

Effect of caffeine on anti-clotting activity of warfarin in healthy male albino rabbits

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Abstract: Drug-drug interactions are most commonly occurring phenomenon in clinical practice. Many physicians are afraid of being involved in an allegation of malpractices due to the occurrence of any severe interaction. These interactions not only occur between drugs but also between any kind of food, tobacco smoke, caffeine and alcohol etc. Therefore, the present study was directed to inspect the effect of caffeine on the anticoagulation activity of warfarin in healthy adult male albino rabbits. Blank blood samples were collected from each rabbit. Rabbits were given warfarin (0.5mg kg⁻¹) orally via stomach tube and blood samples were collected in PT/INR vials at various intervals. After a washout period of 14 days, warfarin was orally administrated at same dose rate along with caffeine (5 mg kg⁻¹ every twelve hours for three days) and same sampling schedule was repeated. Prothrombin time (PT) and the international normalized ratio (INR) of blood samples were determined to estimate changes in the anticoagulation activity of warfarin after its concurrent administration with caffeine. The PT data revealed that R_{max} and AUC increased significantly (P<0.05) from 1991.6 and 60.5 to 2124.8 and 67.5, respectively, before and after co-administration. Similarly, a significant (P<0.05) increase was observed in R_{max} and AUC of INR from 6.42 and 153.7 to 7.4 and 167.5, respectively, alone and along with caffeine. However, no change was observed in T_{max} associated with PT and INR either the drug was administered alone or in combination with caffeine. It was concluded that caffeine has the capacity to inhibit the metabolism of warfarin and enhance its plasma concentration and hence anticoagulant effects. Thus, patients should be advised to limit the frequent use of caffeine-rich products i.e. tea and coffee during warfarin therapy.

Keywords: Anticoagulant therapy, drug interaction, coffee, tea, tobacco smoking, vitamin K.

INTRODUCTION

Drug interactions are most commonly occurring problem in clinical practice. Many physicians are afraid of being involved in an allegation of malpractice due to the occurrence of any severe interaction (Aziz *et al.*, 2016; Sana *et al.*, 2016). In routine medical practice, drug interactions are inevitable. These interactions not only occur between drugs but also between any kind of food, tobacco smoke, caffeine, alcohol and any illegal drug (Haen, 2014). These interactions are explained by the drug pharmacokinetics and pharmacodynamics.

The liver is the major site for clearance of both endogenous (like fatty acids, proteins, cholesterol, steroids etc.) and exogenous chemicals (like drugs). Drugs following their oral administration get absorbed in

the gut and then they are transferred to the liver where they can be extensively metabolized (Gonzalez and Tukey, 2010). Drug-induced hepatotoxicity is life-threatening. Therefore, patients with liver dysfunction and with a liver transplant, who are prescribed a large number of drugs, should be keenly monitored for adverse drug events and drug-drug interactions. So, the drugs which have a high volume of distribution and are extensively metabolized by liver should be discontinued immediately or closely monitored to avoid drug-drug interactions in such patients (Zhu *et al.*, 2014).

Different natural products are available which can be used as coagulants i.e. *Ficus benghalensis* (Rehman *et al.*, 2017a) and anticoagulants i.e. warfarin (Shamaila *et al.*, 2018). Warfarin is used as an oral anticoagulant and is used extensively for the treatment of thrombosis in patients suffering from atrial fibrillation, heart valve

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implants and thromboembolism. However, its use is restricted because it has a narrow therapeutic range and there is large variability among the individuals in its optimum dose requirement for satisfactory anticoagulant effect (Lindh *et al.*, 2009). Although warfarin has complex pharmacodynamic and pharmacokinetic properties it is still being used extensively. However, proper clinical consideration is required to achieve optimal anticoagulative effects of this drug because warfarin has many food and drug interactions. Therefore, over or under optimal dose of warfarin can lead to bleeding or thromboembolic problems, respectively (Nutescu *et al.*, 2006).

International normalized ratio (INR) is a measure of the extrinsic coagulation pathway. It is a ratio between the prothrombin time of a patient and prothrombin time of a normal (control) sample taking ISI value (International Sensitivity Index) as power for the used analytical system. ISI value is usually between 1.0 and 2.0 and this test is called PT/INR or Pro-Time INR which is used to identify the clotting time of blood to adjust the optimum dosage of warfarin, liver damage or vitamin K status (Majerus and Tollefsen, 2010).

Warfarin is the racemic mixture of R and S warfarin. S warfarin is metabolized by a CYP2C9 isoform of cytochrome P450 enzyme system while R warfarin is metabolized by CYP1A2, CYP2C19 and CYP3A4 (Nagui *et al.*, 2001).

