CAMs suspension. Group A received multiple doses of warfarin aqueous solution (0.5mg/kg) orally in combination with CAMs suspension; turmeric (20mg/kg), hawthorn (30 mg/kg) and cinnamon (25mg/kg). As for group B, warfarin solution was administered as a control. Group C received multiple doses of CAMs; turmeric (20mg/kg), hawthorn (30 mg/kg) and cinnamon (25mg/kg). Blood samples were collected from the retro-orbital plexus at specific time intervals: zero, 100 hours (day five). They were drawn into a sodium citrate 3.2% containing micro tubes. The micro tubes were immediately centrifuged at 5000 RPM for 10 minutes to obtain 250-300µl plasma; samples were placed into labelled Eppendorf tubes for PT and INR laboratory analysis. Readings were taken shortly after sample collection ≤4 hours.

Measurement of PT and INR

The reagent pre warmed by placed in the incubation block for one specific measuring channel. Then, 100 µL of plasma sample was placed in a cuvette that has been pre warmed in the incubation block to 37^0 C. The incubation time (60 sec) as selected in the setup will run and begins down. Once the incubation has been completed a long beep can be heard, then the cuvette placed in the appropriate measuring channel. Next, 200 µL of starting reagent to cuvette, the measuring process started automatically. As soon as the clot formation has been recognized the measuring timer display the measured clotting time.

INR was calculated depending on equation 1.

$$\mathbf{INR} = \left(\frac{\mathbf{PTtest}}{\mathbf{PTnormal}}\right)\mathbf{\hat{}ISI} \quad \text{..... Equation 1}$$

ISI: International Sensitivity Index.

Validation

Instrumentation and chromatographic conditions

An HPLC (FINNIGAN SURVEYOR) (Thermo Electron Corporation, San Jose, CA, USA). The detector (UV-VIS Plus Detector), the pump (solvent delivery systems pump) (LC Pump plus) and the autosampler (Autosampler Plus). Computer system used was Windows Xp and the software used was ChromQuest software 4.2.34. HPLC system was set at a wavelength of 310 nm and coupled with a hypersil™ BDS C-18 Column (Thermo Electron Corporation, San Jose, CA, USA); (150 mm x 4.6 mm, 5µm) with a flow rate of 1 ml/min using a 20 µl injection volume. Metronidazole benzoate was used as an internal standard (I.S) in this method.

Preparation of warfarin solution

Warfarin (20.00 mg) dissolved in 50 ml methanol to obtain a final concentration (400µg/ml) stock solution of warfarin.

Preparation of stock solutions of Metronidazole benzoate IS

Metronidazole benzoate (10.0 mg) dissolved in 10 ml methanol to obtain a final concentration of (1000.0 µg/ml) stock solution of metronidazole benzoate.

Preparation of warfarin spiking samples and quality control (QC) samples in plasma

Calibration curve and QC samples were prepared by taking different volumes (µl) from warfarin stock solution (400µg/ml) to reach 1000 µl final volume. Consequently, concentrations of working solutions (µg/ml) were obtained to be used later for plasma spiking solutions which were prepared by taking 25 µl of each working solution to be spiked in 975 µl of plasma (final volume 1000 µl) (Table 1).

Method of extraction

An appropriate number of Eppendorf tubes were placed in a rack and labeled properly, 100 µl aliquots of each test sample (blank, zero, standards, QC low, QC mid, QC high or rat plasma) was pipetted into the appropriate labeled tube followed by the addition of 150µl IS (1.5 µg/ml). Finally, each sample was vortexed vigorously for 1 min

then centrifuged at 14000 rpm for 15 minutes. The supernatant was transferred to flat bottom insert then 20 µl was injected.

Table 1: Spiked plasma of warfarin serial samples and QC samples

	Serial solutions				Plasma spiking			
	Solution No:	Volume taken from stock (µl)	Total volume (µl)	Working solution concentration (µg/ml)	Calibration	Volume taken from working solution (µl)	Total Volume (µl)	Final concentration (ng/ml)
Calibration points	1	10	1000	4	1	25	1000	100
	2	20	1000	8	2	25	1000	200
	3	50	1000	20	3	25	1000	500
	4	100	1000	40	4	25	1000	1000
	5	200	1000	80	5	25	1000	2000
	6	300	1000	120	6	25	1000	3000
	7	400	1000	160	7	25	1000	4000
QC points	8	30	1000	12	QCL	25	1000	300
	9	180	1000	72	QCM	25	1000	1800
	10	350	1000	140	QCH	25	1000	3500

Validation of the method

Method validation was performed in three separate days. In each day, seven standard calibration levels were prepared (not including zero). The prepared plasma samples of the method validation represented blank, zero, standard calibration curve, six replicates of QC samples (LLOQ, Q.C Low, Q.C Mid and Q.C High). The validation parameters (linearity, intra and inter-day accuracy and precision, recovery and stability) should be within the expected limits according to European Medicines Evaluation Agency (EMEA) guidelines by the Food and Drug Administration (FDA) Guidance for Industry and United state pharmacopeia (USP).

