ALTERATION IN DISPOSITION KINETICS OF WARFARIN MEDIATED BY CAFFEINE IN HEALTHY MALE ALBINO RABBITS

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ABSTRACT

Many physicians are afraid of being involved in allegation of malpractices due to occurrence of severe drug interactions. These interactions not only occur between drugs but also between any kind of food, tobacco smoke, caffeine and alcohol etc. Therefore, current study was conducted to inspect the effect of caffeine on the disposition kinetics of warfarin in ten healthy adult male albino rabbits. Blood samples were collected at same time intervals after administration of warfarin (0.5 $mg.kg^{-1}$) alone and then in its combination with caffeine (5 $mg.kg^{-1}$ every twelve hours for three days) following a washout period of 14 days in each rabbit. Warfarin concentration in plasma was measured by high performance liquid chromatographic (HPLC) technique to determine pharmacokinetic parameters. Paired t-test was applied on data for its statistical comparison. Plasma concentration values of warfarin enhanced significantly at each time interval after its co-administration with caffeine. Significant increase was observed in K_{abs} (from 0.4±0.0 hr^{-1} to 0.5±0.0 hr^{-1}), $t_{1/2}\beta$ (from 34.1±4.8 hr to 49.1±2.4 hr), AUC (from 109.3±10.6 $\mu g.h.mL^{-1}$ to 147.2±6.4 $\mu g.h.mL^{-1}$) and MRT (from 52.1±6.7 hr to 72.9±3.4 hr) of warfarin after its concomitant administration with caffeine. Whereas, a significant decrease in $t_{1/2abs}$ (from 2.0±0.2 hr to 1.5±0.1 hr), β (from 0.03±0.0 hr^{-1} to 0.01±0.0 hr^{-1}) and Cl_B (from 0.01±0.0 $L.hr^{-1}kg^{-1}$ to 0.004±0.0 $L.hr^{-1}kg^{-1}$) of warfarin was observed after its combination with caffeine. Caffeine slows down the biotransformation of warfarin resulting in reduced elimination of warfarin which leads to increased concentration of warfarin within the body. Thus, anticoagulant activity of warfarin may enhance in combination with caffeine. So, clinicians should avoid or be cautious while using caffeine during warfarin therapy to avoid any mishap or unwanted event.

Keywords: Pharmacokinetics, Anticoagulant, Interaction, Metabolism.

INTRODUCTION

Drug interactions are most commonly occurring problem in clinical practice. Many physicians are afraid of being involved in allegation of malpractice due to occurrence of any severe interaction. In routine medical practice, drug interactions are inevitable (Aziz *et al*., 2016; Sana *et al*., 2016). These interactions not only occur between drugs but also between any kind of food, tobacco smoke, caffeine, alcohol and any illegal drug. These interactions can be classified as pharmacokinetic and pharmacodynamic (Zafar *et al*., 2018).

Warfarin, an oral anticoagulant, is used extensively for the treatment of thrombosis in patients suffering from atrial fibrillation, heart valve 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 pharmacodynamics and pharmacokinetics, still it is used extensively and proper clinical consideration is required to achieve optimal anticoagulation with this agent because it 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). Warfarin is the racemic mixture of R and S warfarin. S warfarin is metabolized by CYP2C9 isoform of cytochrome P450 enzyme system while R warfarin is metabolized by CYP1A2, CYP2C19 and CYP3A4 (Nagui *et al*., 2001).

Caffeine is a xanthine alkaloid that is bitter in taste and has stimulatory effect on central nervous system (Bielesz *et al*., 2013). Caffeine is also most popular ingredient in over the counter (OTC) fat burning supplements and proprietary blend. 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 (Patui *et al*., 2014). Coffee and tea are the main sources of caffeine in our society.

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) (Chen *et al*., 2011). While, CYP1A2 and CYP3A4 are

involved in the biotransformation of R-isomer of warfarin (Holbrook *et al*., 2005) and caffeine is inhibitor of CYP1A2 (Eugster *et al*., 1993). As both drugs are metabolized by cytochrome P450 enzyme system there is possibility of interaction because of interruption in metabolism of either drug when they are administered concomitantly.

Objectives: The purpose for conduction of this study was evaluation of the effects of caffeine on plasma concentration and disposition kinetics of warfarin in healthy adult male albino rabbits.