Coffee and tea are the main sources of caffeine in society. Caffeine is a xanthine alkaloid that is bitter in taste and has a stimulatory effect on the central nervous system (Bielez *et al.*, 2013). It is a good anti-oxidant agent (Akram *et al.*, 2017; Rehman *et al.*, 2017b). It is regularly consumed by human population in tea, coffee, cocoa, paullinia cupana (guarana), yerba mate, prescribed and non-prescribed form of drugs, coffee plant seeds, tea brush leaves and energy drinks having Kola nut (Hasegawa *et al.*, 2009; Vieira *et al.*, 2010; Patui *et al.*, 2014). Caffeine is also a most popular ingredient in over the counter (OTC) fat burning supplements and proprietary blend (Jeukendrup and Randell, 2011).

Up to 84% of caffeine's metabolism is through CYP1A1 and CYP1A2 liver microsomal enzyme isoforms and complete metabolism is done by xanthine oxidase, CYP2A6 and NAT1 (N-acetyltransferase 1) (Caubet *et al.*, 2002; Chen *et al.*, 2011). CYP1A2 and CYP3A4 are involved in the metabolism of R-isomer of warfarin (Holbrook *et al.*, 2005) and caffeine is an inhibitor of CYP1A2 (Eugster *et al.*, 1993). As both drugs are metabolized by cytochrome P450 enzyme system there is the possibility of interaction because of interruption in the metabolism of either drug when they are administered concomitantly.

Objectives

The purpose for conduction of this research was to evaluate the effect of caffeine on the anti-clotting action of warfarin in male albino rabbits and to see the risk of bleeding through assessment of prothrombin time and INR to check the safety of concomitant use of both drugs (warfarin and caffeine).

MATERIAL AND METHOD

The given protocol was followed to investigate the effects of caffeine on anti-clotting activity and the bleeding risk associated with warfarin in healthy adult male albino rabbits.

Animals

Adult male albino rabbits of an average weight of 1.32 ± 0.082 kg were arranged after an ethical approval from Faculty of Veterinary Sciences, University of Agriculture, Faisalabad. Rabbits were kept in the animal room at room temperature ($22 \pm 3^\circ\text{C}$) with proper ventilation facility and were acclimatized for 1 week. Rabbits were fed with seasonal fodder and drinking water *ad-libitum*.

Methodology

Drugs

A commercial preparation of warfarin tablets 1 mg and caffeine in the form of white crystalline powder (up to 99% pure) were purchased from local market of district Faisalabad.

Drug administration

Warfarin was administered orally at a dose rate of 0.5 mg kg^{-1} body weight in each rabbit through stomach tube during the first treatment period. After a washout period of fourteen days, warfarin (0.5 mg kg^{-1}) was again administered orally along with caffeine (5 mg kg^{-1}) in each rabbit. The dose of caffeine was repeated every twelve hours for three days onward.

Blood sample collection

For determination of prothrombin time and international normalized ratio, blood samples were collected at 0, 4, 8, 12, 24, 48, 72, 96 and 120 hours in PT/INR tubes. After giving fourteen days of washout period, a concomitant administration of warfarin and caffeine was made and the same schedule for blood samples collection was adopted. Blood samples were centrifuged at 4000 rpm for 30 minutes. Plasma was separated and stored at -20°C until further analysis.

Analytical procedure

Measurement of plasma concentration of warfarin

Plasma concentration of warfarin in the blood samples was determined by a sensitive, selective and accurate high-performance liquid chromatography (HPLC) method (Bjornsson *et al.*, 2006; Lomonaco *et al.*, 2013) with following specifications.

Mobile Phase (Methanol and 0.5% Acetic acid 1:1 v/v); Flow Rate (1mL min⁻¹); Wavelength (308 nm); Pressure (36kg cm⁻²); Injection Volume (20µL); Column C₁₈ (250×4.6nm, 5µm); Temperature (20°C); Detector (UV-Visible Detector)

Measurement of coagulation parameters

Following coagulation parameters were measured:

1. Prothrombin time (PT)
2. International normalized ratio (INR)

Methodology

Prothrombin time (PT)

The determination of Prothrombin Time or Quick's Time is a global test to evaluate the extrinsic coagulation, being sensitive to factor II or prothrombin, factor V or proaccelerin, factor VII or proconvertin and factor X or Stuart-Prover Factor. Therefore, this test is used for routine tests in the pre-surgery analysis, detection of alterations in the levels of one or more factors involved in the extrinsic pathway and control of oral anticoagulants therapy. Soluplastin kit was used for measurement of PT (Wiener, 2000).

International Normalized Ratio (INR)

The INR is the ratio of subject's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system being used. INR was calculated after measuring prothrombin time by using following formula:

$$INR = \left(\frac{PT_{test}}{PT_{normal}} \right)^{ISI}$$

The value of ISI from kit used was 1.27.

