IN VITRO THROMBOLYTIC / FIBRINOLYTIC EFFECTS OF RUE AQUEOUS DISTILLED EXTRACT

Sabih M. Jawad Jafar

Department of Chemistry, College of Science, Baghdad University.

E mail: sabhmjj@yahoo.com

Abstract

Ruta graveolens showed several biological and pharmacological actions. In vivo, its effect on the circulating blood is secondary rather than primary. The aim of this work was to study its effect on human blood clot, in vitro experimental model. This work was done in Department of Chemistry, College of Science, Baghdad University in Baghdad, Iraq. Aqueous distilled extract of Rue was prepared by simple distillation and scanned by UV-Visible spectrophotometer. The ability of this extract to generate peroxynitrite was investigated in vitro experimental mode. The effect of 100 μL of 1% Rue extract was tested on human blood clot prepared from five healthy volunteers. The extract was added either before the clot is formed or after it. UV-visible spectra of aqueous Rue extract showed the presence of single peak at 195nm. Rue extract enable to generate peroxynitrite in a concentration dependent manner (r = 0.98). It is significantly reduced the formed clot size by 12.534 ± 5.674% while the simultaneous administration of blood and aqueous extract resulted in reduction of clot size by 4.772 ± 2.207%. Rue aqueous distilled extract has thrombolytic and fibrinolytic effect by the evidence of clot lysis. This effect may be related to donation of nitric oxide.

Introduction

The hemostatic system helps to maintain the integrity of the circulatory system after severe vascular injury, whether traumatic or surgical in origin. Part of these events, in any patient, is stimulation of clot breakdown (fibrinolysis), which may become pathological (hyperfibrinolysis) in some (1).

The common name of Ruta graveolens L. is Rue. All parts of the plant contain the active principles although they are mostly encountered in leaves especially before blooming. The most frequent international use of Rue has been for induction of abortion. Methyl-nonyl-ketones, one of Rue active substances, induced uterine contractions and pelvic congestion leading to uterine hemorrhage and possibly abortion in pregnancy(2).

Several substances were isolated from Rue including rutin (glycoside), frucocoumarins, alkaloids (quinolones), tannin and essential oils. Extracts of Rue proved experimentally to have several beneficial pharmacological actions including antibacterial (3-5), antifungal (6), antihelmintic, antiparasitic(7), antitumor (8), anti-inflammatory (9), and antiandrogenic (10). Also it showed several toxic effects including dermal photosensitization(11,12), cytotoxicity (5), antifertility (13), mutagenicity (14) and abortifacient effect (15).

Its effect on blood, due to its content of coumarin derivatives, is secondary rather than primary. As a result of its toxic effect on the liver, it caused coagulation disorders.

Recently, there is an evidence that peroxynitrite, a molecule formed from nitric oxide and superoxide, decreased the activity of tissue plasminogen activator, i.e., antifibrinolysis (16).

The aim of this study is to investigate the distilled aqueous extract of Rue leaves on human blood clot, in vitro experimental model, in an attempt to show whether such extract has fibrinolytic activity in relation to the peroxynitrite.

Experimental

This study was conducted at Department of Chemistry, College of Science, Baghdad University in Baghdad, Iraq. After obtaining permission from the local ethics committee
and informed consent, five healthy male volunteers were allocated randomly from the college students.

Materials
All the chemicals used in this work were of analar grade. Commercially available *Ruta graveolens* L. leaves was obtained from the north of Iraq.

Herbal preparation
Aqueous extract of *Ruta graveolens* L. leaves was prepared by simple distillation. In brief, one gram of dried *Rue* leaves in 100 mL distilled water (1%) was heated, the vapors separated and recondensed them to obtain a clear extract liquid that was more concentrated in the more volatile components.

UV-visible spectra
UV-visible spectra was recorded on a Aquarius (Cecil series with scanning ability) spectrophotometer (France). The spectra of distilled aqueous *Rue* extract (1%) was measured from 150-900nm at room temperature with a 10 mm path length quartz cell with a scan rate of 600 nm/min.

Determination of peroxynitrite
Peroxynitrite levels in the distilled aqueous extract of *Rue* was determined according to the method described by Beckman et al (1992)(17) cited by VanUffelen et al (1998)(18). Peroxynitrite mediated nitration of phenol, resulting in nitrophenol formation, formed the basis of the peroxynitrite assay.

In brief, serial dilutions of 1% distilled aqueous *Rue* extract were placed in a glass test tubes and then 5 mM phenol in 50 mM sodium phosphate buffer to a final volume of 2 mL were added, mixed well and incubated for 2 hours at 37 C°. Then the reaction was stopped by addition 15 μL of 0.1M sodium hydroxide, mixed and immediately recorded the absorbance of the samples at 412 nm.

Clot lysis
Venous blood samples (3 mL each) were drawn from five healthy human male volunteers. 500 μL of blood was transferred to each of four previously weighed Eppendorff tubes for each subject. In the first series, the transferred 500 μL allowed to form clots at 37 C° for 45 minutes (19). After clot formation, serum was completely removed and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - weight of the tube alone). To each Eppendorff containing pre-weighed clot, 100 μL of distilled aqueous *Rue* extract or 100 μL distilled water as a negative control were added. All the tubes were then incubated at 37 C° for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot stabilization. The obtained difference in weight was expressed as percentage of stabled or lysed clot. In the second series of experiments, simultaneous addition of 500 uL blood and 100 uL either distilled water or distilled aqueous *Rue* extract, incubated at 37 C° for 45 minutes. The obtained clot weight was determined as above.