Pharmacokinetic interaction study

Study design

Study was performed in male and female Wister laboratory rats (weight range 170–200 g) supplied by the animal house of Applied Science University. All animal experiments were performed in compliance with Federation of European Laboratory Animal Science Association (FELASA) guidelines. The rats were divided into multiple dosing groups. Groups of rats in the multiple dosing studies were divided into 3 groups (n=8); A, B, and C in each experiment. For multiple dosing experiments; group A orally received multiple doses of warfarin aqueous solution (0.5mg/kg) orally in combination with CAMs suspension; turmeric (20mg/kg), hawthorn (30mg/kg) and cinnamon (25mg/kg). As for the group B, warfarin solution was administered as a control. For single dosing experiments; group A orally received single dose of warfarin aqueous solution (0.5mg/kg) in combination with CAMs suspension; turmeric (20mg/kg), hawthorn (30 mg/kg) and cinnamon (25mg/kg).

As for group B, single dose of warfarin was administered as a control. Blood samples were collected from the rats retro-orbital plexus at specific time intervals: zero,4,24,28,48,52,72,76,96 and100 hours from group A and B of multiple dosing. While blood samples were taken at the flowing time points: zero, 1,2,3,4,5,7,10,24,30,48 and 78 hours from group A and B of single dosing.

They were drawn into EDTA micro tubes. The micro tubes were immediately centrifuged at 5000 rpm for 10 minutes to obtain the plasma (250-300µl), which was placed into labelled Eppendorf tubes and stored at (-30C°) until further HPLC analysis.

Statistical analysis

Statistical analysis was performed using Independent Students t-test by SPSS software (version 22).

Each data point represents the mean ± SD. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Pharmacodynamics interaction between warfarin and CAMs

Effect of turmeric on warfarin pharmacodynamics

Warfarin- turmeric combination significantly increased ($P<0.05$) mean PT and INR in comparison to warfarin alone. The mean PT was 220.13±33.02 and the mean INR was 27.05±5.44. Mean PT and INR increased (94.41%, 163.91% correspondingly) (Table 2). Significant pharmacodynamic interaction between warfarin (0.5mg/kg) and turmeric extract (20mg/kg) was noticed (Figures1 and 2).

Effect of hawthorn on warfarin pharmacodynamics

There was a significant difference (P<0.05) in PT and INR between warfarin –hawthorn combination and warfarin alone. Mean PT and INR increased 42.16%, 49.87% respectively (Table 3). Significant pharmacodynamic interaction between warfarin (0.5mg/kg) and hawthorn extract (30mg/kg) was noticed (Figures 3 and 4).

Effect of cinnamon on warfarin pharmacodynamics

Significant increase in PT and INR of warfarin (P<0.05) when combined with cinnamon. Mean PT and INR increased was of 69.13%, 110.24% respectively (Table 4). Significant pharmacodynamic interaction between warfarin (0.5mg/kg) and cinnamon extract (25mg/kg) was noticed (Figure 5 and 6).

Table 2: Comparing mean PT and INR of warfarin, turmeric and warfarin -turmeric. The data are presented as mean ± SD (n=8).

	PT (second)	INR	Ratio
Warfarin"control"	113.22±17.87	10.25±2.52	6.22±1.03
Turmeric	28.5±8.12*	1.80±0.66	1.54±0.44
Warfarin-Turmeric combination	220.13±33.02	27.05±5.44	11.89±1.78
Difference between 2 mean (control & combination)	106.9	16.80	5.68
Percent change%	94.41	163.91	91.29
P(t-test)	0.000012*	0.000015*	0.0000018*

Note: Negative control PT=18.5second; *P<0.00001 (significant).

Table 3: Comparing mean PT and INR of warfarin, hawthorn and warfarin- hawthorn. The data are presented as mean ± SD (n=8).

	PT(seconds)	INR	Ratio
Warfarin	113.22±17.87	10.25±2.52	6.22±1.03
Hawthorn	31.27±6.49*	2.0425±0.54	1.73±0.36
Warfarin-Hawthorn combination	160.96±5.18	17.16±8.21	8.92±3.20
Percent change	42.16	49.87	43.44
P(t-test)	0.04*	0.04*	0.04*

Note: Negative control PT=18.5second; *P<0.05 (significant).

Table 4: Comparing mean PT and INR of warfarin, cinnamon and warfarin -cinnamon. The data are presented as mean ± SD (n=8)

	PT(second)	INR	Ratio
Warfarin "control"	113.22±17.87	10.25±2.52	6.22±1.03
Turmeric	28.5±8.12*	1.80±0.66	1.54±0.44
Warfarin-Turmeric combination	220.13±33.02	27.05±5.44	11.89±1.78
Difference between 2 mean (control & combination)	106.9	16.80	5.68
Percent change%	94.41	163.91	91.29
P(t-test)	0.000012*	0.000015*	0.0000018*

Note: Negative control PT=18.5second; *P<0.05 (significant).

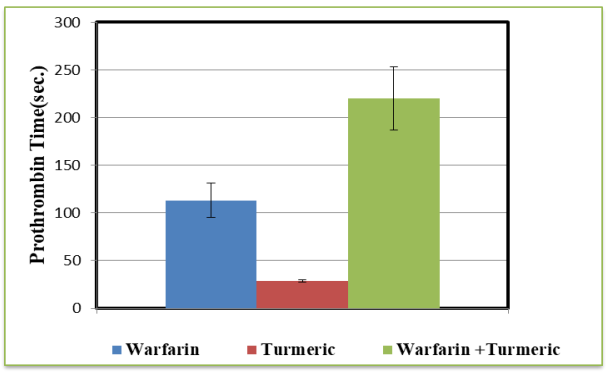

Figure 1: PT values of warfarin, turmeric and combination of both at day five.