STATISTICAL ANALYSIS

Data were collected and their mean values were determined. The pharmacodynamic parameters (PT and INR) were statistically compared using Student's paired t-test (Steel *et al.*, 1997).

RESULTS

Coagulation parameters

Coagulation parameters of ten healthy adult male albino rabbits were determined at specified time intervals after administration of warfarin (0.5 mg kg⁻¹) alone and with caffeine (5mg kg⁻¹, every twelve hours for three days). Coagulation parameters i.e. prothrombin time (PT) and international normalized ratio (INR) of the albino rabbits following a single oral dose of warfarin alone and with caffeine were measured. The results of prothrombin time and international normalized ratio (INR) have been given in table 1 and table 2, respectively. Whereas, comparative mean values of pharmacodynamic parameters of warfarin alone and with caffeine have been given in table 3.

Prothrombin time (PT)

Prothrombin time (PT) is the time taken by blood to clot after it oozes out. It is an important coagulation parameter to evaluate extrinsic pathway of blood coagulation. Maximum response (R_{max}) is the peak response of a drug after its administration and T_{max} is the time at which peak response of administered drug is obtained.

The data of prothrombin time (table 1) of the healthy rabbits (n=10) following a single oral dose of warfarin (0.5 mg kg⁻¹) alone and along with caffeine revealed that at 8 hr (T_{max}) the mean R_{max} values of PT were 60.5 sec and 67.5 sec, respectively. Whereas, the respective AUC values were calculated as 1991.6 and 2124.8 (table 3).

International normalized ratio (INR)

International normalized ratio (INR) is the ratio between the coagulation time of a sample of blood and the normal coagulation time when coagulation takes place in certain standardized conditions. As it is ratio so it has no units.

The data of INR (table 2) for ten healthy rabbits following a single oral dose of warfarin (0.5 mg kg⁻¹) alone and in concomitant administration with caffeine showed that at 8 hr (T_{max}), the mean values of INR (R_{max}) were 6.42 and 7.39 (table 3). So, the value of R max, in this case, was 6.42 and T max was 8 h. The values of AUC were 153.69 and 167.53 for caffeine alone and in combined form with caffeine, respectively (table 3).

Plasma concentration

Plasma concentrations of warfarin determined in each blood sample of albino rabbits have been presented in table 4 and were found significantly (P≤0.05) increased at all time intervals after co-administration with caffeine.

Table 4 comparative mean ± SE plasma concentration (µg mL⁻¹) of warfarin following its single oral administration 0.5 mg kg⁻¹ alone and with multiple doses of caffeine (5mg kg⁻¹ every twelve hours for three days) in ten healthy adult male albino rabbits

DISCUSSION

The effect of caffeine on the anticoagulation effects of warfarin was studied in ten healthy adult male albino rabbits. The results of coagulation parameters i.e. prothrombin time (PT) and the international normalized ratio (INR) of warfarin alone and in combination with caffeine at different time intervals after administration in healthy albino rabbits have been discussed below:

Coagulation parameters

Warfarin is an anticoagulant drug which has a narrow therapeutic range so, its monitoring is very essential. For purpose of warfarin's anticoagulant monitoring, coagulation parameters (PT and INR) are checked time to time to avoid the risk of bleeding or thrombosis (Lane *et al.*, 2012).

Table 1: Comparative mean prothrombin time of ten healthy adult male albino rabbits following a single oral dose of 0.5 mg kg⁻¹ of warfarin alone and with caffeine (5 mg kg⁻¹ every twelve hours for three days)

Time(hours)	0	4	8	12	24	48	72	96	120
PT (seconds) warfarin	14 ^{NS}	22 ^{NS}	60.5*	21.2 ^{NS}	16.6 ^{NS}	14*	14*	14 ^{NS}	14 ^{NS}
PT (seconds) warfarin with caffeine	14	23.6	67.5	22.6	17.4	15.6	15	14	14

Table 2: Comparative mean international normalized ratio (INR) of ten healthy adult male albino rabbits following a single oral dose of 0.5 mg kg⁻¹ of warfarin alone and with caffeine (5 mg kg⁻¹ every twelve hours for three days)

Time(hrs)	0	4	8	12	24	48	72	96	120
INR (warfarin)	1	1.78 ^{NS}	6.42*	1.70 ^{NS}	1.18*	1*	1*	1	1
INR (warfarin with caffeine)	1	1.94	7.39	1.84	1.32	1.15	1.09	1	1

Table 3: Comparative mean values for the coagulation parameters of ten healthy adult male albino rabbit following single oral dose of warfarin (0.5 mg kg⁻¹) alone and with caffeine (50 mg kg⁻¹ every 12 hours for three days)

Parameters	1-PT	AUC _(0, 120)	R _{max} (s)	T _{max} (h)	2-INR	AUC _(0, 120)	R _{max}	T _{max} (h)
Warfarin only		1991.6*	60.5*	8 ^{NS}		153.6912*	6.42*	8 ^{NS}
Warfarin + caffeine	2124.8	67.5	8	167.53	7.39	8		

