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Research Article

Possible Drug–Drug Interaction between Warfarin and Azithromycin in Infected Rats with Hypercoagulable State

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Abstract. *Background*: Warfarin is frequently used in clinical practice in the treatment of deep vein thrombosis and as a prophylactic agent against thromboembolism. Unfortunately, many medical, herbal, and dietary agents affect the anticoagulant activity of warfarin. Azithromycin is one of the most frequently used antimicrobial agents mainly in respiratory tract infections. This work was designed to assess the possible interaction between azithromycin on the anticoagulant activity of warfarin and the effect of warfarin on the antibacterial activity of azithromycin with subsequent readjustment of the dose of both drugs when they are coadministered. *Methodology*: The current study used 72 male adult albino rats weighing 200–250 g (3–4 months old) that were randomly subdivided equally into nine groups. Hypercoagulable state in rats was induced by a single intraperitoneal injection of ellagic acid at a dose of 10.5 mg/kg for 5 min before collection of blood samples. Anticoagulant activity of warfarin was assessed by prothrombin time (PT), bleeding time (BT), and clotting time (CT). The parameters for the antibacterial activity of azithromycin were the viable bacterial count, myeloperoxidase enzyme activity in the blood, neutrophil enumeration, and determination of serum C-reactive protein and serum tumor necrosis-α. *Results*: No significant differences were noted on PT, CT, and BT of hypercoagulable rats treated with azithromycin compared with hypercoagulable rats only. Moreover, azithromycin did not affect the anticoagulant activity of warfarin, and no significant change was noted in PT, CT, and BT between rats treated and not treated with azithromycin. The antibacterial activity of azithromycin had not been affected by warfarin. Furthermore, no significant differences were noted between infected and warfarin-treated rats only concerning antibacterial activity. *Conclusion*: Warfarin and azithromycin can be safely used together without cross-interaction between each other. Moreover, dose adjustment is not needed if both drugs are co-administered.

Keywords: Warfarin; anticoagulant; Azithromycin; Hypercoagulable state, Interaction

1. Introduction

Warfarin is a frequently used oral anticoagulant used in the treatment of deep vein thrombosis. Moreover, it has been used as a prophylactic agent of thromboembolism associated with orthopedic surgery, atrial fibrillation, cerebrovascular disease, and prosthetic heart valves [1]. Consequently, many pharmacokinetic and pharmacodynamic interactions with drugs, herbs, and medicinal foods were noted. Also, several medicinal products have a possible impact on its anticoagulant effect [2].

Patients administered long-term warfarin may be subjected to bacterial infection. Thus, a practitioner must routinely undergo conflicting antibacterial and anticoagulant treatment. Successful prevention of intravascular thrombosis and the result of antibacterial and anticoagulant care in warfarinised patients should be accordingly managed [3].

Azithromycin is an antibiotic macrolide orally used for the prevention of upper and lower respiratory tract bacterial infections, skin, and uncomplicated chlamydial urinary tract diseases. The prototype macrolide antibiotic erythromycin is similar to azithromycin. Azithromycin has a longer half-life permitting once-a-day dosages, effectiveness over a shorter 5-day treatment period, and improved antibiotic range [4].

Drug reactions with warfarin, including serious hemorrhage or thrombosis, can lead to severe consequences. Medicines should be considered for co administration in warfarinised patients while administering antibacterial drugs like azithromycin to avoid such complications [3].

This work was designed to assess the possible interaction between azithromycin on the anticoagulant activity of warfarin and the effect of warfarin on the antibacterial activity of azithromycin with subsequent readjustment of the dose of both drugs when they are administered.

2. Materials and Methods

2.1. Animals. Male adult albino rats weighing 200–250 g (3–4 months old) have been used in this study. Animals were obtained from the animal house of the Faculty of Medicine, Assiut University, with room temperature maintained at 22˚C–24 ˚C. Animals were fed on a commercial pellet diet and kept under a normal light/dark cycle. Animals were given free access to food and water ad libitum. The experimental protocol was approved by the Ethical Committee of the Faculty of Medicine, Assiut University.

2.2. Chemicals and drugs. Warfarin and azithromycin (AK scientific, USA) were dissolved in water sterile saline, respectively, before administration. Moreover, ellagic acid (AK scientific, USA) was available in a yellow powder (5 g in a bottle) and dissolved in distilled water before administration.

2.3. Induction of infection. The current study used *Escherichia coli* (ATCC 8739) strain (a gift from the Microbiology Department, Faculty of Medicine, Assiut University). Infection degree was evaluated by (1) myeloperoxidase enzyme (MPO) activity by MPO kit (Sigma-Aldrich, Cairo, Egypt), (2) C-reactive protein (CRP) by CRP-latex agglutination test kits (LTA, Bussero, Italy), and (3) tumor necrosis factor-α (TNF-α) concentration by TNF-α ELISA kits (LTA, Italy).

2.4. Experimental design. The current study had two sets of experiments, and rats were randomly divided into nine groups (eight rats in each group).