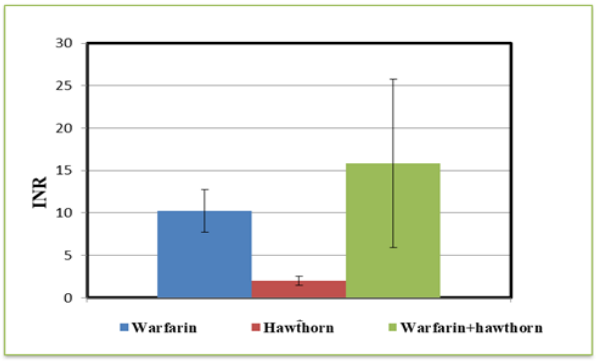

Figure 2: INR values of warfarin, turmeric and combination of both at day five.

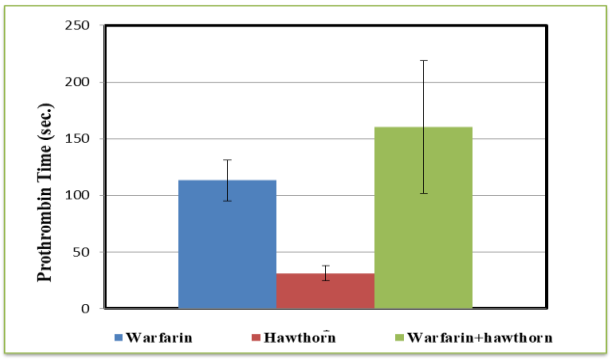

Figure 3: PT values of warfarin, hawthorn and combination of both at day five.

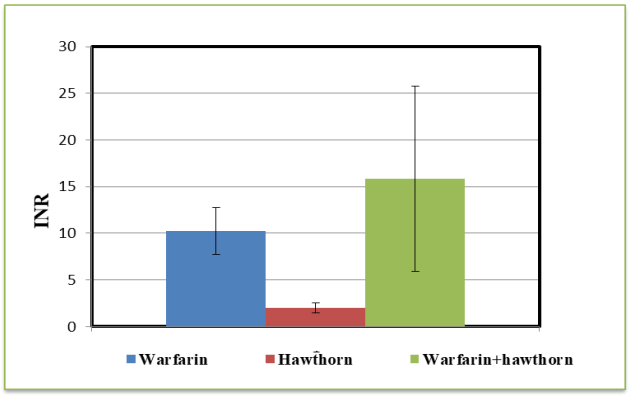

Figure 4: INR values of warfarin, hawthorn and combination of both at day five

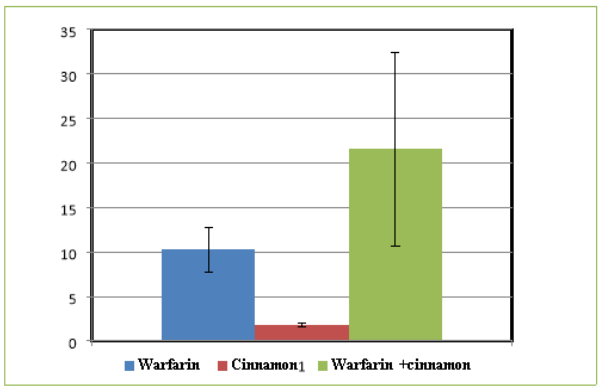

Figure 5: PT values of warfarin, cinnamon and combination of both at day five.

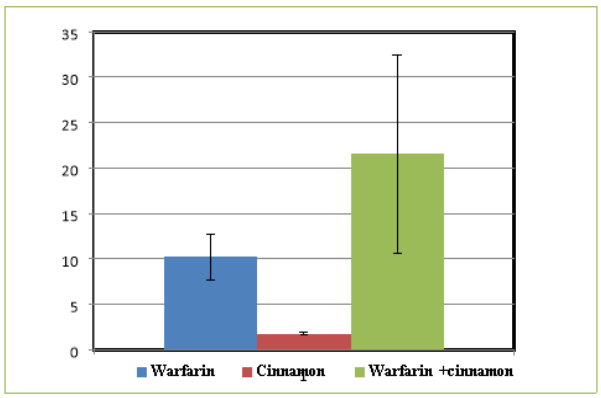

Figure 6: INR values of warfarin, cinnamon and combination of both at day five.

Validation

Validation of this analytical HPLC-UV method was performed in order to be evaluated in terms of recovery, linearity of response, precision, accuracy, and stability for quantification of warfarin. Inter-day precision and accuracy with CV% range (1.22-4.85) and accuracy % range (97.95-107.76) with a linear relationship which was observed between the concentration and the peak area of warfarin (correlation coefficient, R^2 = 0.999). In addition, the recovery of warfarin from its biological matrix in this bioanalytical method was 98.74-98.81%). The plot of linearity of calibration curve levels for warfarin quantification against their analytical response and regression linear equation that represents all three days of validation was done by plotting the calculated mean of the measured concentrations versus the calculated mean of the AUC ratio for each and (%) standard point (Figure 7).

