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Possible Pharmacological Basis for Antithrombotic Effect of Glycyrrhiza *Glabra* in Sprague Dawley Rats

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ABSTRACT
It has been previously identified that 3-aryl comarin derivative, GU-7 isolated from Liquorice posses Antiplatelet activity. It inhibits platelet aggregation by increasing intraplatelet cyclic AMP concentration. Here we report the in-vivo effects of extract of *Glycyrrhiza glabra* and also the combined effect with Vitamin K and Heparin. Extract of *Glycyrrhiza glabra* increased the bleeding time when given in the doses of 180 mg/kg and 360 mg/kg. Blood loss was evaluated 60 minute later as a function of absorbance at 540 nm due to hemoglobin content in water solution. Altogether data indicates that *Glycyrrhiza glabra* is an effective anti thrombotic agent in vivo, which may account for its known pharmacological properties.

Keywords: *Glycyrrhiza glabra*, Thrombin, Venous thrombosis, Rats.

INTRODUCTION

Thrombin is a 308 amino acid protease that cleaves peptide bonds in selective substrate including fibrinogen, f V, f VIII & f XIII. Thrombin also participates in platelet, endothelial cell & leukocyte activation. [1-2] Beside this it acts as a catalyst for converting fibrinogen to fibrin, which subsequently cross links to from the mesh that creates a thrombus. [3] Thrombin is a serine protease with high substrate specificity. [4] Therefore the pivotal role of thrombin in the pathogenesis of these diseases makes this enzyme the main target for antithrombotics.

Antithrombin

Antithrombin (AT) is one of the most important physiological inhibitors of science proteases involved in blood coagulation. Inhibition of thrombosis has become a key therapeutic strategy. While unfractionated Heparin remains the most commonly used antithrombotic agent Thrombin surface possess two positively charged regions named exosites that play key roles in the specificity of thrombin towards macromolecules substrate such as fibrinogen, cofactors and some inhibitors such as Hirudin peptide or Heparin

Plant Profile

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Glycyrrhiza *glabra* is a plant of family leguminosae. Aqueous extract was prepared from the root of this plant. It contains many important constituents which include Coumarins Flavanoids Terpenoids:Volatile oils:Amino acids, Amines, Gums, Lignins, Starch, Sterols (beta sitosterol, stigmasterol), Sugars and Wax.

Glycyrrhiza *glabra* is known for its anti oestrogenic action, activity,

Antimicrobial activity Anti-viral activity and anti-ulcer activity. [5]

Objective

The present study was done to evaluate the in-vivo effects of extract of glycyrrhiza *glabra* on the bleeding effect and the combined effect of heparin and vitamin K.

MATERIAL AND METHOD

The powder of root of plant *Glycyrrhiza glabra* was provided as a gift sample from Baidyanath Ayurved Bhawan pvt. Ltd., Vitamin K was procured from glaxo smith klime. Other equipments used were UV spectrophotometer and pH meter. Jhansi. Aqueous extract of *Glycyrrhiza glabra* was taken out.

Solvent	Colour and consistency	% Extractive value
Distilled water	Black and pilular	12 %

Extract of *Glycyrrhiza Glabra* was dissolved in 0.05M NaOH at 37°C and pH was adjusted to 7.4 using 1M Hydrochloric Acid. For evaluation of the bleeding effect Wistar rats, were anaesthetized by using xylazin (16 mg/kg, intra muscularly) followed by ketamin, (100 mg/kg)

Eight groups of animals were employed in the present study and each group consists of five animals.

Control group (group 1): Rats of group 1 were treated with normal saline (1ml/I.V.), five minutes before the evaluation of bleeding effect.

***Glycyrrhiza glabra* extract treated group:** Rats of group 2 were treated with *Glycyrrhiza glabra* extract (180 mg/I.V.), five minutes before the evaluation of bleeding effect.

Vitamin K treated group: Rats of group 3 were treated with Vitamin k (1µg/kg /I.M.), five minutes before the evaluation of bleeding effect.

Heparin treated group: Rats of group 4 were treated with Heparin (85.714 IU/kg /I.V.), five minutes before the evaluation of bleeding effect.

Heparin + *Glycyrrhiza glabra* extract treated group: Rats of group 5 were treated with Heparin (85.714 IU/kg /I.V.), + *Glycyrrhiza glabra* extract (180 mg/I.V.), five minutes before the evaluation of bleeding effect

Heparin + Vitamin k: Rats of group 6 were treated with Heparin (85.714 IU/kg /I.V.), + Vitamin k (1µg/kg /I.M.), five minutes before the evaluation of bleeding effect.

Vitamin k + *Glycyrrhiza glabra*: Rats of group 7 were treated with Vitamin k (1µg/kg /I.M.) + Glycyrrhiza *glabra* extract (180 mg/I.V.), five minutes before the evaluation of bleeding effect.

Heparin + vitamin k + *Glycyrrhiza glabra*: Rats of group 8 were treated with Heparin (85.714 IU/kg /I.V.), + Vitamin k (1µg/kg /I.M.) + *Glycyrrhiza glabra* extract (180 mg/I.V.), five minutes before the evaluation of bleeding effect.