The first set of experiments were carried out to determine the effect of azithromycin on the anticoagulant activity of warfarin. It included:

- *First group* (control group): received only physiological saline orally by gastric tube for 5 days consecutively.
- *Second group* (rats in the hypercoagulable state): rats were injected with intraperitoneal (IP) ellagic acid (EA; 10.5 mg/kg) as a single dose 5 min before blood sample collection.
- *Third group*: rats in the hypercoagulable state were orally given warfarin (0.05 mg/kg/day) daily as a single dose by gastric tube for 5 days before induction of hypercoagulable state.
- *Fourth group*: rats in the hypercoagulable state were orally treated with azithromycin as a single dose of 300 mg/kg at 24 h before induction of hypercoagulable state.
- *Fifth group*: rats in the hypercoagulable state were treated with a combination of warfarin and azithromycin. Both agents were given treatments similar to the third and fourth groups.

The second set of experiments was carried out to determine the effect of warfarin on the antibacterial activity of azithromycin. It included:

- *Sixth group* (infected rats): rats were injected IP with *E. coli* (1 × 108) to induce infection.
- *Seventh group*: infected rats were orally treated with azithromycin as a single dose of 300 mg/kg at 24 h before infection induction.
- *Eighth group*: infected rats were treated orally with warfarin (0.05 mg/kg/day) as a daily single dose by gastric tube for 5 days consecutively before infection induction.

- *Ninth group*: infected rats were treated with a combination of warfarin and azithromycin. Both agents were given treatments similar to the seventh and eighth groups.

2.5. Induction of hypercoagulable state. The hypercoagulable state was induced by intraperitoneal injection of a single dose of EA (10. 5 mg/kg). The development of the hypercoagulable state in rats was confirmed by prothrombin time (PT) and concentration as well as measurement (blood samples were collected from orbital venous plexus of the eye, 1 mL/each rat) 10 min after EA injection. Animals had shorter PT (10–15 s) and were considered in the hypercoagulable state compared with the control group PT (20–25 s) [5].

Preliminary experiments which were conducted to adjust the dose of warfarin in rats

One group was used to evaluate the effect of warfarin in rats in the hypercoagulable state.

In the first trial, rats were orally administered with warfarin (0.1 mg/kg) for 5 consecutive days. The animals were observed daily for any signs of bleeding or other adverse effects. Animals expired on day 4 due to internal hemorrhage. Thus, the current study considered that the dose which was documented by Zaghloul et al. [6] (0.1 mg/kg) is not suitable for the experiment.

In the second trial, animals orally received warfarin (0.05 mg/kg) using a gastric tube as a single dose for 5 consecutive days. Moreover, the animals were observed daily for any signs of bleeding or other adverse effects. On day 5, blood samples were collected. Consequently, PT was determined to be 30–35 s.

In the third trial, animals orally received warfarin at a dose of 0.075 mg/kg using a gastric tube as a single dose for five consecutive days. The animals were observed daily for any signs of bleeding or other adverse effects. On day 5, blood samples were collected. PT was determined to be 40–50 s.

From previous trials, the current study considered the dose of 0.05 mg/kg as the submaximal dose for prolongation of PT compared with the control group.

To determine the effect of azithromycin on the induced hypercoagulable state, azithromycin was orally given into rats as a single dose of 300 mg/kg at 24 h before induction of the hypercoagulable state. Moreover, rats were administered with combined treatment of warfarin and azithromycin to determine the effect of azithromycin on the anticoagulant activity of warfarin. Warfarin was orally given (0.05 mg/kg/day) daily for five consecutive days before induction of the hypercoagulable state.

Azithromycin was given orally into rats as a single dose of 300 mg/kg at 24 h before induction of the hypercoagulable state (day 4 after 2-h administration of warfarin). Moreover, the hypercoagulable state was induced on day 5 of the warfarin treatment by IP injection of EA (10.5 mg/kg) 5 min before blood sample collection.

2.6. Induction of infection. Infection was induced by intraperitoneal injection of 1×10^8 colony-forming unit (CFU) of *E. coli* [7]. The development of infection in animals was confirmed by a viable count (blood samples were collected from the orbital venous plexus at 0.5 mL/rat 24 h after *E. coli* injection with diarrhea as a sign of infection). Azithromycin was orally given as a single dose (300 mg/kg) 24 h before induction of infection to evaluate the antibacterial activity of azithromycin according to Retsema et al. [8]. In general, the bactericidal activity was slow to develop, requiring >8 h to reduce the CFU to >99%–9% [8,9] Considering the high and sustained tissue levels achieved with azithromycin in animals [10, 11] and humans [12], the pathogen would be continuously exposed to bactericidal levels of azithromycin at the infection site.

Preliminary tests which were conducted to adjust the dose of azithromycin in rats.

One group was used to evaluate the antibacterial activity of azithromycin and was given orally as a single dose (200 mg/kg) 24 h before induction of infection according to Retsema et al. [8]. Animals were observed for any signs of infection. Diarrhea with less severity was noted in the infected group and the viable count was less than the infected group.

In the second trial, animals were orally treated with a higher dose of azithromycin (400 mg/kg) as a single dose 24 h before infection induction. Moreover, animals were observed for any signs of infection. Diarrhea and a significant number of bacteria in the viable count were not noted compared with the group treated with azithromycin dose (200 mg/kg).

