ANALYSIS

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Assessment of *Spinacia oleracea* extract on the reversal of Edoxaban anticoagulation development in a rabbit model

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ABSTRACT

Background: The disadvantage of Edoxaban use is the availability of definitive antidotes with many unfavorable effects. *Objectives:* This study was planned to determine whether the leaves of *Spinacia oleracea* ethanol extract can promising the effect of prothrombin complex concentrate (PCC) in the reversal of the anticoagulation development of Edoxaban. *Methods:* Anesthetized rabbits (n=5) were divided in to following groups, Group I: Normal Saline (negative control); other groups treated with pretreatment of 01 mg/kg Edoxaban followed by Group II: Normal Saline; Group III: 50 IU kg^{-1} PCC; Group IV: *Spinacia oleracea* leaves ethanol extract test doses of 150 mg/kg + 50 IU kg^{-1} PCC. After a standardized kidney incision, the volume of blood loss and time to

hemostasis was determined. *Conclusion:* Leaves of *Spinacia oleracea* may reduce the prothrombin time and blood loss and potentiate the effect of prothrombin complex concentrate in reversing Edoxaban. The additional probe is warranted.

Keywords: Edoxaban, oral anticoagulant, reversal, vitamin K, prothrombin complex concentrate

1. INTRODUCTION

Spinacia oleracea is commonly called as Spinach a leafy green flowering plant indigenous to central and western Asia belongs to family Amaranthaceae. Its leaves are a common edible, may be eaten cooked or raw. A quantity of 3.5 ounces of spinach contains over four times the recommended daily intake of vitamin K. For this reason, individuals taking the anticoagulant warfarin, which acts by inhibiting vitamin K are instructed to minimize consumption of spinach (as well as other dark green leafy vegetables) to avoid blunting the effect of warfarin. The uses of direct-acting oral anticoagulants are common without documented reversal agents to minimize the life-threatening adverse consequence. Our aim of this study to assess the *Spinacia oleracea* leaves extract which is source of vitamin k on reversing the effect of Edoxaban anticoagulant by boosting the effect of prothrombin complex concentrate (Gijsbers et al., 1996; Pedersen et al., 1991; Schurgers et al., 2004; Kamao et al., 2007; Booth 2012; Rustandi et al., 1990; Franchini et al., 2010; Almarshad et al., 2018).

2. METHODS

Animal Groups

Anesthetized rabbits (n=5) were divided in to following groups, Group I: Normal Saline (negative control); other groups treated with pretreatment of 01 mg/kg Edoxaban followed by Group II: Normal Saline; Group III: 50 IU kg-1 PCC; Group IV: *Spinacia oleracea* leaves ethanol extract test doses of 150 mg/kg + 50 IU kg-1 PCC. After a standardized kidney incision, the volume of blood loss and time to hemostasis were determined.

Animals

Female CHB rabbits, 3–4 months old, weighing 2.6–3.2 kg were housed individually in wire steel cages at 21–23 0C and 50% relative humidity under a 12 h/12 h light-darkness cycle. The animals had free access to tap water and were fed rabbit pellets *ad libitum*. The rabbits received care in compliance with the European Convention on Animal Care, and the study was approved by the institutional ethical committee of College of Medicine at Shaqra, Saudi Arabia (Approval Number: SUCOM/LIRB/2019-05).

Endpoints

Prothrombin time (PT) as Surrogate markers of bleeding diathesis served as secondary endpoints. In this open-label, placebo-controlled rabbit study, and the primary endpoints were the volume of blood loss and time to hemostasis up to 30 min following a standardized kidney incision injury (Pragst et al., 2012).

Drugs

Edoxaban (Savaysa, Daiichi Sankyo), prothrombin complex concentrate (Kcentra, CSL Behring), Ketamine (Delphis Pharma), Xylazine (Xylamed, Bimeda), Ethanol (Merk Ethanol)

Dose-finding

Edoxaban (Daiichi Sankyo) was reconstituted in-vehicle solution and administered to rabbits as a 1 mg/kg intravenous (IV) bolus injection over approximately 30 to 60 seconds into the marginal ear vein. The edoxaban dose was selected based on data from previous rabbit pharmacokinetic studies that determined the dose required to cause approximately a 2-fold increase in blood loss compared with vehicle. The edoxaban vehicle solution was composed of 90%/8%/2% v/v PEG 300/H20/DMSO (Lu et al., 2018).