Statistical analysis
Data are expressed as mean ± SE of number of experiments (n=5). The significance (p < 0.05) between % clot weight changes induced by distilled water or *Rue* extract was tested by paired and unpaired one tailed "t" test.

Results
UV-visible spectra of aqueous *Rue* extract showed the presence of one peak at 195nm with optic density 0.141 Fig.(1). Fig.(2) showed that *Rue* extract had the ability of generation peroxynitrite in a concentration dependent manner (r = 0.98). One hundred microliters of *Rue* extract, the volume that was used in clot lysis experiments, generated 15.9 uM of peroxynitrite.

Distilled water did not show any significant lytic effect on the formed clot. The clot weight is decreased by 0.229 ± 0.447 %. Aqueous extract of *Rue* was significantly (p=0.0495) showed thrombolytic effect. It reduced the clot size by 12.534 ± 5.674%. The difference between distilled water and aqueous extract of *Rue* reached to the level of significant (p = 0.05) Fig.(3). The simultaneous administration of blood and aqueous extract of *Rue* reached to the level of significant (p = 0.05) Fig.(3). The simultaneous administration of blood and aqueous extract resulted in reduction of clot size by 4.772 ± 2.207% as compared with the effect of distilled water. Such effect was approximated the level of significant (p = 0.06) Fig.(3).
Fig.(1) : UV-Visible spectra of aqueous distilled extract of Rue.

Fig.(2) : The relationship between the volume of aqueous Rue extract and the formation of peroxynitrite.
Discussion

The present results clearly show that aqueous extract of Rue has thrombolytic and/or fibrinolytic effect(s) as it reduces the clot weight. Its effect seems to be solely related to the active substance demonstrated by UV-visible spectra.

Although the detected active substance generates peroxynitrite but its effect on the clot seems to be not related to peroxynitritite i.e. exerts direct effect. Its fibrinolytic and/or thrombolytic effect(s) is reported here for the first time. Ethanolic extract of Ruta chalepenis had significant inhibitory effect on the number of circulating red cells in mice (20).

Few herbal medicines exert thrombolytic or fibrinolytic effects like Fangonia Arabica (Dhamasa) (21), Artmisiae folium (Gaiyoh) (22), Hemidesmus indicus (23), and garlic (24).

Some plants/extracts products exert their thrombolytic or fibrinolytic effects via their content of certain fibrinolytic proteases enzymes like Pleurotus ostreatus (25), Spirodel polyrhiza (26) and others. The thrombolytic/fibrinolytic effect(s) of Rue in this work is not related to the effect of proteases enzymes because the aqueous distilled extract was used not crude solvent extract.

The interesting finding in this study is the concentration dependent formation of peroxynitrite by Rue aqueous distilled extract. This means that Rue extract behaves as nitric oxide donor, since peroxynitrite is the result of non enzymatic reaction of nitric oxide and superoxide anion in solution. Therefore, the effect of Rue extract on clot lysis may be related to the vital role of nitric oxide on the blood platelets (27).

In conclusion, Rue aqueous distilled extract shows thrombolytic and fibrinolytic effect by the evidence of clot lysis. Further study is recommended to identify the chemical structure of its active ingredient and to elucidate the exact mechanism of action. Also it is recommended to study the Rue extract as nitric oxide donor.

References


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الخلاصة

تتولى عشبة السذاب العديد من الفعالية البيولوجية والدوائية. ويبدو أن تأثيرها في جهاز الدوران داخل الجسم الحي ثانوي وليس أوليا أي بصورة مباشرة. تهدف هذه الدراسة إلى معرفة عملها في تحلل الدم البشري خارج الجسم الحي. تم تحضير المستخلص المائي المعمق للعشبة بوساطة عملية التقطير البسيط وإجراء مسح طيفي للمحلول بوساطة المطياف الضوئي المرئي فوق الأشعة البنفسجية. كما تم التجربة على قابلية المستخلص في توليد بيروكسي نتروت في أنموذج خارج الجسم الحي. تم اختبار فعالية المستخلص (100 ميكرومتر من 1%) في خثرة الدم البشري المحضر من خمسة متطوعين أصحاء، حيث أضيف المستخلص قبل أو بعد تكون الخثرة. أظهرت نتائج المسح الطيفي وجود ذروة مفردة عند طول موجي 195 نانومتر. كما واستطاع المستخلص من توليد بيروكسي نتروت بتراكيز تزايدت بتزايد حجوم المستخلص المستخدم. عمل المستخلص على تقلص حجم الخثرة بدرجة نوعية متميزة بمقدار 12.534 ± 5.674% عند إضافة المستخلص بعد تكوين الخثرة بينما عمل على تقلص تكوين الخثرة بمقدار 4.772 ± 2.207% عند إضافته قبل تكوين الخثرة.

يستنتج من ذلك أن المستخلص صفة تحلل الخثرة وتحلل الفابرين بدالا حصول تحلل الخثرة. ومن المحتمل أن يزيد هذا التأثير إلى قابلية المستخلص على ويب أوكسيد النترك.