In the third trial, animals were orally treated by azithromycin as a single dose (300 mg/kg) 24 h before infection induction. Moreover, animals were observed for any signs of infection. Diarrhea was not noted, and the viable count was less than that of the infected group and less than the group treated with azithromycin dose (200 mg/kg) but more than the group treated with azithromycin dose (400 mg/kg).

From the previous trials, the current study considered the submaximal dose of azithromycin at 300 mg/kg which was suitable for the antibacterial activity compared with the control group.

To determine the effect of warfarin on the induced infection, warfarin was orally given (0.05 mg/kg/day) as a single dose daily for consecutive 5 days before infection induction. Infection was induced on day 5 of warfarin treatment by *E. coli* (1×10^8 CFU) injection after 2-h warfarin administration.

Rats were administered with the combined warfarin and azithromycin treatment to determine the effect of warfarin on the antibacterial activity of azithromycin. Moreover, warfarin

Table 1: Effect of warfarin and azithromycin on PT in hypercoagulable rats

Group	Treatment	Prothrombin time (s)
1	Control	24.2 ± 0.8
2	Ellagic acid (10.5 mg/kg)	11 ± 0.8*
3	Warfarin + ellagic acid	20.9 ± 0.9**
4	Azithromycin + ellagic acid	11.2 ± 0.6
5	Warfarin + azithromycin + ellagic acid	22.5 ± 1.01

Results are represented as mean ± SE ($n = 10$); $^{*}p < 0.01$, highly significant result compared with the control group; $^{**}p < 0.01$, highly significant result compared with the hypercoagulable group

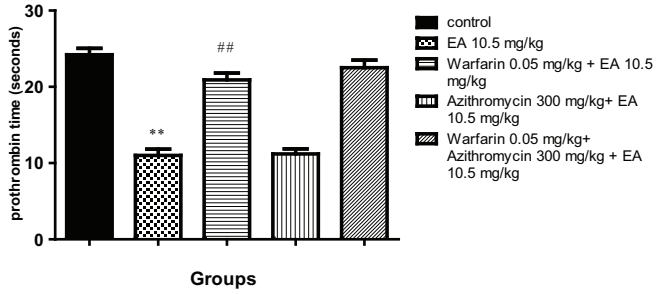

Figure 1: Effect of warfarin and azithromycin on PT in hypercoagulable rats.

was orally given (0.05 mg/kg/day) daily for consecutive 5 days before infection induction.

Azithromycin was orally given into rats as a single dose of 300 mg/kg at 24 h before infection induction (day 4 after 2-h administration). Infection was induced on day 5 of warfarin treatment by *E. coli* (1 × 108 CFU) injection after 2-h warfarin administration.

2.7. Sample collection, preparation, and storage. Plasma blood samples were collected in centrifuge tubes using a one-tenth volume of 0.1 M sodium citrate as an anticoagulant. The blood was centrifuged at 3,500 rpm for 15 min and was immediately used. Serum blood samples were collected in centrifuge tubes, the blood was centrifuged at 3,500 rpm for 15 min, and serum was separated and stored in Eppendorf tubes in the deep freezer at (−20 °C).

The following parameters were measured:

Parameters for anticoagulant effects

- PT and concentration were determined by commercial kits supplied by Biomed Diagnostics, Inc. (Cairo, Egypt) [13].

- CT, which is two drops of blood, was obtained from the rat's tail 5 min after EA injection at a diameter of 5 mm at both ends on a glass slide to record CT. Simultaneously, the drops were poked up from the edges with a sterile needle at intervals until a blood streak appeared [14].

Platelet function tests

- *Bleeding time (BT)*

The tail was warmed for 1 min in water at 40 °C and then dried. A small cut was made in the middle of the tail with a scalpel 5 min after EA injection. Bleeding time started when the first drop touched the circular filter paper. It was checked at 30-s intervals until THE bleeding stopped [15].

Parameters for antibacterial effects

- *Viable bacterial count*

Blood (0.5 mL) was obtained from retro-orbital sinus 1 day after infection induction. Viable bacteria in the specimen were counted using the pour plate method. The counting of bacterial CFU in each sample was calculated using the formula:

Viable bacterial count = CFU/plate × dilution factor

Results were expressed as the number of bacterial CFU per milliliter of blood [16].

- *Enumeration of neutrophils*

Differential cell counts were determined by light microscopy following Leishman staining of whole blood smears collected on ethylenediaminetetraacetic acid [17].

Table 2: Effect of warfarin and azithromycin on clotting time in rats in the hypercoagulable state.

Group	Treatment	Clotting time (s)
1	Control	127.3 ± 8.2
2	Ellagic acid (10.5 mg/kg)	33.6 ± 4.5*
3	Warfarin + ellagic acid	111.3 ± 5.1**
4	Azithromycin + ellagic acid	35.8 ± 4.2
5	Warfarin + azithromycin + ellagic acid	111.5 ± 5.04

Results are represented as mean ± SE ($n = 10$); $^{*}p < 0.01$, highly significant result compared with the control group; $^{**}p < 0.01$, highly significant result compared with the hypercoagulable group

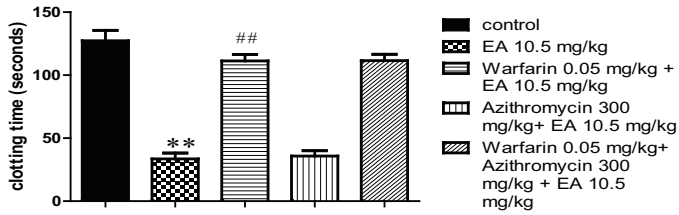

Figure 2: Effect of warfarin and azithromycin on clotting time in rats the hypercoagulable state.

