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Effect of two sesquiterpene lactones from *Capparis decidua* (Forsk.) on arachidonic acid and adenosine diphosphate-induced platelets aggregation

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Abstract

Variable responses among individual patients, in addition to adverse effects are the main challenges facing most commonly used antiplatelet therapies like aspirin for example. Therefore, there is a need to seek naturally occurring, plant-derived substances which show minimal side effects. *Capparis decidua* is an indigenous shrub widely distributed in desert and semi-desert area of northern and central Sudan. Phytochemical investigation of aerial parts of *Capparis decidua* resulted in isolation of two new sesquiterpine lactones: MW-6 (germacr-3β-ol-7,9-dien-6,14-olide-15-oic acid) and MW-11 (Germacr-3β-ol-12-ene-6,14-olide-15-oic acid) along with several known compounds. The two compounds have shown potent antiplatelets activity evaluated by using of guinea-pig platelets rich plasma mode. These compounds may provide a chemical moiety for more potent analogues.

Keywords: Sesquiterpene lactones, *Capparis decidua*, Anti platelets..

Introduction

Platelets are central component of both homeostatic and thrombotic processes. Haemostatic plug that normally limits blood loss during injury are formed through regulated interaction between platelets, vascular walls and plasma proteins.[1] Three categories of substances are involved in the regulation of platelet function. The first group represents substances generated outside the platelet and interacts with platelet membrane receptors, e.g, catecholamines. The second one involved agents that formed within the platelet and interact with membrane receptors, eg, adenosine diphosphate (ADP), while, the third group comprises substances produced within the platelet and act within the platelet, eg, thromboxane A2 (TXA2).[2]

Several agents have been identified in vivo as activators of platelet aggregation; one of them is TXA2. So Platelets function will be impair if this agent and/or their receptor is defective.[3,4]

Arachidonic acid converted in platelets to potent aggregating agent TXA2 via both COX-1 and thromboxane A2 synthase enzyme. ADP also converted to the same agent, but via thromboxane A2 synthase enzyme only.[3]

Many plants having platelet inhibitory effects[5] due to present of bioactive secondary metabolites such as: flavonoids, polyphenols, alkaloids and carotenoids.[6,7]

Capparis decidua (Family: Capparidaceae) is a xerophytic shrub[8], it is widely distributed in desert and semi-desert area of northern and central Sudan especially on sandy soils and in low rainfall savanna on clays.[9] It is also found in Blue Nile, Upper Nile, western and eastern Sudan as well as northern areas of the country.[10] Various names have been attributed to *Capparis decidua*, In Sudan it is locally known as Al Tundub.[11] It has been widely used in folk medicines to cure various illnesses. Biological studies reveal important antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral properties.[12]

Variable responses among individual patients, in addition to adverse effects are the main challenges facing most commonly used antiplatelet therapies like aspirin for example. Therefore, there is a need to seek naturally occurring, plant-derived substances which show minimal side effects.

Materials and Methods

Plant materials

Capparis decidua (aerial parts) was collected from Shambat, Khartoum north-Sudan. The plant was authenticated at the Medicinal and Aromatic Plant Research Institute (MAPRI), Sudan and voucher specimens deposited in the Herbarium at the pharmacognosy department- Faculty of Pharmacy-University of Khartoum.

Animals

Albino guinea-pigs were used in this study. The animals were obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh. The animals were housed under constant temperature (22 ± 2ºC) and light/dark cycle (12/12 h).

Extraction and isolation of the two Sesquiterpene lactones

Collected plant was dried under shade and then powdered. The powdered plant material (200 g) was extracted using cold maceration method with sufficient quantity of 80% methanol at room temperature for 48 hour. The process of extraction was repeated twice to complete extraction. The extract was filtered using Whatman filter paper and the filtrates were concentrated under reduced pressure which afforded 39 g of a concentrated extract.

Methanolic extract of the plant was subjected to normal phase column chromatography for separation of compounds. Various factions have been collected and purified to get the crystals of pure compounds (MW-6 and MW-11). The chemical structure of the compounds was elucidated with the help of 500/125 MHz NMR using 1D and 2D spectral methods viz. ^{1}H and ^{13}C NMR, ESIMS and FABMS aided by IR spectroscopy and then confirmed by mass spectroscopic analysis.