MATERIALS AND METHODS

Animals: Ten adult male albino rabbits of an average weight of 1.3±0.1 kg were arranged after an ethical approval from University of Agriculture, Faisalabad in 2005 under document number DGS/22696-7. Rabbits were kept in the animal room at room temperature (22±3°C) with proper ventilation facility and were acclimatized for 1 week. Rabbits were fed with seasonal fodder and drinking water *ad-libitum*. Clinical investigations on the rabbits were carried out in Institute of Pharmacy, Physiology and Pharmacology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan.

Methodology

Drugs: A commercial preparation of warfarin tablets 1 mg and caffeine in the form of white crystalline powder (up to 99% pure) was arranged from local market of district Faisalabad. Reference standard powder of warfarin was procured from Lahore Chemical and Pharmaceutical Works (PVT) LTD, Lahore, Pakistan.

Drug administration: Warfarin was administered in each rabbit through stomach tube at dose rate of 0.5 $mg.kg^{-1}$. 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: First blood sample was collected before the administration of warfarin as a control sample from each rabbit. Following drug administration, the blood samples were collected at 0.5, 1, 2, 3, 5, 7, 12, 18, 24, 36, 48, 72, 96 and 120 hours for purpose of analysis of plasma concentration of warfarin.

Analytical procedure

Warfarin analysis: Warfarin in plasma samples was determined by using High Performance Liquid Chromatography (HPLC) method (Bjornsson *et al*., 2006) with some modifications as described by Lomonaco *et al*. (2013).

Chromatographic conditions

Mobile Phase	:	Methanol and 0.5% Acetic acid (1:1 v/v)
Flow Rate	:	1 $mL.min^{-1}$
Wavelength	:	308 nm
Pressure	:	36 $kg.cm^{-2}$
Injection Volume	:	20 µL
Column	:	C_{18} (250×4.6nm, 5µm)
Temperature	:	20 ° C
Detector	:	UV-Visible Detector

Stock solutions and standards: Standard stock solution of warfarin was prepared in methanol to a concentration of 1.0 $mg.ml^{-1}$ and stored at 4 ^{0}C. Working solutions of different concentrations ranging from 500 $\mu g.ml^{-1}$ to 1 $\mu g.ml^{-1}$ were prepared by dilutions of stock solution with deionized water to construct calibration curve. Working solutions were prepared freshly for daily analysis. The solutions were filtered through pheomenex membrane of 0.45 mm pore size (25mm filter) and 20 µL was injected into HPLC for analysis. Calibration graph was prepared by using peak area versus concentration of working solutions.

Sample preparation: Plasma samples were thawed just prior to the extraction of drug at room temperature. Then samples were thoroughly agitated in vortex mixer and centrifuged at 800 g for 10 min. From each sample, 0.5 mL of Plasma was taken and mixed with 0.5 mL of acetonitrile in apendrof tube. The solution was vortex mixed for 30 seconds and then centrifuged at 5000 rpm for 5 min at room temperature. Supernatant was transferred to second tube. The samples were filtered through a millipore filter 0.45 µm (Gelman Sciences, Ann Arbor, MI, USA). Aliquots of each sample (20 µL) were injected in chromatograph.

Preparation of mobile phase: Mobile phase was a mixture of methanol and 0.5% acetic acid (1:1, v/v). After mixing both these solvents, the mobile phase was passed through filtration assembly, having the filter paper size 0.45 µm white nylon HNWP 47 mm. Then, the filtered mobile phase was sonicated to remove any air bubble for 30 minutes.

Standard curve: Working standards having warfarin concentrations 200, 100, 40, 10 and 1 $\mu g.ml^{-1}$ were prepared. These working standards were analyzed by using HPLC and concentration versus peak area data was plotted on a graph to construct the calibration curve (Fig. 1). The curve was linear over the range of 1 to 200 $\mu g.ml^{-1}$ (R^2 =1).