- *MPO* in the blood, *CRP*, and *TNF-α* were analyzed using MPO staining kit, CRP-latex agglutination test [18], and ELISA [19] kits, respectively.

2.8. Statistical analysis. Statistical analysis was done using Prism software, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). The variability of samples was expressed as mean ± standard deviation. Moreover, analysis of variance and chi-square tests for trends was done to compare between the studied groups. Analysis of the difference was insignificant, significant, and highly significant at $P > 0.05$, $P < 0.05$, and $P < 0.01$ (20).

3. Results

3.1. Effect of warfarin and azithromycin on PT in hypercoagulable rats. Table 1 and Figure 1 showed a highly significant decrease in PT of hypercoagulable rats compared with control rats at 11.0 s ± 0.8 and 24.2 s ± 0.8, respectively. Animals treated with warfarin before induction of the hypercoagulable state had a highly significant increase in PT compared with hypercoagulable animals only at 20.9 s ± 0.9 and 11.0 s ± 0.8, respectively. Moreover, animals treated with azithromycin before induction of the hypercoagulable state had no significant change in PT compared with hypercoagulable animals at 11.2 s ± 0.6 and 11 s ± 0.8, respectively. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in PT compared with animals treated with warfarin only at 22.5 s ± 1.01 and 20.9 s ± 0.9, respectively.

3.2. Effect of warfarin and azithromycin on CT in hypercoagulable rats. Table 2 and Figure 2 showed a highly significant decrease in CT of the hypercoagulable rats compared with the control rats at 33.6 s ± 4.5 and 127.3 s ± 8.2, ± 4.566 respectively. In addition, animals treated with warfarin before induction of the hypercoagulable state had a highly significant increase in CT compared with the hypercoagulable animals only at 111.3 s ± 5.1 ± 4.263 and 33.6 s ± 4.5 ± 4.566, respectively.

Animals treated with azithromycin before induction of the hypercoagulable state had no significant change in CT compared with the hypercoagulable animals only at 35.8 s ± 4.2 ± 4.263 and 33.6 s ± 4.5 ± 4.566, respectively. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in CT compared with animals treated with warfarin only at 111.5 s ± 5.04 and 111.3 s ± 5.1 ± 5.145, respectively.

3.3. Effect of warfarin and azithromycin on BT in hypercoagulable rats. Table 3 and Figure 3 showed a highly significant decrease in BT of the hypercoagulable rats compared with control rats at 48.7 s ± 4.8 and 97.8 s ± 5.3 ± 4.566, respectively. Moreover, animals treated with warfarin before induction of the hypercoagulable state had a highly significant increase in BT compared with the hypercoagulable animals only at 101.5 s ± 8.8 ± 4.263 and 48.7 s ± 4.8 ± 4.566, respectively.

Animals treated with azithromycin before induction of hypercoagulable state had no significant change in BT compared with the hypercoagulable animals at 47.9 s ± 2.4 and 48.7 s ± 4.8, respectively. Similarly, animals treated with a combination of warfarin and azithromycin had no

Table 3: Effect of warfarin and azithromycin on bleeding time in rats in the hypercoagulable state.

Group	Treatment	Bleeding time (s)
1	Control	97.8 ± 5.3
2	Ellagic acid (10 mg/kg)	48.7 ± 4.8*
3	Warfarin + ellagic acid	101.5 ± 8.8**
4	Azithromycin + ellagic acid	47.9 ± 2.4
5	Warfarin + azithromycin + ellagic acid	105.3 ± 7.4

Results are represented as mean ± SE ($n = 10$); $^{*}p < 0.01$, highly significant result compared with the control group; $^{**}p < 0.01$, highly significant result compared with the hypercoagulable group

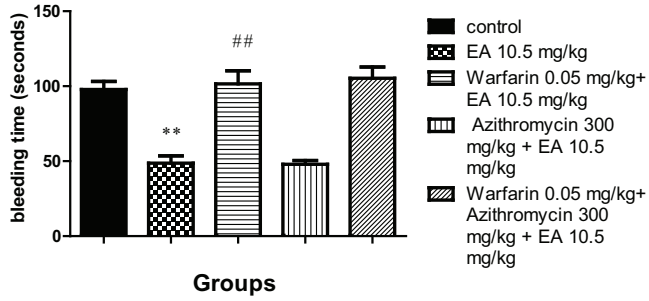

Figure 3: Effect of warfarin and azithromycin on bleeding time in the hypercoagulable rats.

significant change in BT compared with animals pretreated with warfarin only at 105.3 s ± 7.4 and 101.5 s ± 8.8, respectively.