Bleeding Time: After five minutes of drug administration the rat-tail was cut 3mm from the tip. The tail was carefully immersed in 40 ml of distilled water at room temperature. Blood loss was evaluated 60 minute later as a function of absorbance at 540 nm due to hemoglobin content in water solution. The absorbance detected for a control group that received normal saline instead of *Glycyrrhiza glabra* was taken as a normal blood loss.

RESULT

In order to determine whether *Glycyrrhiza glabra* could exert its anti-thrombotic activity by increasing its bleeding time we first ruled out the effect of solvent i.e. normal saline (Control Group). The bleeding effect of *Glycyrrhiza glabra* was assessed based on blood loss from a *rat* cut tail, after intra vascular administration of the drug it was observed that *Glycyrrhiza glabra* in the dose of 180 mg/kg body weight produce strong hemorrhagic effect, as showed by 2-4 fold enhancement of blood loss, as compared to control values.

Effect of normal saline on thrombosis (bleeding Effect): Rats treated with Normal saline produce mean λmax - 0.238 (Table 1)

Effect of *Glycyrrhiza glabra* extract on thrombosis (bleeding Effect): *Glycyrrhiza glabra* extract (180 mg/I.V.), treatment enhances the mean λmax as compared to normal saline. It indicates that *Glycyrrhiza glabra* extract reduces the viscosity of blood (Table 1) Effect of Vitamin K on thrombosis (bleeding Effect). Treatment with Vitamin k (1µg/kg /I.M.) reduces the mean λmax as compared to normal saline. It indicates that Vitamin k reduces the release of blood from tail vein (Table 1)

Effect of Heparin on thrombosis (bleeding Effect) Treatment with Heparin (85.714 IU/kg /I.V.) enhances the mean λmax as compared to normal saline. It indicates that Heparin increases the release of blood from tail vein (Table 1). Effect of Heparin + *Glycyrrhiza glabra* extract on thrombosis (Bleeding Effect). Treatment with Heparin (85.714 IU/kg /I.V.) + *Glycyrrhiza glabra* extract (180 mg/I.V.), enhances the mean λmax as compared to normal saline, Heparin, and *Glycyrrhiza glabra* extract. It indicates that *Glycyrrhiza glabra* extract potentiates the bleeding effect of heparin (Table 1) Effect of Heparin + Vitamin k (on thrombosis (Bleeding Effect). Treatment with vitamin k decreases the mean λmax in the animals previously treated with heparin it indicates that vitamin k reduces the release of blood from tail vein (Table 1). Effect of Vitamin k+ *Glycyrrhiza glabra* extract on thrombosis (Bleeding Effect). Treatment with *Glycyrrhiza glabra* extract enhances the mean λmax in the animals previosly treated with vitamin K it indicates that *Glycyrrhiza glabra* extract overcome the reduced blood flow (Table 1). Effect of Heparin + vitamin k + *Glycyrrhiza glabra* extract on thrombosis (bleeding Effect). Glycyrrhiza glabra extract enhances the mean λmax in the animals previosly treated with vitamin K and heparin in the group 5 it indicates that *Glycyrrhiza glabra* extract potentiate the effect of heparin and also reduces the effect of vitamin K (Table 1).

Table 1:

AVERAGE BODY WEIGHT (gm)	COMPOUND	DOSE	MEAN λmax.
151	NORMAL SALINE	1ml (0.9%)	0.238
152	GLYCYRRHIZA *GLABRA*	180mg/kg	0.450
151	VITAMIN K	1µg/kg	0.203
149	HEPARIN	85.714 (IU/kg)	0.658
148	HEPARIN + GLYCYRRHIZA *GLABRA*	85.714 IU/kg + 180mg/kg	1.363
149	HEPARIN + VITAMIN K	85.714 IU/kg + 1µg/kg	0.341
151	VITAMIN K + GLYCYRRHIZA *GLABRA*	1µg/kg + 180mg/kg	0.631
150	HEPARIN + VITAMIN K + GLYCYRRHIZA *GLABRA*	85.714 IU/kg + 1µg/kg + 180mg/kg	0.937

DISCUSSION

Present work show that *Glycyrrhiza glabra* exhibits significant antithrombitic activity *in vivo*. *Glycyrrhiza glabra* was able to increase the bleeding when given intravenously to rats. A common feature between glycyrrhizin and Heparin is the presence of glucouronic acid units in their structures. At this point the ability of glycyrrhizin to potentiate the inhibitory action of serpins towards thrombin has never been examined. Though distinct mechanism of action, Heparin greatly enhances the inhibitory effect of the serpins anti thrombin III and Heparin cofactor II towards thrombin. Since both glycyrrhizin and Heparin contain glucouronic acid units in their structures, we further attempted to identify a Heparin like anticoagulant activity for glycyrrhizin. Glycyrrhizin does not require the presence of antithrombin for its action, confirming a direct effect on thrombin exosite I. The use of *Glycyrrhiza glabra* as a anti coagulant drug should not be

followed in a view of the broad specificity of this molecule. That GL, provokes a significant bleeding effect, which is a great disadvantage for the indication of GL, as a drug and also when compared with other effective thrombin exosite inhibitors. Rather we believe that GL structure might be used as a model for searching new antithrombotic drugs. More ever a number of studies have described the involvement of thrombin in leukocyte function and cancer metastasis. At this point, although there are no reports of haemostatic changes due to GL administration, it is possible that its mild effect towards coagulation system, especially towards thrombin, is responsible for some of its known biological studies.

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