Plant Material

The fresh leaves of *Spinacia oleracea* were collected from the farm area and authenticated by the Botanist of the Institution and sample specimen were stored for further reference (specimen number FMA-255).

Anesthesia

5 mg kg-1 i. v. ketamine and 0.5 mg kg-1 i. v. xylazine, 2% was used for induction of anesthesia. Inhaled anesthesia was maintained with isoflurane. After a 20-min stabilization period, a carotid artery catheter was used to make measurements of hemodynamic, coagulation and hematological parameters (Pragst et al., 2012; Lu et al., 2018).

Kidney incision

When 5 min had elapsed after PCC infusion, a standardized kidney injury was created in the form of a 15-mm long and 5-mm deep scalpel incision at the lateral kidney pole. The 30- min observation period for blood loss and time to hemostasis began immediately after the incision. Blood samples were collected at baseline, prior to PCC infusion, just before kidney incision and at the end of the 30-min observation period (Pragst et al., 2012; Lu et al., 2018; Godier et al., 2012; Eerenberg et al., 2011; Pragst et al., 2010).

Measurements

Assays of PT were performed with a Schnitger & Gross coagulometer (Heinrich Amelung GmbH, Lemgo, Germany) using the Thromborel (Dade Behring, Marburg, Germany) reagents. Blood loss was measured as the volume of blood collected from the kidney incision site with a syringe. Time to hemostasis was recorded as the interval from the kidney incision until the cessation of observable bleeding or oozing (Pragst et al., 2012; Lu et al., 2018; Godier et al., 2012; Eerenberg et al., 2011; Pragst et al., 2010).

3. RESULTS

Edoxaban dose selection

Edoxaban doses in *vivo* progressively extinguished thrombin generation. Nearly maximal inhibition was observed at the 01 mg/kg Edoxaban dose. That finding is consistent with results from a rabbit model of venous thrombosis, in which dabigatran was shown to exhibit approximately maximal inhibition of clot formation at a 01 mg/kg dose.

Laboratory measurements of coagulation

PT at 10 min ranged from 8.2 to 14.1 s in the negative control group. PT was prolonged in all animals receiving 01 mg/kg Edoxaban plus saline. There was a decrease in PT in group IV containing *Spinacia oleracea* ethanol extract test doses of 150 mg/kg + 50 IU kg-1 PCC as compared to group III containing 50 IU kg-1 PCC (Figure 1).