3.4. Effect of warfarin and azithromycin on viable bacterial count in infected rats. Table 4 and Figure 4 showed a highly significant increase in the viable bacterial count of infected rats compared with the control rats at 152.8 ± 42.6 and 0 CFU/mL, respectively. Animals treated with azithromycin before the induction of infection had a highly significant decrease in viable bacterial count compared with infected animals at 5.3 ± 1.6 and 152.8 ± 42.6 CFU/mL ± 4.566, respectively. However, animals treated with warfarin before infection induction had no significant change in viable bacterial count compared with infected animals at 140.7 ± 38.2 and 152.8 ± 42.6 CFU/mL, respectively. Animals treated with a combination of warfarin and azithromycin had no significant change in viable bacterial count compared with animals treated with azithromycin only at 5.4 ± 1.8 and 5.3 ± 1.6 CFU/mL, respectively.

3.5. Effect of warfarin and azithromycin on neutrophils count in infected rats. Table 5 showed a highly significant increase in neutrophils count of infected rats compared with control rats at 74.8 ± 2.8 and 40.6 ± 2.5/mL, respectively. Animals treated with azithromycin before infection induction had a highly significant decrease in neutrophils count compared with the infected animals at 41.9 ± 2.6 and 74.8 ± 2.8/mL ± 4.566, respectively.

Animals treated with warfarin before infection induction had no significant change in neutrophils count compared with the infected animals at 71.6 ± 3.1 and 74.8 ± 2.8/mL, respectively. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in neutrophils count compared with animals treated with azithromycin only at 42.6 ± 2.8 and 41.9 ± 2.6/mL, respectively.

3.6. Effect of warfarin and azithromycin on MPO activity in infected rats. Table 6 shows that the MPO activity of the infected animals was significantly increased compared with the corresponding control animals. Moreover, the MPO activity of animals treated with azithromycin was significantly decreased compared with the corresponding infected animals.

Animals treated with warfarin before infection induction had no significant change in MPO activity compared with the corresponding infected animals. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in MPO activity compared with the animals treated with azithromycin only.

3.7. Effect of warfarin and azithromycin on C-reactive protein (CRP) in infected rats. Table 7 and Figure 5 show that the CRP of infected animals was significantly high

Table 4: Effect of warfarin and azithromycin on the viable bacterial count in infected rats.

Groups	Treatment	Viable bacterial count × 10^5 (CFU/mL)
1	Control	0
2	Infected by *E. coli* 1 × 10^8 CFU	152.8 ± 42.6*
3	Infected by *E. coli* + azithromycin	5.3 ± 1.6**
4	Infected by *E. coli* + warfarin	140.7± 38.2
5	Infected by *E. coli* + warfarin + azithromycin	5.4 ± 1.8

Results are represented as mean ± SE ($n = 10$); *$p < 0.01$, highly significant result from the control group; **$p < 0.01$, highly significant result from the infected group

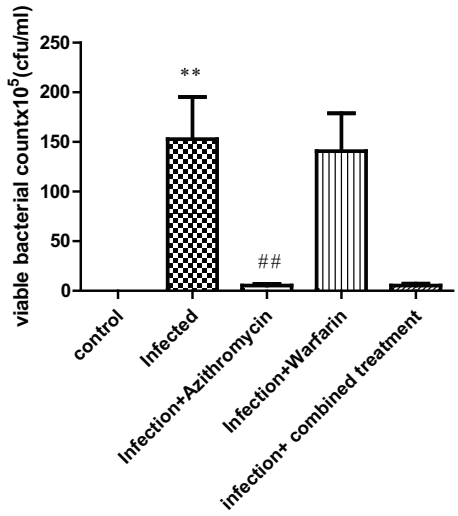

Figure 4: Effect of warfarin and azithromycin on the viable bacterial count in infected rats.

compared with the corresponding control animals. Moreover, the CRP of animals treated with azithromycin was negative in all serum samples. Animals treated with warfarin before infection induction had no significant change in serum CRP compared with infected animals. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in serum CRP compared with animals treated with azithromycin only.

3.8. Effect of warfarin and azithromycin on TNF-α in infected rats. Table 8 and Figure 6 shows that TNF-α of infected animals was significantly increased compared with the control animals. The serum TNF-α of animals treated with azithromycin was significantly lower than the corresponding infected animals.

Animals treated with warfarin before induction of infection had no significant change in serum TNF-α compared with the infected animals. Similarly, animals treated with a combination of warfarin and azithromycin had no significant change in serum TNF-α compared with animals treated with azithromycin only.

4. Discussion

Warfarin has many forms of a serious drug interaction that may be life-threatening. Thus, clinicians should think about medication interactions when prescribing any drug, especially antibiotics and anti-inflammatories, to a patient taking warfarin [1].

In the present study, the first set of experiments was carried out to determine the effect of azithromycin on the anticoagulant activity of warfarin. The parameters used to evaluate the anticoagulant activity of warfarin were PT, CT, and BT [5]. In the current study to assess warfarin efficiency, the hypercoagulable state in rats was induced by a single IP injection of EA at a dose of 10.5 mg/kg at 5 min before the collection of blood samples. Moreover, the induction of the hypercoagulable state by EA significantly reduced the PT, CT, and BT of rats. These results are consistent with Liu et al. [5].