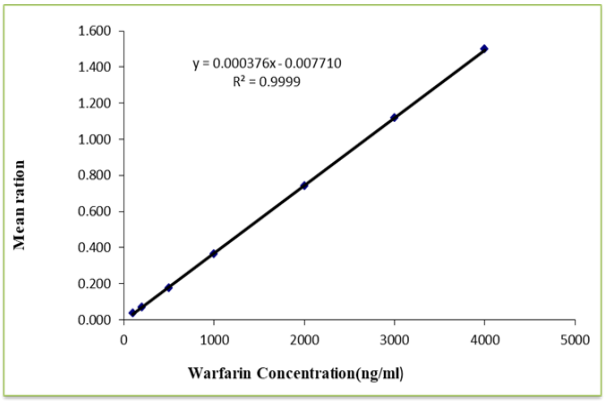

Figure 7: Warfarin concentration verses mean ratio for each standard point.

Pharmacokinetic interaction between warfarin and CAMs

In the current study, the pharmacokinetic profile of warfarin in single dose and multiple doses was evaluated on rats. Furthermore, warfarin was studied in combination with turmeric, hawthorn and cinnamon.

Effect of turmeric combination on warfarin pharmacokinetics

Multiple doses

No notable differences were found in mean plasma concentration between samples obtained from rats fed with warfarin (0.5mg/kg) and in combination with turmeric (20mg/kg) in multiple doses (Table 5, Figure 8).

Single dose

No statistically significant difference (P>0.05) in warfarin pharmacokinetic parameters (Cmax, AUC, t0.5, Kel) was noticed in combination with turmeric (20mg/kg) in single dose (Table 6). Furthermore, an overview of pharmacokinetic profile of warfarin in presence of turmeric; showed no change in AUC and Cmax (Figure 9).

Effect of hawthorn combination on warfarin pharmacokinetics

Multiple doses

No notable differences were found in mean plasma concentration between samples obtained from rats fed with warfarin (0.5mg/kg) and in combination with hawthorn (30mg/kg) in multiple doses (Table 7, Fig.10).

Single dose

No significant differences (P>0.05) were found in Cmax, AUC,t0.5 and Kel between warfarin (0.5mg/kg) alone, and combination with hawthorn (30mg/kg) in single dose. No Changes were detected in warfarin pharmacokinetics when used with a single dose of hawthorn (Table 8, Figure 11).

Effect of cinnamon combination on warfarin pharmacokinetics

Multiple doses

No notable differences were found in mean plasma concentration between samples obtained from rats fed with warfarin (0.5mg/kg) and in combination with cinnamon (25mg/kg) in multiple doses (Table 9, Figure 12).

Single dose

When warfarin was administered alone, it reached its mean maximum plasma level (1652.68ng/ml) after 5 hour. But when administered with cinnamon; it is reached maximum plasma level (1729.10ng/ml) at 5 hours. So no significant effect was detected (Table 8, Figure 11).

Table 5: Plasma concentration of warfarin after administration with turmeric in multiple doses. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin "control"(ng/ml)	Concentration of warfarin-turmeric combination (ng/ml)
4	3343.44±483.43	3115.92±551.20
24	1413.19±367.31	1337.09±423.69
28	4148.86±717.63	3872.33±684.53
48	1941.47±595.58	1738.52±558.22
52	4539.67±915.56	4195.85±619.44
72	2352.54±681.19	2056.48±608.09
76	4530.11±710.41	4315.81±852.77
96	2561.08±490.01	2527.37±597.87
100	4907.58±701.21	4731.82±926.02

**P>0.05(insignificant).

Table 6: Plasma concentration of warfarin and warfarin- turmeric in single dose. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin "control" (ng/ml)	Concentration of warfarin-turmeric combination (ng/ml)
1	1339.06±414.85	1522.52±509.93
2	1579.69±369.75	1791.44±501.95
3	1698.30±347.71	1987.20±439.15
4	1785.90±371.11	1958.30±363.88
5	1919.61±412.92	1943.22±406.58
7	1948.73±472.14	1930.78±552.58
10	1837.20±488.03	1731.04±589.60
24	1156.30±374.89	1119.19±584.99
30	913.26±301.54	837.45±453.83
48	427.49±200.06	345.09±189.81
78	128.76±69.71	119.21±65.50
AUC 0-78 (ng/ml*hr)	**64483.53**	**61026.83****
AUMC 0-78 (ng/ml*hr^2)	**1470794.35**	**1321514.57****
Tmax (hr)	**7.00**	**3.00**
Cmax(ng/ml)	**1948.73**	**1987.20****
Kel (hr^{-1})	**0.041**	**0.041**
t 0.5(hr)	**17.01**	**16.73****
AUC 0-∞ (ng/ml*hr)	**67643.17**	**63904.37****
AUMC 0-∞ (ng/ml*hr^2)	**1794784.36**	**1615418.82****

Table 7: Plasma concentration of warfarin and warfarin-hawthorn in multiple doses. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin as a control (ng/ml)	Concentration of warfarin- hawthorn combination (ng/ml)
4	3060.69±595.65	3296.08±554.46
24	1138.72±344.06	1380.57±519.04
28	3577.55±550.25	4518.56±1243.51
48	1631.88±600.69	1952.57±819.03
52	3578.77±537.07	4537.44±1454.83
72	2035.13±836.59	2374.02±873.31
76	3419.93±692.50	4009.11±1052.01
96	2164.51±71.51	1921.52±798.53
100	5112.22±519.98	4116.26±1235.66

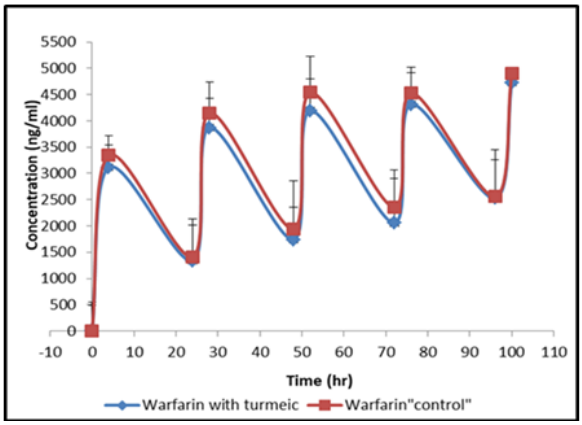

Figure 8: *In vivo* mean plasma concentration of warfarin and warfarin+ turmeric (multiple doses).