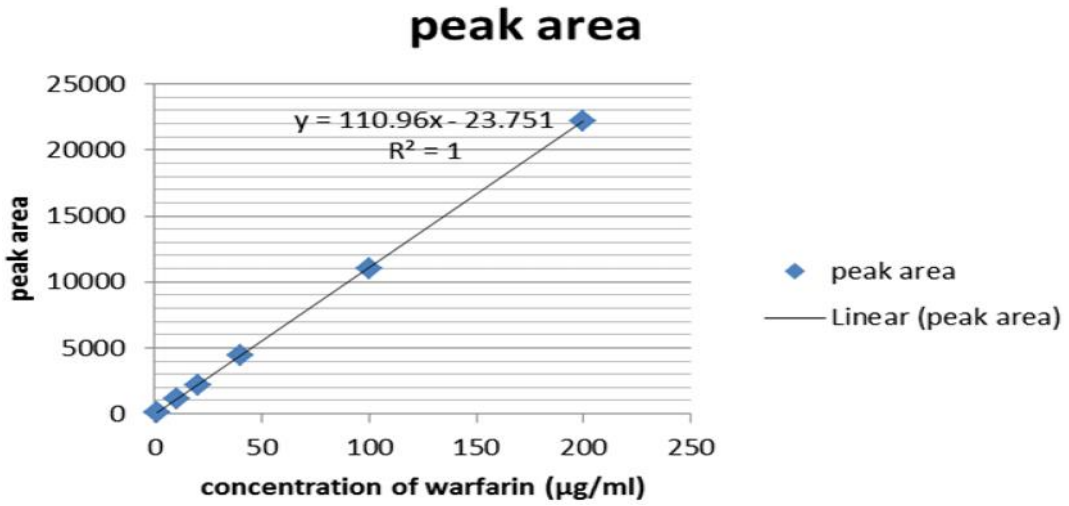

Fig. 1. Peak area versus concentration standard curve of warfarin

Determination of warfarin in plasma: Warfarin concentration in plasma samples was calculated by comparison with peak area obtained from the standard solutions. The concentration of warfarin in plasma was determined by using the given regression equation:

$Y = a + bx$

Where, Y = Peak area of warfarin (for unknown concentration); a = Intercept; b = Slope of regression line; x = concentration of warfarin

Calculations

Pharmacokinetics: Concentration versus time data of each rabbit was manipulated by American Pharmacology Organization (APO) software version 3.02 for the calculation of different pharmacokinetic parameters i.e. maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), plasma half-life ($t_{1/2}$), volume of distribution (V_d), total body clearance (Cl_B), area under curve (AUC) and mean residence time (MRT)

Statistical analysis: After determination of warfarin in the plasma, the plasma drug concentration versus time curve was plotted on a semi-logarithmic scale. The mean values and standard error of mean (± SEM) of each concentration and pharmacokinetic parameter were calculated. The pharmacokinetic parameters were statistically compared using student's paired t test (Steel *et al*., 1997).

RESULTS

Plasma concentration: It was observed in Table 1 that plasma concentration of orally administered warfarin alone reached its maximum 2.4±0.1 µg.ml^{-1} (mean±SE) at 2 hr and then declined slowly with the passage of time to 0.2±0.1 µg.ml^{-1} at 120 hr. Whereas, the peak mean±SE value of plasma concentration of warfarin along with multiple doses of caffeine was 2.5±0.1 µg.ml^{-1} at 2 hr which dropped with the passage of time to 0.31±0.03 µg.ml^{-1} at 120 h (Table 1). The mean±SE values for plasma concentration of warfarin alone and in combination with caffeine following administration in ten healthy adult rabbits have been schemed against time in Fig. 2. A significant increase in the peak plasma concentration of warfarin in the presence of caffeine was revealed at all time intervals.

Table 1. 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
0.5	0.1± 0.0*	0.2±0.0
1	1.6±0.0*	1.6±0.1
2	2.4±0.1*	2.5±0.1
3	2.0±0.0*	2.1±0.0
5	1.8±0.0*	1.9±0.0
7	1.7±0.0*	1.8±0.0
9	1.6±0.0*	1.6±0.0
12	1.4±0.0*	1.5±0.0
18	1.3±0.0*	1.4±0.0
24	1.3±0.0*	1.3±0.0
36	1.1±0.0*	1.2±0.0
48	0.9±0.1*	1.1±0.0
72	0.6±0.1*	0.9±0.0
96	0.4±0.1*	0.6±0.0
120	0.2±0.1*	0.3±0.0