Preparation of guinea-pig platelets rich plasma

The method used was similar to that described previously by El Tahir *et al*[13] except that guinea-pig was used instead of rats. In brief:

Albino guinea pigs were anaesthetized with ether and blood was obtained via cardiac puncture and mixed with 3.6% aqueous solution of trisodium citrate in a ratio of 1:9 (citrate blood). The blood was centrifuged at 1600 rpm for 10 minutes at room temperature and the platelets-rich plasma (PRP) was then separated, pooled and distributed into siliconized glass cuvettes in volumes of 0.45 ml. Platelets poor plasma (PPP) was obtained by further centrifuging 2 ml of the PRP. Aggregation was conducted using Bio/Data Corporation Aggregometer (Horsham, PA, USA).

Preparation of Arachidonic acid and ADP

The arachidonic acid and ADP used to induce aggregation were 40 and 20 µg respectively contained in a volume of 20 µl per ml PRP (i.e 20 and 10 µg/cuvette). Each sample of PRP was used once to induce control responses or to test the effect of the different doses of the tested compounds.

Anti-platelets activity

The anti-aggregatory effects of the tested compounds were studied by incubating the tested compounds with aliquots of PRP and stirred at 37ºC for 6 minutes, followed by the addition of the aggregating agent. The resulting aggregation was recorded for 6 min after the challenge, by the change in light transmission as a function of time. All test compounds were dissolved in PPP with excessive vortexing. The compounds were tested in doses of 0.1, 0.5 and 1 mg/ml PRP.

Results and Discussion

Platelets dysfunctions significantly contribute to the development and progression of cardiovascular diseases, so reducing platelet hyperactivity was linked to reduce incidence of primary and secondary coronary events related to cardiovascular diseases.[14,15]

Inhibition of platelet aggregation by blocking the ADP pathway considered as a target for development of new antiplatelet drugs which have no effect on prostaglandin metabolism.

Thromboxane A2 plays a vital role in platelet adhesion, to change their shape, release their granules, and aggregate, thus agents that antagonize this pathway will interfere with platelet aggregation in vitro and prolong the bleeding time in vivo.

The two sesquiterpene lactones MW-6 and MW-11 as shown in figure 1 (a and b) did not inhibit ADP-induced platelets aggregation, but they did inhibit Arachidonic acid induced aggregation in a dose dependent manner figure-2 (a,b and c). Figure-2(a) showed that the aggregation % of arachidonic acid alone is 37% (transmission of light increases with time) which considered as control test. On the other hand, the aggregation % of arachidonic acid after its addition to compound MW-11 and MW-6 (1 mg/ml PRP) was reduced to 1% and 10% respectively (figure 2(b) and 2(c), indicating that compound MW-11 in a dose of 1 mg/ml PRP inhibited arachidonic acid-induced aggregation by 97% whereas compound MW-6 inhibited the aggregation by 73%, The effective dose 50 (ED_{50}) values for compound MW-11 was found to be 0.47 mg/ml PRP (N=4) and that for MW-6 was 0.72 mg/ml PRP (N=4).

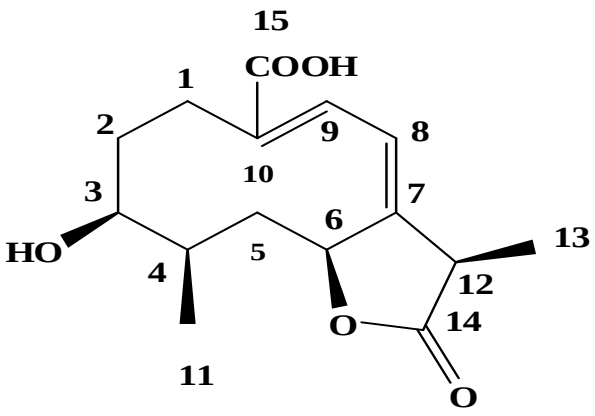

Figure 1(a): MW-6 (Germacr-3β-ol-7,9-dien-6,14-olide-15-oic acid)

