PharmacologyOnLine
April 30, 2012
Special ISSUE • 2012 • vol.1 • 124 - 127

Anti-platelet activity of infusion of *Tithonia diversifolia*'s leaves

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Abstract

Tithonia diversifolia, also known as "Mexican arnica", has been used in traditional medicine to treat inflammatory refractory with absence of citotoxicity. The possible health risks associated with the consumption of ingestion of the infusion (tea) plant makes it is necessary to identify the potential pharmacological activity or toxicity to prove certain plants that are acclimated in Brazil. Considering the limited number of pharmacological studies regarding the *Tithonia diversifolia*, the aim of this study was evaluate the effects of this infusion in platelet aggregation. Venous blood was collected with informed consent from healthy volunteers who denied taking any medication in the previous 14 days. Whole blood was transferred into polypropylene tubes containing one-tenth of final volume of acid citrate dextrose (ACD-C; citric acid 3%, trisodium citrate 4%, glucose 2%; 1:9 v/v) and centrifuged at 200g for 15 min. Platelet rich plasma was added of wash buffer solution (NaCl 140mM, KCl 5mM, sodium citrate 12mM, glucose 10mM and saccharose 12mM; pH 6; 5:7 v/v) and centrifuged at 800g for 12 min at 20°C. Platelet pellet was gently resuspended in Krebs–Ringer solution and counts were performed on a Neubauer chamber. Aggregation assay was carried out with 400 µL of platelet suspension ($1.2x10^8$ platelets/mL) in a cuvette at 37°C with constant stirring. Platelet suspension was incubated for 3 min with aqueous extract infusion (0.6-20µg/mL) prior to addition of thrombin (100 mU/mL). Percentage of platelet aggregation was recorded with an aggregometer (Chrono-log Lumi-Aggregometer model 560-Ca, USA). Our results show an inhibition of thrombin induced platelet aggregation in the presence of 0.6-20 ug/mL *Tithonia diversifolia* infusion leaves. The *Tithonia diversifolia* infusion leaves inhibits thrombin induced washed platelet aggregation.

Keyword: Tithonia diversifolia; Aqueous extract and Platelet aggregation

Introduction

An extract of *T. diversifolia* has been traditionally used for the treatment of a variety of pathologies. Pharmacological studies of *T. diversifolia* showed that it has anti-diabetic, anti-malarial, anti-inflammatory, analgesic and cancer chemopreventive activity, some of which account for the folkloric claims of this medicinal plant (1).

The role of platelets is well established in primary hemostasis by promoting thrombus formation and vessel wall repair. Antiplatelet drugs are used in stroke prevention and management of coronary syndrome. Besides, increased platelet activation is reported to participate in many pathological conditions, for instance in atherosclerosis (2), diabetes (3), tumor metastasis (4), asthma (5) and other types of inflammation. Therefore, the study of anti-aggregating platelet agents represents new perspectives of therapeutic intervention in platelet- and platelet-correlated disorders.

Platelets are activated by agonists such as thrombin, adenosine diphosphate (ADP) and thromboxane (TXA_2). Thrombin occupancy to protein G-coupled receptor in platelet plasma membrane activate phospholipase Cβ (PLCβ) resulting in the formation of IP_3 and diacyl glycerol, leading to an elevation of free cytoplasmic [Ca^{2+}] and activation of protein kinase C (PKC), respectively (Offermans, 2006). These events induce TXA_2 generation, by aracdonic acid-cyclooxygenase 1 (COX-1) pathway, reorganization of the cytoskeleton and GPIIb/IIIa integrin activation, bridging platelets to each other, culminating in platelet aggregates formation (6).

In the pharmaceutical market there are few drugs that can be used in platelet disorders. Extracts that have this purpose are of commercial interest. Another important aspect is the drug, infusions with antiplatelet activity may have interaction with other drugs that can be used by patients.

Materials and methods

Materials

The leaves of *Tithonia diversifolia* Helms. (A Gray) was collected from adjoining areas of Paulista State University (UNESP – Araraquara), in May, 2011, and was authenticated by carrying out macroscopic and microscopic evaluation.

Volunteers

Venous blood was collected with informed consent from healthy volunteers who denied taking any medication in the previous 14 days.

Preparation of the leaves extract

The infusion was prepared with 15g of fresh leaves and this crude aqueous extract was used for pharmacological platelet aggregation.