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

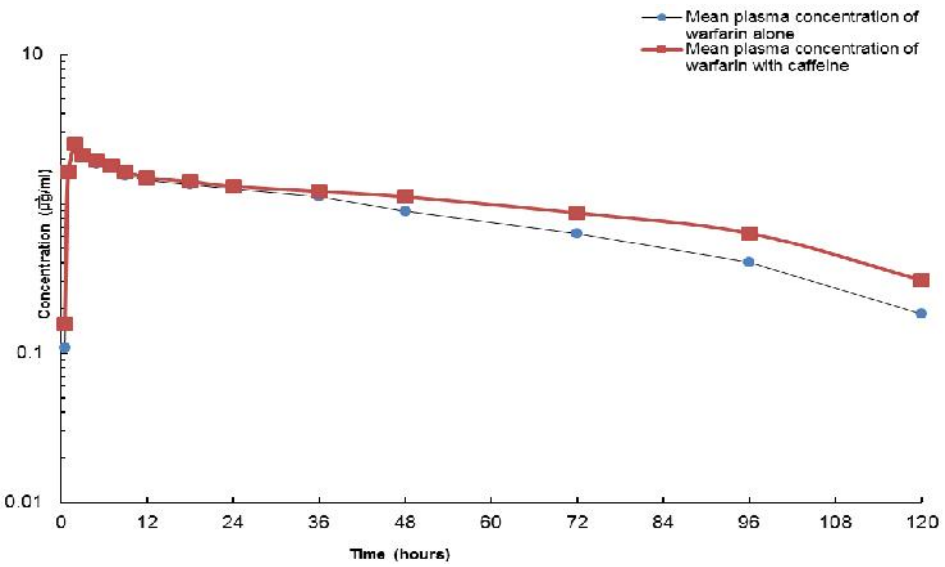

Fig. 2. Comparative mean plasma concentrations of warfarin on a semi-logarithmic scale versus time following oral administration 0.5 mg.kg^{-1} alone and with multiple doses of caffeine (5 mg.kg^{-1} every twelve hours for three days) in ten healthy adult male albino rabbits

Pharmacokinetics: The results showing pharmacokinetic parameters of warfarin alone and in combination with caffeine in ten rabbits have been presented in Tables 2 along with statistical analysis comparison of these values by "t" test.

The maximum plasma concentration (C_{max}) is the highest concentration of a drug in specified compartment after it has been administrated and was found to be 1.9±0.1 µg.mL^{-1} for warfarin alone and 1.9±0.0 µg.mL^{-1} for warfarin in in combination with caffeine in present study. Whereas, the time required for achieving peak plasma concentration (T_{max}) of warfarin alone in albino rabbits was 8.1±0.4 hr and of warfarin in combination with caffeine was 7.6±0.4 hr.

Absorption rate constant (K_{abs}) is the fractional rate of drug absorption from the site of drug administration into the systemic circulation and absorption half-life ($t_{½}$abs) is the time taken for half of the drug to reach the systemic circulation. Table 2 showed the mean±SE K_{abs} and $t_{½}$abs of warfarin alone as 0.4±0.0 hr^{-1} and 2.0±0.2 hr, respectively. On the other hand, mean±SE values of K_{abs} and $t_{½}$abs of warfarin in combination with caffeine was found as 0.5±0.0 hr^{-1} and 1.5±0.1 hr, respectively.

The elimination rate constant (β) is the proportionality constant which relates the rate of elimination of the drug and the amount of drug remaining to be eliminated. Mean±SE overall elimination rate constant (β) of warfarin alone and in combination with caffeine was determined as 0.03±0.0 hr^{-1}and 0.01±0.0 hr^{-1}. The elimination half-life ($t_{1/2}$ β) of a drug refers to the time required for a drug to be reduced to half of its original concentration in the body through various elimination processes. The data regarding elimination half-life ($t_{1/2}$ β) of warfarin alone and in combination with caffeine in ten healthy adult male albino rabbits was 34.1±4.8 hr and 49.1±2.4 hr, respectively.