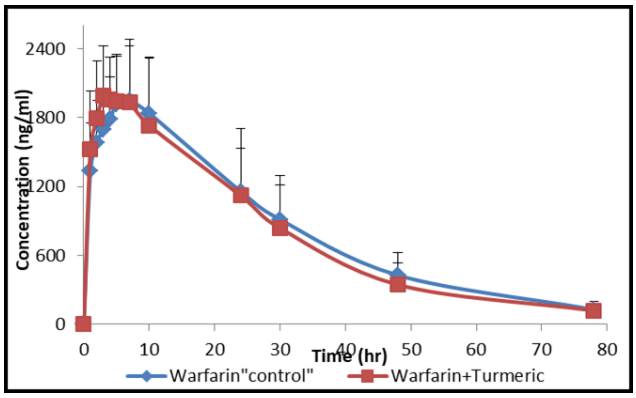

Figure 9: *In vivo* plasma concentration of warfarin and warfarin+ turmeric (single dose).

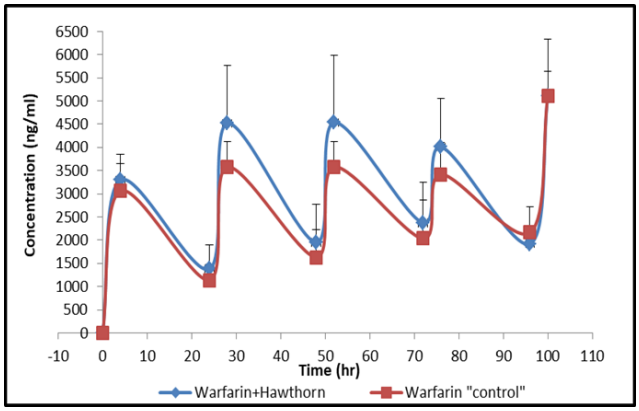

Figure 10: *In vivo* plasma concentration of warfarin and warfarin+hawthorn (multiple doses).

Table 8: Plasma concentration of warfarin and warfarin +hawthorn in single dose. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin "control" (ng/ml)	Concntration of warfarin-hawthorn combination (ng/ml)
1	1423.09± 301.02	1018.38±335.13
2	1608.50±419.24	1203.10±354.96
3	1751.52±427.58	1443.17±452.50
4	1718.06±401.91	1607.71±485.60
5	1792.30±424.86	1696.67±482.06
7	1856.66±318.71	1864.78±525.46
10	1857.32±331.09	1770.96±546.62
24	1005.18±329.69	1139.76±535.82
30	726.27±237.84	880.50±426.72
48	329.82±124.79	357.06±226.51
78	104.38±27.16	116.50±83.92
AUC 0-78 (ng/ml*hr)	**57866.75**	**59813.16****
AUMC 0-78 (ng/ml*hr^2)	**1225342.54**	**1348243.06****
Tmax (hr)	**10.00**	**7.00**
Cmax(ng/ml)	**1857.32**	**1864.78****
Kel (hr^{-1})	**0.042**	**0.042**
t 0.5(hr)	**16.70**	**16.33****
AUC 0-∞ (ng/ml*hr)	**60381.71**	**62557.90****
AUMC 0-∞ (ng/ml*hr^2)	**1482106.98**	**1626997.82****

**P>0.05 (insignificant).

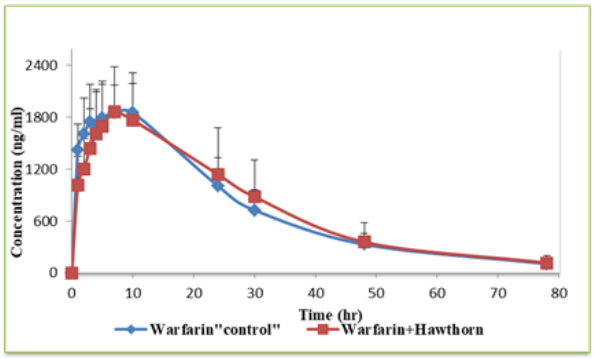

Figure 11: *In vivo* plasma concentration of warfarin and warfarin+hawthorn (single dose).

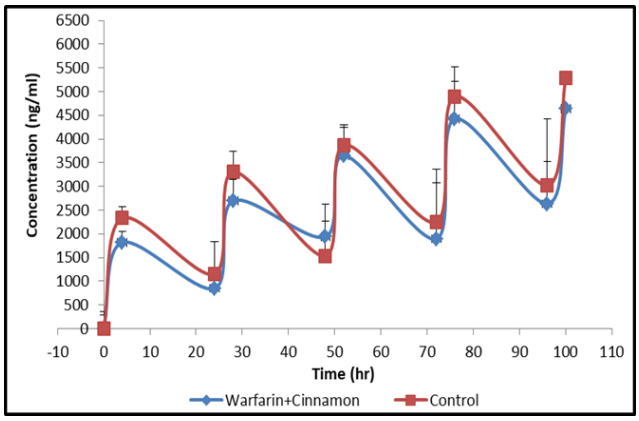

Figure 12: *In vivo* plasma concentration of warfarin and warfarin+ cinnamon (multiple doses).