Platelet aggregation

Venous blood was collected with informed consent from healthy volunteers who denied taking any medication in the previous 14 days. Whole blood was transferred into polypropylene tubes containing one-tenth of final volume of acid citrate dextrose (ACD-C; citric acid 3%, trisodium citrate 4%, glucose 2%; 1:9 v/v) and centrifuged at 200g for 15 min. Platelet rich plasma was added of wash buffer solution (NaCl 140mM, KCl 5mM, sodium citrate 12mM, glucose 10mM and saccharose 12mM; pH 6; 5:7 v/v) and centrifuged at 800g for 12 min at 20°C. Platelet pellet was gently resuspended in Krebs–Ringer solution and counts were performed on a Neubauer chamber. Aggregation assay was carried out with 400 µL of platelet suspension ($1.2x10^8$ platelets/mL) in a cuvette at 37°C with constant stirring. Platelet suspension was incubated for 3 min with aqueous extract infusion (0.6-20µg/mL) prior to addition of thrombin (100 mU/mL). Percentage of platelet aggregation was recorded with an aggregometer (Chrono-log Lumi-Aggregometer model 560-Ca, USA).

Statistical Analysis

All data are shown as means±SD. A One-way ANOVA (and non parametric) test was used for data analysis and P < 0,05 significantly different for samples.

Results

Our results show an inhibition of thrombin induced platelet aggregation in the presence of 0.6-20 ug/mL *Tithonia diversifolia* infusion leaves.

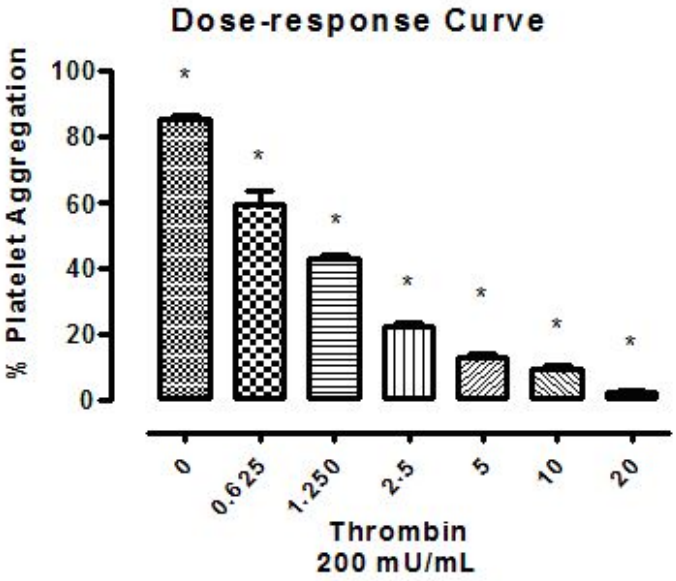

Figure 1. Dose response curve for inhibition of platelet aggregation for different concentrations of infusion of T. diversifolia (* P <0.05).

Discussion

The antiplatelet potential was investigated in human blood against platelet aggregation induced by agonist thrombin. This study attempts to explore the aqueous extract of *T. diversifolia*.

Thrombin was used to induce platelet aggregation, because it plays an important role in maintaining homeostasis. Thrombin (activated Factor II) is a protein-type serine protease (EC 3.4.21.5) and therefore acts breaking down proteins in certain locations. Its main function is to convert fibrinogen to fibrin (protein filaments) making role in the coagulation process (7).

Thrombin in addition to transforming fibrinogen to fibrin, it also slows down the coagulation process (probably, so this does not occur wildly). Genetic factors such as Factor V Leiden can interfere directly in this system of self-control. In these cases the inefficiency of this self-coagulation (via protein C) may cause a hypercoagulability blood, favoring the formation of thrombi (clots) in veins, whose clinical condition is thrombosis (8).

Because thrombin considered the central mediator of thrombogenesis (hypothesis of thrombin) it is believed that inhibition of its activity and / or its receptor should interrupt thrombus formation and reduce the vascular injury (9).

Our results showed that the infusion of *T. diversifolia* was able to inhibit platelet aggregation at very low concentrations. The phytochemical studies reveal that extracts of *T. diversifolia* are composed of sesquiterpenes such as Tagitinina C, one of the active ingredients most isolated and identified from the aerial parts of the plant (10). A study performed with sesquiterpenes obtained from the essential oil of the stems of *L. martiniano*, showed that this substance was able to inhibit platelet aggregation in 37.0% (11). However, the inhibition mechanism of action remains unknown.

The potential infusion of *T. diversifolia* stimulates further tests to determine the mechanism of action and effective dose to keep up with in vivo testing.

Acknoledgments

Financial Support: FAPESP, CNPq, CAPES.

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