The apparent volume of distribution (V_d) relates the drug concentration in plasma to total amount of drug in body after distribution equilibrium has been established. The mean±SE value for V_d was 0.2±0.0 L.kg^{-1} in case of 0.5 mg.kg^{-1} of warfarin alone and 0.2±0.0 L.kg^{-1} when warfarin and caffeine was given at same time to ten healthy rabbits. The total body clearance (Cl_B) represents the sum of metabolic and all excretory processes and is the volume of blood completely cleared of a drug per unit time. Mean±SE value for Cl_B was 0.01±0.0 mL.min^{-1}.kg^{-1} and 0.004±0.0 mL.min^{-1}.kg^{-1} for warfarin alone and in concomitant administration with caffeine.

Area under the curve (AUC) of warfarin (0.5 mg.kg^{-1}) after its single oral administration in albino rabbits was 109.3±10.6 µg.hr.L^{-1} and after its combination with caffeine was 147.2±6.4 µg.hr.mL^{-1}. The mean±SE value of B (Zero time drug concentration of elimination phase) was 2.4±0.2 µg.mL^{-1} and 2.1±0.1 µg.mL^{-1}, respectively, after single oral administration of warfarin alone and in concomitant administration with caffeine in ten healthy adult male albino rabbits. Mean residence time (MRT) is the average total time taken by molecules of a given dose of drug to spend in the body. The mean±SE residence time (MRT) of warfarin (0.5 mg.kg^{-1}) after its administration alone and in co-administration with caffeine in ten healthy male albino rabbits appeared to be 52.1±6.7 hr and 2.9±3.4 hr, respectively.

Statistical comparison of pharmacokinetic parameters of wasrfarin following its single oral

administration alone and alongwith caffeine has shown a non-significant difference in C_{max}, T_{max}, V_d and B. Whereas a significant increase in K_{abs}, $t_{1/2}\beta$ and MRT was observed after concomitant administration of warfarin with caffeine and a significant deccrease was observed in $t_{1/2abs}$, β and Cl_B, when warfarin was combined with caffeine.

Table 2. Comparative mean±SE values for the disposition kinetics of warfarin (0.5 mg.kg^{-1}) following a single oral administration alone and with caffeine (50 mg.kg^{-1} every 12 hours for three days) in healthy adult male albino rabbits.

Parameters	Units	Warfarin	Warfarin with Caffeine
C_{max}	µg.mL^{-1}	1.9±0.1 ^{NS}	1.9±0.0
t_{max}	.hr	8.1±0.4 ^{NS}	7.6±0.4
K_{abs}	hr^{-1}	0.4±0.0*	0.5±0.0
.$t_{1/2abs}$	.hr	2.0±0.2*	1.5±0.1
.β	hr^{-1}	0.03±0.0*	0.01±0.0
$t_{1/2}\beta$	.hr	34.1±4.8*	49.1±2.4
V_d	L.kg^{-1}	0.2±0.0 ^{NS}	0.2±0.0
Cl_B	L.hr^{-1}kg^{-1}	0.01±0.0*	0.004±0.0
AUC	µg.h.mL^{-1}	109.3±10.6*	147.2±6.4
B	µg.mL^{-1}	2.4±0.2 ^{NS}	2.1±0.1
MRT	hr	52.1±6.7*	72.9±3.4

* = Significantly (P≤0.05) different from the respective value
NS = Nonsignificantly (P>0.05) different from the respective value

DISCUSSION

The change in peak plasma concentrations can be influenced by different environmental conditions and by the difference in human race or by consumption of diverse food items during study. Genetic make-up of man and animals as well as environmental conditions is different in different regions of this planet. An original term "geonetics" has been used to describe the environmental influence on the genetics which ultimately affect disposition kinetics and fate of drugs in a population (Shehzad *et al.*, 2017; Naz *et al.,* 2017; Ashraf *et al*., 2015; Anwar *et al*., 2015).