The use of warfarin as a pretreatment single dose for five consecutive days before induction of the hypercoagulable state by EA in rats showed that warfarin protected animals from the hypercoagulable state wherein PT, CT, and BT in

Table 5: Effect of warfarin and azithromycin on neutrophils count in infected rats

Groups	Treatment	Neutrophils count/mL
1	Control	40.6 ± 2.5
2	Infected by *E. coli* 1×10^8 CFU	$74.8 \pm 2.8^*$
3	Infected by *E. coli* + azithromycin	$41.9 \pm 2.6^{**}$
4	Infected by *E. coli* + warfarin	71.6 ± 3.1
5	Infected by *E. coli* + warfarin + azithromycin	42.6 ± 2.8

Results are represented as mean ± SE ($n = 10$); $^*p < 0.01$, highly significant result from the control group; $^{**}p < 0.01$, highly significant result from the infected rats

Table 6: Effect of warfarin and azithromycin on MPO activity in infected rats.

Group	Treatment	Myeloperoxidase enzyme activity
1	Control vs. infected group	4.81^*
2	Infected group vs. infected group treated with azithromycin	16.9^*
3	Infected group vs. infected group treated with warfarin (0.05 mg/kg)	0.11
4	Infected group treated with azithromycin (300 mg/kg) vs. infected group treated with combination of azithromycin (300 mg/kg) and warfarin (0.05 mg/kg)	0.15

Results are represented by chi-square for trend values ($n = 10$); $^*p < 0.01$, highly significant result

Table 7: Effect of warfarin and azithromycin on C-reactive protein in infected rats.

Groups	Treatment	C-reactive protein (mg/L)
1	Control	Negative
2	Infected by *E. coli* 1×10^8 CFU	Positive (210 ± 42.9)
3	Infected by *E. coli* + azithromycin	Negative
4	Infected by *E. coli* + warfarin	Positive (198 ± 44.8)
5	Infected by *E. coli* + warfarin + azithromycin	Negative

Results are represented as mean ± SE ($n = 10$)

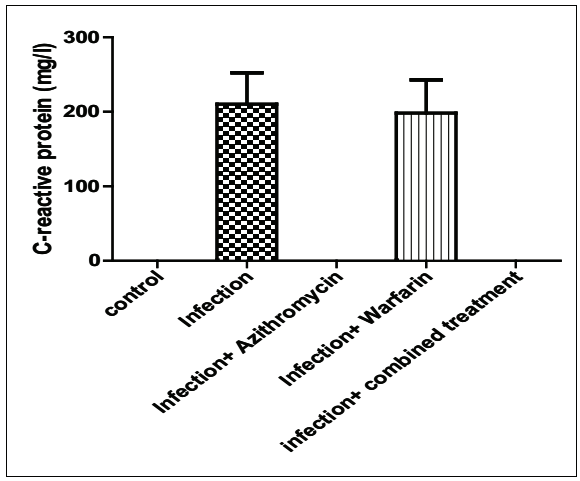

Figure 5: Effect of warfarin and azithromycin on C-reactive protein in infected rats.

Table 8: Effect of warfarin and azithromycin on tumor necrosis factor-α in infected rats

Groups	Treatment	TNF-α (ng/mL)
1	Control	8.01± 0.4
2	Infected by *E. coli* 1×10^8 CFU	$40.2 \pm 8.1^*$
3	Infected by *E. coli* + azithromycin	$8.2 \pm 0.2^{**}$
4	Infected by *E. coli* + warfarin	44.1 ± 7.7
5	Infected by *E. coli* + azithromycin + warfarin	8.9 ± 0.5

Results are represented as mean ± SE ($n = 10$); $^*p < 0.01$, highly significant result from the control group; $^{**}p < 0.01$, highly significant result from the infected group

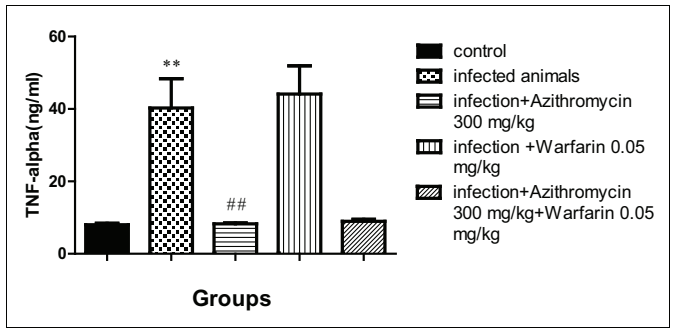

Figure 6: Effect of warfarin and azithromycin on TNF-α in infected rats.

rats was significantly higher than the corresponding animals in the hypercoagulable state only. Moreover, in this study, no significant change was noted on PT, CT, and BT of the hypercoagulable rats treated with azithromycin compared with hypercoagulable rats only.

In addition, the results revealed that no significant effect of azithromycin was noted on the anticoagulant activity of warfarin. Moreover, no significant change in PT, CT, and BT of animals exists compared with rats treated only with warfarin before induction of the hypercoagulable state.

Interaction between warfarin and other macrolide group members was clinically studied. Erythromycin and clarithromycin were extensively studied clinically and all results documented that erythromycin and clarithromycin enhance warfarin hypoprothrombinemia activity.