Table 9: Plasma concentration of warfarin and warfarin – cinnamon in multiple doses. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin "control" (ng/ml)	Concentration of warfarin – cinnamon combination (ng/ml)
4	2330.98± 366.09	1815.63± 278.44
24	1155.19±246.16	847.56± 236.05
28	3307.94±683.44	2694.66± 416.60
48	1530.43±424.86	1937.63± 457.02
52	3872.45±735.98	3651.91± 685.64
72	2244.90±364.25	1893.41±645.46
76	4899.28±1118.57	4425.66±1182.17
96	3021.00± 630.15	2633.22±793.80
100	5281.15± 495.05	4641.34±1793.92

Table 10: Plasma concentration of warfarin after administration with cinnamon in single dose. The data are presented as mean±SD (n=8).

Time (hr)	Concentration of warfarin "control" (ng/ml)	Concentration of warfarin- cinnamon combination (ng/ml)
1	1025.93±154.05	1334.46±225.95
2	1385.79±228.95	1584.79±304.74
3	1516.81±237.16	1682.40±276.99
4	1607.74±242.60	1719.12±268.35
5	1652.68±248.00	1729.10±286.41
7	1642.91±255.11	1667.24±268.98
10	1561.58±231.51	1628.94±304.96
24	968.54±150.00	1050.73±241.81
30	697.33±106.57	799.52±213.69
48	374.04±57.68	430.89±108.81
78	109.23±24.25	115.53±34.75
AUC 0-78 (ng/ml*hr)	**54064.56**	**59104.30****
AUMC 0-78 (ng/ml*hr^2)	**1230833.30**	**1368591.47****
Tmax (hr)	**5.00**	**5.00**
Cmax(ng/ml)	**1652.68**	**1729.10****
Kel (hr^{-1})	**0.040**	**0.040**
t 0.5(hr)	**17.48**	**17.12****
AUC 0-∞ (ng/ml*hr)	**56819.33**	**61957.53****
AUMC 0-∞ (ng/ml*hr^2)	**1515182.20**	**1661606.59****

**P>0.05(insignificant).

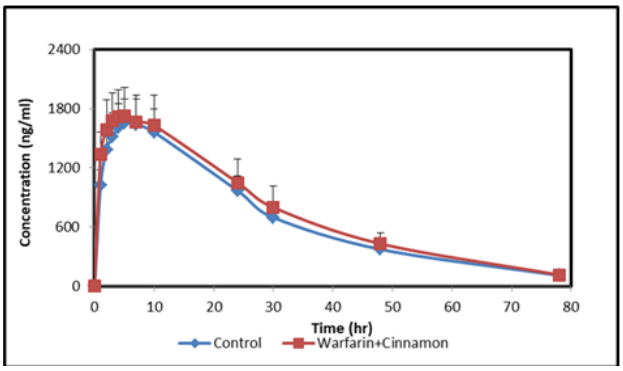

Figure 13: *In vivo* plasma concentration of warfarin and warfarin +cinnamon (single dose).

DISCUSSION

The present study was conducted to investigate the impact of co-administration of some commonly used CAMs on warfarin. In the current study; turmeric, hawthorn and cinnamon were investigated due to their therapeutic and large sales on both national and international markets. To study such interaction, a validated, sensitive, precise and simple reversed phase HPLC method was developed for the estimation of in rat plasma. It was validated using EMEA guidelines with acceptable ranges of accuracy, precision, linearity, recovery, limits of quantification, and detection.

The combination between turmeric and warfarin revealed a significant pharmacodynamic interaction. The reason for increasing PT values might be due to that *In vitro* and *In vivo* activity of curcumin and bis-demethoxycurumin in human plasma prolonged PTT and PT significantly and inhibited thrombin and Fxa activities [15].The combination between hawthorn and warfarin revealed a significant pharmacodynamic interaction. This may be associated to antiplatelet activity of flavonoids of hawthorn that may potentially increase the risk of bleeding or potentiate the effects of warfarin therapy. The studies showed that quercetin and rutin which had an anti-thrombotic effect, prolonged PTT[16]. Qurecetin and rutin are major flavonoids present in hawthorn[17]. One study showed that hawthorn extract (100 – 500 mg/kg) yielded significant platelet inhibition [18]. In our findings hawthorn extract (30 mg) showed significant change in PT.

The combination between cinnamon and warfarin revealed a significant pharmacodynamic interaction.It was reported that examined 13 compounds obtained from cinnamon; e.g. eugenol, amygdalactone, cinnamic alcohol, 2-methoxycinnamaldehyde and coniferaldehyde showed 1.5–73-fold larger inhibitory effects on arachidonic acid induced aggregation than acetylsalicylic acid .The other compounds; coumarin, cinnamaldehyde and cinnamic acid also inhibited aggregation for up to 2.5 -4 times in comparison with acetylsalicylic acid [19].