Theoretically, coffee (a rich source of caffeine) has been listed in those foods which are considered to interact with the metabolism of warfarin. Warfarin and caffeine both the drugs were metabolized by CYP450 enzyme system (Nagui *et al*., 2001). Warfarin occurs as pair of enantiomers R and S. Where, R warfarin is metabolized primarily by CYPlA2, CYP3A4 and by carbonyl reductase and S warfarin is metabolized primarily by CYP2C9 (Kaminsky and Zhang, 1997). Expression of the enzyme CYP1A2 is induced by exposure to caffeine (Preissner *et al*., 2010). However, at higher concentration of caffeine, the contribution of CYP1A2 in metabolism of caffeine reduces in favor of CYP2C9 (Kot and Daniel, 2008). Such situation could affect S warfarin metabolism. Moreover, CYP450 polymorphisms (Sachse *et al*., 1999) could explain variability in responses to caffeine in users of warfarin. Plasma concentration of warfarin alone and with caffeine describing its pharmacokinetic parameters in present study have been briefly discussed below:

Plasma concentration: To achieve optimal anticoagulant effect in body, the drug must be maintained to a certain therapeutic level in plasma or serum. The therapeutic concentration of warfarin has been reported as 0.6±0.1 µg.ml^{-1} (Sun *et al.,* 2006) and 0.6-2.6 µg.ml^{-1} (Kwon *et al*., 2009).

From results (Table 1) it was revealed that peak concentration of warfarin in the presence of caffeine rose up from 2.4 µg.ml^{-1} to 2.5 µg.ml^{-1}. Thus, a 3.8% increase in peak concentration of warfarin was observed in the presence of caffeine. Time of peak concentration remained same in the presence of caffeine. Minimum therapeutic concentration (MTC) of warfarin in presence of caffeine was achieved at 1 hr in the presence of caffeine which remained in its MTC limits up to 96 hr. But in the absence of caffeine, the minimum therapeutic concentration of warfarin was maintained up to 72 hr after which it declined below therapeutic concentration. Mean±SE values of plasma concentrations of warfarin at different time intervals following its oral administration along with caffeine were significantly high as compared to mean±SE values of plasma concentrations of warfarin alone. These results showed that caffeine moderately affected the plasma concentration, hence, disposition of warfarin in the body.

Pharmacokinetics: Pharmacokinetic parameters play very important role in the evaluation of health risks of a drug as adverse events are observed commonly during anticoagulation oral therapy.

The C_{max} in present study was found to be 1.9±0.1 µg.ml^{-1} at 8.1±0.4 hr for warfarin alone and 1.9±0.0 µg.ml^{-1} at 7.6±0.4 hr for warfarin with caffeine. The statistical comparison of C_{max} and T_{max} of warfarin alone and along with caffeine showed that caffeine has a non-significant effect on C_{max} and T_{max} of warfarin (Table 2). In literature, peak concentration of warfarin was achieved within 2 to 8 hours (Majurus and Tollefson, 2010), 2.2 µg.ml^{-1} at 1 hr (Walker *et al*., 2009). Data of present study showed that value of C_{max} of warfarin corresponded to reported value but value of T_{max} may vary. Whereas, in another previous research on human being the reported values of C_{max} and T_{max} for warfarin were 1.5 µg.ml^{-1} and 0.5 hr, respectively (Dieterle *et al*., 2004). Similarly, peak concentration (1.45 µg.ml^{-1}) of warfarin was achieved at 1.2 hr after administration in human (Kwon *et al*., 2009). As present study was

conducted in rabbits so species difference may be an important factor behind variations observed in the plasma concentrations (Fayyaz *et al.*, 2017; Aziz *et al.,* 2016; Ashraf *et al.,* 2015).

Table 2 showed that absorption rate constant (K_{abs}) and absorption half-life ($t_{½}$ abs) of warfarin alone was 0.4±0.0 hr^{-1} and its as 2.0±0.2 hr, respectively, and of warfarin with caffeine was 0.5±0.0 hr^{-1} and 1.5±0.1 hr. Previously reported value of absorption rate constant for warfarin was 1.6 hr^{-1} (Lane *et al*., 2012). Another study which was conducted in rats showed rapid absorption of warfarin with apparent absorption rate constant of 0.3 min^{-1} (Julkunen *et al*., 1980). Statistical comparison of both these parameters (K_{abs} and $t_{½\ abs}$) after administration of warfarin alone and with caffeine in present study appeared to be significant. This comparison revealed that on concomitant administration of warfarin and caffeine, absorption of warfarin became more rapid.