The results of the current study were supported by a clinical study carried out by McCall et al. [21] to determine the effect of adding azithromycin to patients receiving stable doses of warfarin. They concluded that no interaction was noted between azithromycin and warfarin observed in patients receiving both drugs concurrently. However, Mergenhagen et al. [22] found a significant increase in the international normalized ratio (INR). This result is not in agreement with the result of the current study which may be due to the absence of the control group, or the unassessed adherence to azithromycin and warfarin therapy.

The interaction between warfarin and azithromycin was reported to lead to the elevation of the INR of an elderly patient. Elevated INR in such a state may be due to many factors including the patient's age, fever, and acetaminophen–codeine prescription. Moreover, a reduction in metabolic hepatic capacity to the drugs occurred with aging [23,24].

Lane [25] found the interaction between warfarin and azithromycin elevated INR and attributed this secondary to combined azithromycin treatment. In addition, Seamans [26] reported that macrolides and metronidazole may increase INR by inhibiting warfarin's metabolism. However, these suggestions are not compatible with the result of the current study.

Many factors that have increased the patient's sensitivity to warfarin exist. These factors include vitamin E which has been reported to increase the hypoprothrombinemia effects of warfarin [27]. In addition, pneumonia and viral infections have been shown to reduce hepatic drug metabolism. Furthermore, low albumin [28] and fever have been shown to increase the catabolism of clotting factors [29].

All these factors can contribute to the elevations of the INR in patients receiving oral anticoagulants. In addition, the severity of the condition and the numerous underlying disease processes in this patient that could have contributed to the elevated INR makes the association between warfarin and azithromycin questionable [29].

Two elderly patients were reported to experience significantly elevated INR during combined warfarin and azithromycin therapy. This may be due to receiving

acetaminophen, concomitant antibiotic therapy, and a complex hospital course in the first case. In the second case, confounding factors consisted of a 50% increase in warfarin dosage before the start of azithromycin therapy and a hepatocellular injury of unknown origin [13]. Moreover, Rao et al. stated that the initial increase in the INR could have been multifactorial either due to combined warfarin with azithromycin or the patient received prednisone therapy [29].

The second set of experiments was carried out to determine the effect of warfarin on the antibacterial activity of azithromycin. The parameters used in this study to evaluate this effect were the viable bacterial count, myeloperoxidase enzyme activity in the blood, enumeration of neutrophils, determination of serum CRP, and serum TNF-α.

According to Diniz et al. [7] who showed a highly significant increase in viable bacterial count, myeloperoxidase enzyme activity in the blood, enumeration of neutrophils, serum CRP, and serum TNF-α of animals, diarrhea was noted. From different trials which were conducted in this study to adjust the appropriate dose for azithromycin, azithromycin at a dose of 300 mg/kg is the submaximal dose.

The results of this study displayed that azithromycin protected animals from infection with a significant decrease in the viable bacterial count, myeloperoxidase enzyme activity in blood, enumeration of neutrophils, serum CRP, and serum TNF-α compared with the infected animals. Moreover, no significant change was noted in the viable bacterial count, neutrophils count, serum TNF-α, CRP, and myeloperoxidase enzyme activity of infected animals treated with warfarin compared with the infected animals only.

The results also revealed that no significant effect of warfarin was noted on the antibacterial activity of azithromycin without significant change in the viable bacterial count, neutrophils count, TNF-α, CRP, and myeloperoxidase enzyme activity of infected animals treated with the combination of azithromycin and warfarin compared with the infected animals treated with azithromycin.

The main strengths of the current work are (1) the unification of all factors related to animals were used in this study, (2) all animals received the same doses by the same routes at the same time of drug administration, (3) the pharmacokinetics of the drugs of the current study were considered (e.g., the onset of drug action, blood peak concentration, and duration of drug action). In conclusion, no cross-interaction was noted between azithromycin and warfarin without the need for readjustment in the dose of both drugs when they are coadministered.

Competing Interests

The authors declare no competing interests.

Abbreviations

BL: bleeding time;
PT: prothrombin time;
CT; clotting time;
CFU: colony-forming unit;
IP: intraperitoneal;
MPO: myeloperoxidase;
TNF: tumor necrosis factor;
CRP: C-reactive protein.

References

[1] J. Hirsh, J. Dalen, D. R. Anderson, L. Poller, H. Bussey, J. Ansell, D. Deykin. "Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range" *Chest* 2001; 119 z(1); Suppl:8S-21S. Review.

[2] N. A. Buckley, A. H. Dawson. "Drug interactions with warfarin", *Med J Aust*, 1992; 157(7):479-483.

[3] O. Rehulková. "Interactions of warfarin", *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 2001; 145(2):27-38.

[4] S. C. Piscitelli, L.H. Danziger, K.A. Rodvold. "Clarithromycin and azithromycin: new macrolide antibiotics", *Clin Pharm* 1992; 11(2):137-152.

[5] L. Na, L. Jun-tian, Z. Qiang-zong. "Ellagic acid-induced hypercoagulable state in animals: a potentially useful animal hypercoagulable model for evaluation of anticoagulants", *Chin Med Sci J*, 2010 Dec; 25(4):237-242.

[6] I.Y. Zaghloul, Z. H. Aly, M. A. Radwan. "Effect of ciprofloxacin on the hypoprothrombinemia activity of warfarin in rats", *Int J Pharm* 1996; 131(2):131-136.