Overall, the combinations of warfarin with CAMs provide an insight about the use of turmeric, hawthorn and cinnamon with conventional antithrombotic drugs.

The pharmacokinetic profile of warfarin was evaluated in multiple doses and in single dose, in presence of turmeric, hawthorn and cinnamon. Warfarin is rapidly and totally absorbed from the stomach. It is nearly 100% bioavailable (F = 1) when taken orally [20]. S-warfarin is approximately 90% oxidized, primarily by CYP2C9; whereas R-warfarin is approximately 60% oxidized by CYP1A2[21] and approximately 92% of orally administered doses are recovered in the urine[22]. Furthermore, warfarin reached maximum plasma concentration between 5-10 hour after single dose with a biological half life about 17 hr .These results are in line with those of previous studies [23- 24].

Turmeric had no significant effect on mean plasma concentration, and pharmacokinetic parameters of warfarin, in multiple doses and single dose**.** These results are likely to be related to poor systemic bioavailability of curcumin after oral administration. Also efficient first-pass and some grade of intestinal metabolism of curcumin, might lead to its poor systemic availability when administered via the oral route [25].As for metabolism, curcumin undergoes metabolic O-conjugation to Curcumin glucuronide and curcumin sulfate and bio reduction to tetra -hydrocurcumin, hexa -hydrocurcumin, octa-hydrocurcumin[26].

Furthermore, curcumin was well excreted in the bile [27]. Turmeric extract dose the in current study was lower than reported doses (20mg/kg). A study illustrated that curcumin and its metabolites could not be detected in serum and plasma at doses below 4 g/day. In the current study, the dose of turmeric was lower than 4g/day. So, that might lead to insignificant of pharmacokinetic interaction between warfarin and turmeric [28].

The results of this study did not find that hawthorn (30mg/kg) significantly changed (P>0.05) pharmacokinetic profile of warfarin in multiple doses and in single dose. A possible explanation for these results may be the limited permeability of the main active flavonoids hawthorn extract; hyperoside, isoquercitrin and epicatechin. Epicatechin and isoquercitrin established more extensive metabolism in the rat in situ intestinal perfusion model and in vivo study [29]. So poor permeability of flavonoid components of hawthorn and difference in metabolic pathway from warfarin might lead to these results.

The results of this study did not find that significant effect (P<0.05) in mean plasma concentration of warfarin was found when administered with cinnamon in multiple doses and single dose. A possible explanation for these results may be due to pharmacokinetics of cinnamon bioactive compounds. A study indicated that relative amount of absorption of coumarin from powder of *Cassia cinnamon* was considerably lower than that of isolated coumarin[30]. Procyanidin is one of the content of cinnamon; it shows lower bioavailability (8-11%) after oral administration in rats [31].

CONCLUSION

The effect of single and multiple doses of commonly used CAMs; turmeric, hawthorn and cinnamon on pharmacodynamic and pharmacokinetic of warfarin was investigated. Multiple doses of these CAMs lead to significant increase in PT and INR of warfarin. Therefore, they increased the anticoagulant effect of warfarin. Plasma warfarin level was not affected by turmeric, hawthorn and cinnamon in combination; the difference between Cmax (single vs. combined administration in single and multiple doses) was insignificant. A simple, reproducible analytical method with high resolution and sensitivity was used for simultaneous quantification of warfarin in rat plasma. The method was validated and all of obtained data was within the acceptance criteria according to EMEA validation guidelines. The type of interaction between warfarin and CAMs is only a pharmacodynamic interaction. Thus, awareness must be raised among public about the use of CAMs, especially patients using anticoagulants like warfarin. Moreover, awareness on significant warfarin interaction with these CAMs must be spread among pharmacists and physicians.

This study can lead to many possible future studies on drug –herb interaction in general. Moreover, combinations of commonly used CAMs with drugs of low therapeutic index are crucial.