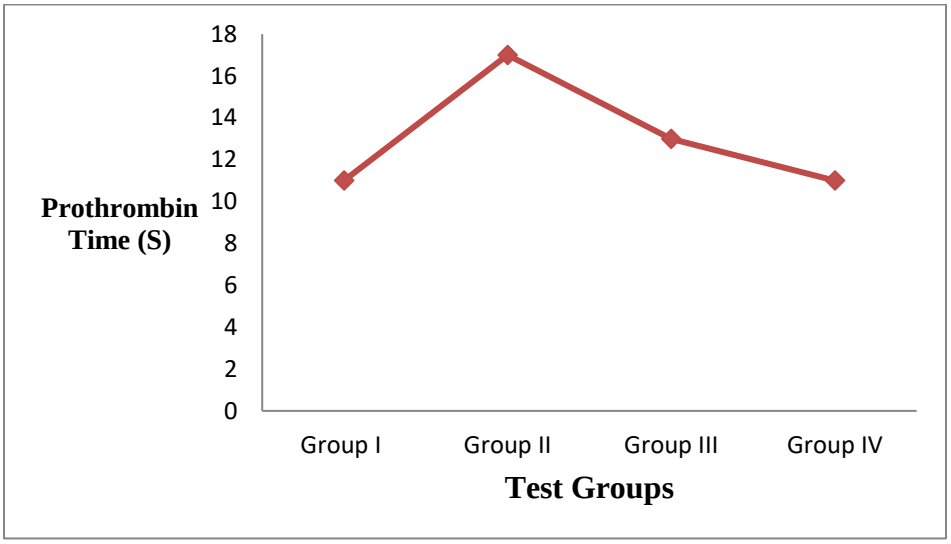

Figure 1 Effect of Test groups on Prothrombin time

Blood loss

In the negative control group receiving no normal saline, blood loss during the 30-min period after kidney incision ranged from 1.5 to 7.4 ml. In all animals treated with Edoxaban and 5 min thereafter with saline, blood loss within the 30-min observation period was higher, ranging from 14 to 43 ml. There was a decrease in blood loss in group IV containing *Spinacia oleracea* ethanol extract test doses of 150 mg/kg + 50 IU kg-1 PCC as compared to group III containing 50 IU kg-1 PCC (Figure 2).

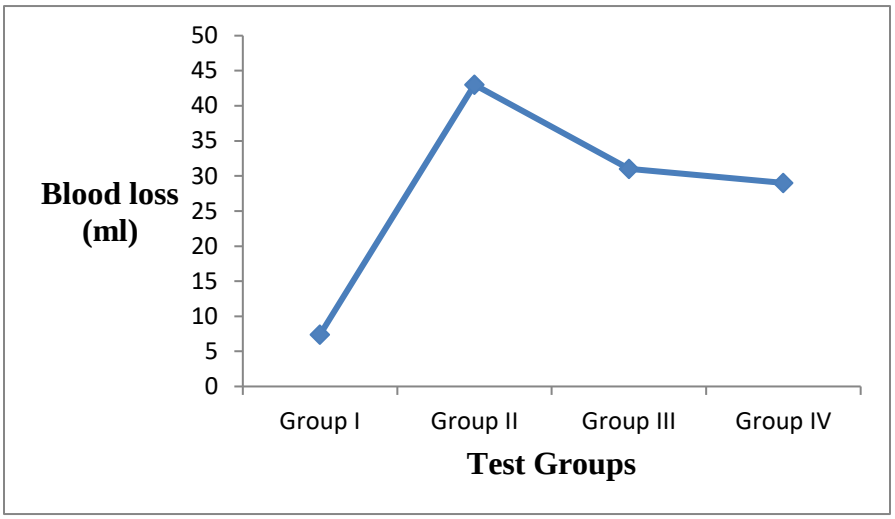

Figure 2 Effects of test groups on blood loss (ml)

4. DISCUSSION

Vitamin K is the reversal of oral anticoagulants that are widely used for the treatment and prophylaxis of thromboembolic disease. Vitamin K is an essential micronutrient. The clinical effectiveness of OACs derives from their ability to block posttranslational γ-carboxylation of the 4 vitamin K–dependent pro-coagulants (II, VII, IX, and X). Hence treatment with OACs results in the production of dysfunctional, under-carboxylated species of coagulation factors (Hirsh et al., 2003; Furie et al., 1999). Our previous study indicated the ethanolic extract the leaves of *Spinacia oleracea* with the combination of prothrombin complex concentrate potentiate the effect prothrombin complex concentrate and therefore reduces the prothrombin time and blood loss on the reversal of Apixaban anticoagulation in rabbit model (Almarshad et al., 2019). Our aim of the study was to compare the reversal effect of PCC alone and with the combination of ethanol extract of *Spinacia oleracea* leaves and PCC by using standard Edoxaban as an anticoagulant. There was a reduction of PT and blood loss in a group where ethanol extract of *Spinacia oleracea* leaves is combined with PCC as compared to PCC alone.

5. CONCLUSION

Leaves of *Spinacia oleracea* may reduce the prothrombin time and blood loss and potentiate the effect of prothrombin complex concentrate in reversing Edoxaban anticoagulation. The additional probe is warranted.

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Conflicts of Interest: The authors declare no conflict of interest.

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