[7] S. O. F. Diniz, C. F. Siqueira, D. L. Nelson, J. M. Martin-Comin, V. N. Cardoso. "Technetium-99 m ceftizoxime kit preparation", *Braz Arch Biol Technol* 2005; 48(spe2):89-96.

[8] J. A. Retsema, A. E. Girard, D. Girard, W. B. Milisen. "Relationship of high tissue concentrations of azithromycin to bactericidal activity and efficacy in vivo", *J Antimicrob Chemother* 1990; 25; Suppl A:83-89.

[9] E. Yourassowsky, M. P. van der Linden, M. J. Lismont, F. Crokaert, Y. Glupczynski. "Rate of bactericidal activity for Branhamella catarrhalis of a new macrolide, CP-62,993, compared with that of amoxicillin-clavulanic acid" *Chemotherapy*, 1988;34(3):191-4.

[10] A. E. Girard, D. Girard, A. R. English, et al. "Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution", *Antimicrob Agents Chemother*. 1987;31(12):1948-1954.

[11] R. M. Shepard, F. C. Falkner. Pharmacokinetics of azithromycin in rats and dogs. *J Antimicrob Chemother*, 1990 Jan;25 Suppl A:49-60.

[12] G. Foulds, R. M. Shepard, R. B. Johnson. The pharmacokinetics of azithromycin in human serum and tissues. *J Antimicrob Chemother*, 1990 Jan;25 Suppl A:73-82.

[13] J. Hirsh, J. E. Dalen, D. Deykin, L. Poller, H. Bussey. Oral anticoagulants; Mechanism of action. Clinical Effectiveness and optimal therapeutic range. *Chest* 1995; 108(4); Suppl:231S-246S.

[14] K. R. Wu. Hemostatics in: Chen Q editor Methodology in pharmacological study on Chinese materia medica. 1st ed. Beijing: People's Medical Publishing House, 1993. p. 484.

[15] C. Zoja, M. Noris, D. Corna, G. Viganò, N. Perico, G. de Gaetano, G. Remuzzi. "L-arginine, the precursor of nitric oxide, abolishes the effect of estrogens on bleeding time in experimental uremia", *Lab Invest* 1991; 65(4):479-483.

[16] W. H. Holms. "Viable bacterial counts of bacteria - a new method or facultative anaerobic bacteria", *J Gen Microbiol* 1968; 54(2):255-260.

[17] International Committee for Standardization in Haematology. Recommendations for reference method for hemoglobinometry in human blood (ICSH standard EP 6/2:1977) and specifications for international hemiglobincyanide reference preparation (ICSH standard EP 6/3:1977). *J Clin Pathol* 1978; 31(2):139-143.

[18] L.-O. Hansson, L. Lindquist. "Immunology and infectious diseases". *Curr Opin Infect Dis* 1997; 10(3):196-201. Chetana Vaishnavi.

[19] P. C. Taylor. "Anti-TNF therapy for rheumatoid arthritis and other inflammatory diseases", *Mol Biotechnol* 2001; 19(2):153-168. Review.

[20] G. Snedcor and W. Cochan. "Statistical methods", 1989; 6 th. Lowa state university press, lowa, USA.

[21] K. L. McCall, H. G. Anderson Jr, A. D. Jones. "Determination of the lack of a drug interaction between azithromycin and warfarin", *Pharmacotherapy* 2004 Feb; 24(2):188-194.

[22] K. A. Mergenhagen, P. M. Olbrych, A. Mattappallil, M. P. Krajewski, M. C. Ott. "Effect of azithromycin on anticoagulation-related outcomes in geriatric patients receiving warfarin", *Clin Ther* 2013; 35(4):425-430.

[23] N. P. Clark, T. Delate, C. S. Riggs, D. Pharm et al. "Warfarin interactions with antibiotics in the ambulatory care setting", *JAMA Intern Med* 2014; 174(3):409-416.

[24] D. R. Foster, N. L. Milan. "Potential interaction between azithromycin and warfarin", *Pharmacotherapy* 1999; 19(7):902-908.

[25] G. Lane. "Increased hypoprothrombinemic effect of warfarin possibly induced by azithromycin", *Ann Pharmacother* 1996; 30(7-8):884-885.

[26] E. Seamans. 2018 Antibiotic and anticoagulation: watching warfarin Levels. The Neuriva Brain Health Challenge is sponsored by RB Health. Also, (Azithromycin [prescribing information]. New York, NY: Pfizer Labs; 2017.

[27] A. M. Holbrook, D. Pharm, J. A. Pereira, R. Labiris, H. McDonald, J. D. Douketis, M. Crowther, P. S. Wells. "Systematic overview of warfarin and its drug and food interactions", *Arch Intern Med* 2005; 165(10):1095-1106.

[28] W. Ageno, A. G. Turpie. "Exaggerated initial response to warfarin following heart valve replacement", *Am J Cardiol* 1999 Oct 15; 84(8):905-908.

[29] K. B. Rao, M. Pallaki, S. R. Tolbert, T. R. Hornick. "Enhanced hypoprothrombinemia with warfarin due to azithromycin", *Ann Pharmacother* 2004; 38(6):982-985.