Table 4: Comparative mean ± SE plasma concentration (µg mL⁻¹) of warfarin following its single oral administration 0.5 mg kg⁻¹ alone and with multiple doses of caffeine (5 mg kg⁻¹ every twelve hours for three days) in ten healthy adult male albino rabbits

Time in hours	Warfarin	Warfarin along with caffeine
4	1.9 ± 0.04*	2.1 ± 0.04
8	1.6 ± 0.01*	1.7 ± 0.02
12	1.42 ± 0.03*	1.50 ± 0.03
24	1.25 ± 0.03*	1.31 ± 0.03
48	0.89 ± 0.09*	1.11 ± 0.02
72	0.64 ± 0.08*	0.87 ± 0.04
96	0.41 ± 0.07*	0.64 ± 0.01
120	0.18 ± 0.05*	0.31 ± 0.03

* = Significant (P≤0.05) differ ent from the respective value
 NS = Non significantly (P>0.05) different from the respective value

Prothrombin time (PT)

Prothrombin time (PT) of the blood of rabbits is usually 12-14 seconds. When warfarin is administered to healthy subjects it increases the subject’s PT. In the present study, the mean value of PT of ten rabbits before administration of warfarin was 14 Sec (table 1). The data of PT of ten healthy rabbits following a single oral dose of warfarin (0.5mg kg⁻¹) alone showed that PT level increased progressively and reached its maximum (60.5 sec) at 8 hr. Then a slow decline was observed leading to 14 Sec at 120 hrs. A similar trend of rising and then decline in PT was observed after co-administration of warfarin and caffeine. The PT values observed at 8, 48 and 72 hours after drug administration alone and along with caffeine were found to be significant (P≤0.05). The area under response curve of PT versus time data was also measured and found to be 1991.6 and 2124.8 for warfarin alone and for its combination with caffeine, respectively.

Statistical comparison of mean values of R_{max}(PT) and AUC_{0, 120} (PT) following an oral dose of warfarin alone and with caffeine also appears to be significant (P≤0.05). The results indicate that caffeine moderately affects the anticoagulant effect of warfarin and increases the prothrombin time.

International normalized ratio (INR)

Warfarin, a narrow therapeutic index drug, has a tendency of having many drug interactions so conscientious monitoring of warfarin’s concentrations is done by assessing its effect on the international normalized ratio (INR) in order to maintain it within therapeutic range i.e. 2 and 3. INR depends upon prothrombin time and the normal value of INR for rabbits is 1.

The data of INR of healthy rabbits (n=10) following a single oral dose of warfarin (0.5mg kg⁻¹) alone and in combination with caffeine showed a progressive increase

in the mean value of INR reaching at its maximum to 6.42 and 7.39, respectively, at 8 hrs. Thereafter a declining trend in INR values of warfarin alone and its combination was observed which ended at 1 after 120 hours. The observations of INR values of warfarin alone and in concomitant use with caffeine were significant ($P \leq 0.05$) at 8, 24, 48 and 72 hours (table 2). The area under response curve of INR versus time data was measured as 153.69 and 167.53 for warfarin alone and along with caffeine, respectively (table 3).

Statistical comparison of mean values of R_{\max} (INR) and $AUC_{0, 120}$ (INR) following an oral dose of warfarin alone and with caffeine also appears to be significant ($P \leq 0.05$). It indicates that caffeine moderately affects the anticoagulant effect of warfarin and increases the value of INR.

Thus, caffeine tends to increase anti-clotting effects of warfarin by increasing prothrombin time and international normalized ratio as caffeine interacts with the metabolism of warfarin and alters the plasma level of warfarin. So, the anticoagulant response of warfarin is increased in the presence of caffeine.

CONCLUSION

It is concluded from the results of coagulation parameters that caffeine has the capacity to reduce the metabolism of warfarin by blocking CYP1A2 and CYP3A4 and also interacting with CYP2C9 isoenzymes decreases the elimination of warfarin from the body and enhances its anticoagulant activity. It is also concluded from findings of this experiment that plasma concentration of warfarin and value of INR are directly proportional to each other. As on increasing plasma concentration of warfarin, the INR of the individual was also increased.

Caffeine does not affect the C_{\max} of warfarin on initiation of therapy but the administration of caffeine for multiple times during warfarin therapy can increase the plasma level of warfarin. It is also concluded from findings of this experiment that there is a direct relationship between plasma concentration of warfarin and value of INR. On increasing plasma concentration of warfarin, the INR of individual will also be increased.

The findings of this experiment suggest that patients should be advised to limit the frequent use of tea and coffee during warfarin therapy as tea and coffee are the rich sources of caffeine.

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