The value of elimination half- life ($t_{1/2\beta}$) of warfarin (0.5 $mg.kg^{-1}$) in the absence and presence of caffeine was 34.1±4.8 hr 49.1±2.4 hr, respectively and were found to be significant. It indicates that caffeine with its multiple doses increases the $t_{1/2\beta}$ of warfarin. The elimination ha lf-life of warfarin varies greatly among individuals and its range was 35-45 hours (Palareti and Legnani, 1996). A study conducted in 2009 reported half-life of warfarin as 35.4 hr (Walker *et al*., 2009). Thus, elimination half- life of warfarin in present study corresponded to its previously reported values.

The mean±SE value of B (Zero-time drug concentration of elimination phase) of warfarin alone and along with caffeine was 2.4±0.2 and 2.1±0.1 $\mu g.ml^{-1}$, respectively. Whereas, the volume of distribution of warfarin alone and in combination with caffeine in present study was calculated as 0.2±0.0 $L.kg^{-1}$ and 0.2±0.0 $L.kg^{-1}$, respectively. The statistical comparison of B and V_d of warfarin with and without caffeine was to be non-significant indicating that caffeine does not affect the distribution of warfarin. Literature cited value of volume of distribution is almost 0.1 $L.kg^{-1}$ (Majurus and Tollefson, 2010) and 15.2 L (Lane *et al*., 2012). Results of another study revealed that the volume of distribution of S warfarin and R warfarin were 16.6 L and 10.9 L, respectively, in patients of 70 kg body weight (Lane *et al*., 2012). Warfarin is highly plasma protein bound drug so its volume of distribution is very small (Palareti and Legnani, 1996). The value of V_d in present study was in accordance with the literature cited value.

Overall elimination rate constant (β) was recorded as 0.03±0.0 h^{-1} in case of administration of warfarin alone and 0.01±0.0 h^{-1} after administration of warfarin with caffeine in healthy adult male albino rabbits. The total body clearance (Cl_B) of warfarin when administered alone was 0.01±0.0 $mL.min^{-1}.kg^{-1}$ and with caffeine was 0.004±0.0 $mL.min^{-1}.kg^{-1}$. The statistical comparison with t-test showed a significant decrease in β and Cl_B values before and after warfarin-caffeine combination therapy. It was evident from results that the presence of caffeine reduced elimination of warfarin from body. Whereas, the results of a study conducted in 70 kg women with average age of 69.8 years revealed that the clearance of S warfarin was estimated to be 0.14 $L.hr^{-1}$ and that of the R warfarin clearance was 0.13 $L.hr^{-1}$ (Lane *et al*., 2012). Thus, Cl_B of warfarin in present study was found lesser than previous reported values.

Area under the curve (AUC) of warfarin after alone oral administration was 109.3±10.6 $\mu g.h.mL^{-1}$ and with caffeine was 147.2±6.4 $\mu g.h.mL^{-1}$. After applying t-test, the values of AUC of warfarin alone and with caffeine appears to be significant (P>0.05). It indicates that caffeine raises the value of AUC of warfarin. Increased value of AUC of warfarin in the presence of caffeine is manifestation of inhibitory effect of caffeine on metabolism of warfarin when caffeine is administered for multiple times. Similarly, the mean residence time (MRT) of warfarin was 52.1±6.7 hr and 72.9±3.4 hr in the absence and presence of caffeine, respectively and was statistically significant. It indicates that caffeine reduces the metabolism of warfarin and increases its residence time in body.

Conclusion: It was concluded from the results of present pharmacokinetic study that caffeine has capacity to reduce the metabolism of warfarin by blocking CYP1A2, CYP 3A4 and also interacting with CYP2C9 isoenzymes, thus, decrease the elimination of warfarin from the body and thus may enhance anticoagulant effects of warfarin. Caffeine does not affect the C_{max} of warfarin on initiation of therapy but administration of caffeine for multiple times during warfarin therapy can increase the plasma level of warfarin. It was also concluded from findings of this experiment that plasma concentration of warfarin increased significantly after its combination with caffeine. Increased concentration of a warfarin in body may result into its increased anti-coagulation activity. Thus, clotting parameters i.e. prothrombin time and international normalized ratio values may also change. The findings of this experiment in rabbits suggest confirming its conclusion in human subjects also to verify that should human patients be advised to limit the frequent use of tea and coffee during warfarin therapy as tea and coffee are the rich source of caffeine.

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