REFERENCES

1. Heinrich M, Barnes J, Prieto-Garcia J, Gibbons S, Williamson EM.Fundamentals of Pharmacognosy and Phytotherapy E-Book. Elsevier Health Sciences, 2017 Nov 24.
2. Kuhn MA, Doenges ME. Pharmacotherapeutics: a nursing process approach. Philadelphia: FA Davis, 1998 Jan.
3. Li CG, Yang L, Zhou SF. Interactions between Chinese herbal medicines and drugs. Australian journal of acupuncture and Chinese medicine. 2(1), 2007, 17.
4. Ringman JM, Frautschy SA, Cole GM, Masterman DL, Cummings JL. A potential role of the curry spice curcumin in Alzheimer's disease. Current Alzheimer Research. 1, 2(2), 2005 Apr, 131-6.
5. Shakibaei M, Mobasheri A, Lueders C, Busch F, Shayan P, Goel A. Curcumin enhances the effect of chemotherapy against colorectal cancer cells by inhibition of NF-κB and Src protein kinase signaling pathways. PloS one. 8(2), 2013 Feb 22, e57218.
6. Mahady GB, Pendland SL, Yun G, Lu ZZ. Turmeric (Curcuma longa) and curcumin inhibit the growth of Helicobacter pylori, a group 1 carcinogen. Anticancer research. 22(6C), 2002, 4179-81.
7. Kumar V, Lewis SA, Mutalik S, Shenoy DB, Udupa N. Biodegradable microspheres of curcumin for treatment of inflammation. Indian journal of physiology and pharmacology. 46(2), 2002 Apr, 209-17.
8. Shatoor AS. In vivo hemodynamic and electrocardiographic changes following Crataegus aronia syn. Azarolus (L) administration to normotensive Wistar rats. Saudi medical journal. 34(2), 2013 Feb 3, 123-34.
9. Bernatonienė J, Masteikova R, Majienė D, Savickas A, Kėvelaitis E, Bernatonienė R, Dvoráčková K, Civinskienė G, Lekas R, Vitkevičius K, Pečiūra R. Free radical-scavenging activities of Crataegus monogyna extracts. Medicina. 44(9), 2008, 706-12.
10. Pittler MH, Schmidt K, Ernst E. Hawthorn extract for treating chronic heart failure: meta-analysis of randomized trials. The American journal of medicine. 114(8), 2003 Jun 1, 665-74.
11. Kirkham S, Akilen R, Sharma S, Tsiami A. The potential of cinnamon to reduce blood glucose levels in patients with type 2 diabetes and insulin resistance. Diabetes, obesity and metabolism. 11(12), 2009 Dec, 1100-13.
12. Wang JG, Anderson RA, Graham III GM, Chu MC, Sauer MV, Guarnaccia MM, Lobo RA. The effect of cinnamon extract on insulin resistance parameters in polycystic ovary syndrome: a pilot study. Fertility and sterility. 88(1), 2007 Jul 1, 240-3.
13. Khasnavis S, Pahan K. Cinnamon treatment upregulates neuroprotective proteins Parkin and DJ-1 and protects dopaminergic neurons in a mouse model of Parkinson's disease. Journal of Neuroimmune Pharmacology. 9(4), 2014 Sep 1, 569-81.
14. Bye A, King HK. The biosynthesis of 4-hydroxycoumarin and dicoumarol by Aspergillus fumigatus Fresenius. Biochemical Journal. 117(2), 1970 Apr 1, 237-45.
15. Kim DC, Ku SK, Bae JS. Anticoagulant activities of curcumin and its derivative. BMB reports. 45(4), 2012, 221-6.
16. Mozzicafreddo M, Cuccioloni M, Eleuteri AM, Fioretti E, Angeletti M. Flavonoids inhibit the amidolytic activity of human thrombin. Biochimie. 88(9), 2006 Sep 1, 1297-306.
17. Liu P, Kallio H, Yang B. Phenolic compounds in hawthorn (Crataegus grayana) fruits and leaves and changes during fruit ripening. Journal of agricultural and food chemistry. 59(20), 2011 Sep 26, 11141-9.
18. Shatoor AS, Soliman H, Al-Hashem F, El-Gamal B, Othman A, El-Menshawy N. Effect of Hawthorn (Crataegus aronia syn. Azarolus (L)) on platelet function in albino Wistar rats. Thrombosis research. 130(1), 2012 Jul 1, 75-80.
19. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food and Chemical Toxicology. 40(8), 2002 Aug 1, 1091-7.
20. Stirling Y, Howarth DJ, Stockley R, Bland R, Towler CM, Harding SM. Comparison of the bioavailabilities and anticoagulant activities of two warfarin formulations. British journal of haematology. 51(1), 1982 May, 37-46.

21. Lewis RJ, Trager WF, Robinson AJ, Chan KK. Warfarin metabolites: the anticoagulant activity and pharmacology of warfarin alcohols. Translational Research. 81(6), 1973 Jun 1, 925-31.

22. Hignite C, Uetrecht J, Tschanz C, Azarnoff D. Kinetics of R and S warfarin enantiomers. Clinical Pharmacology & Therapeutics. 28(1), 1980 Jul, 99-105.

23. Yacobi A, Levy G. Comparative pharmacokinetics of coumarin anticoagulants XIV: Relationship between protein binding, distribution, and elimination kinetics of warfarin in rats. Journal of pharmaceutical sciences. 64(10), 1975 Oct, 1660-4.

24. Holford NH. Clinical pharmacokinetics and pharmacodynamics of warfarin. Clinical pharmacokinetics. 11(6), 1986 Dec 1, 483-504.

25. Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. Inthe molecular targets and therapeutic uses of curcumin in health and disease 2007 (pp. 453-470). Springer, Boston, MA.

26. Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug metabolism and disposition. 27(4), 1999 Apr 1, 486-94.

27. Holder GM, Plummer JL, Ryan AJ. The metabolism and excretion of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione) in the rat. Xenobiotica. 8(12), 1978 Jan 1, 761-8.

28. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clinical Cancer Research. 10(20), 2004 Oct 15, 6847-54.

29. Zuo Z, Zhang L, Zhou L, Chang Q, Chow M. Intestinal absorption of hawthorn flavonoids–in vitro, in situ and in vivo correlations. Life sciences. 79(26), 2006 Nov 25, 2455-62.

30. Abraham K, Pfister M, Wöhrlin F, Lampen A. Relative bioavailability of coumarin from cinnamon and cinnamon-containing foods compared to isolated coumarin: A four-way crossover study in human volunteers. Molecular nutrition & food research. 55(4), 2011 Apr, 644-53.

31. Stoupi S, Williamson G, Viton F, Barron D, King LJ, Brown JE, Clifford MN. In vivo bioavailability, absorption, excretion and pharmacokinetics of [14C] procyanidin B2 in male rats. Drug Metabolism and Disposition. 2009 Jan 1, dmd-109.

Source of Support: Nil, **Conflict of Interest:** None.