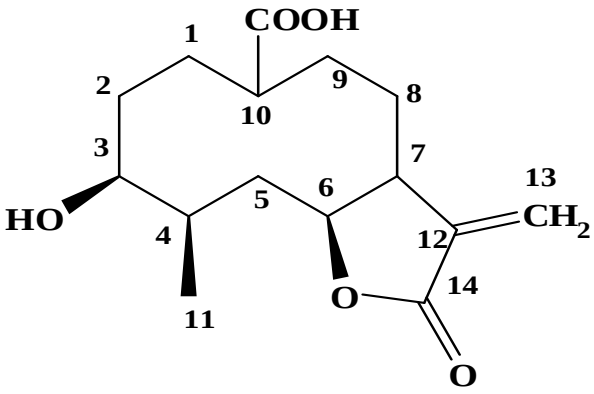

Figure 1(b): MW-11 (Germacr-3β-ol-12-ene-6,14-olide-15-oic acid)

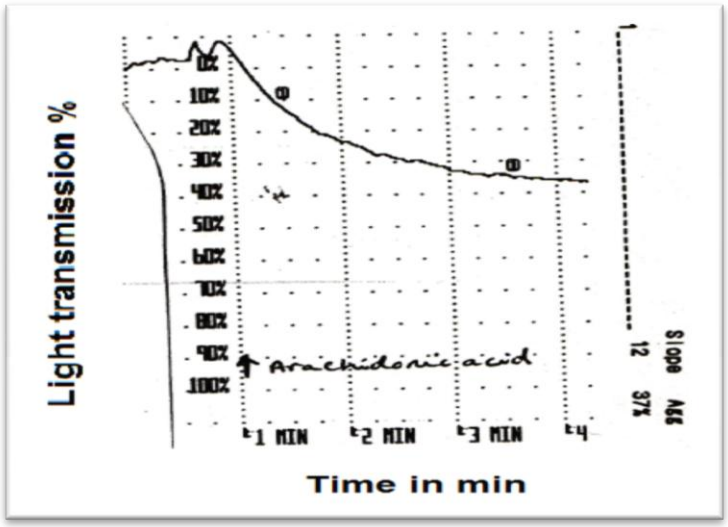

Figure 2(a)

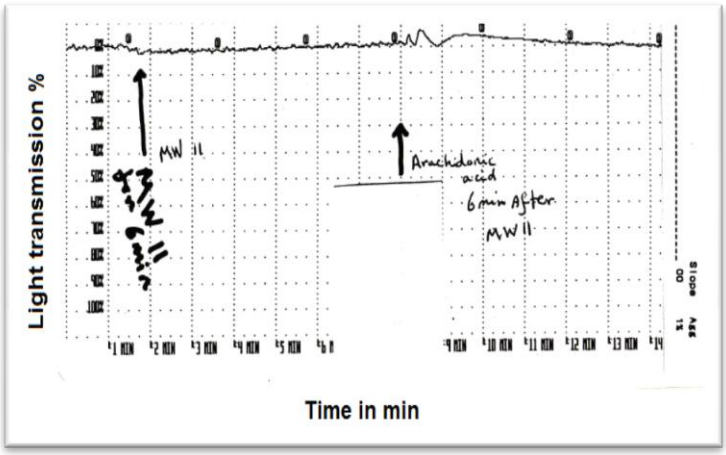

Figure 2(b)

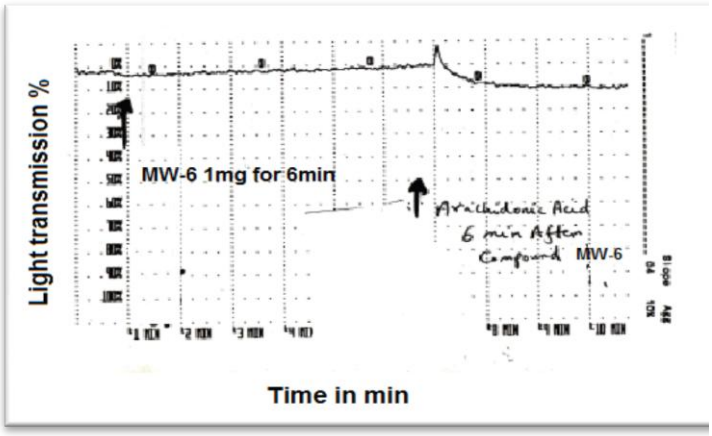

Figure 2(c)

The two compounds seemed to inhibit prostaglandin COX-1 and have no activity on TXA2 synthase enzyme. Therefore, the two compounds under investigation may be a good candidate for development of antiplatelet agents targeting the arachidonic acid cascade. However compound MW-11 has showed greater potency than compound MW-6.

Conclusion

The two compounds have shown potent antiplatelet activity, thus those compounds may provide a chemical moiety for more potent analogues.

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