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Article

Antiplatelet activity of white and pink *Nelumbo nucifera Gaertn* flowers

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Nelumbo nucifera Gaertn (Nelumbonaceae) a plant used in Ayurvedic medicine (common name: lotus), is a perennial, large and rhizomatous aquatic herb most prevalent in South India. Preliminary phytochemical screening of both white and pink *Nelumbo nucifera* flowers revealed the presence of phytochemical constituents (flavonoids, alkaloids, phenols etc,). Hence, an attempt has been made to screen the effect of *Nelumbo nucifera* flowers (both types) on platelet aggregation. The antiplatelet activity of hydroethanolic extract of both types of flowers was studied using platelet-rich plasma in different concentrations (100-500µg/ml). Both white and pink *Nelumbo nucifera* flower extracts showed dose-dependent effective antiplatelet activity with maximum activity at 500µg/ml concentration; prevention of platelet aggregation was 50% of that achieved with standard aspirin. Furthermore, the antiplatelet activity of white flowers was relatively high ($p<0.05$; ANOVA) compared to pink flowers. In conclusion, these observations suggest that both varieties of *Nelumbo nucifera* flower extracts exert different levels of inhibitory action on platelets *in vitro* (secretion and platelet aggregation suppression) due to differences in phytochemical content (alkaloids, flavonoids, phenols, tannins, phytosteroids, glycosides and saponins).

Uniterms: *Nelumbo nucifera* Gaertn./phytochemical constituents. *Nelumbo nucifera* Gaertn./pharmacognosy. *Nelumbo nucifera* Gaertn./Antiplatelet activity. Ayurvedic medicine. Natural products. Aspirin.

Nelumbo nucifera Gaertn (Nelumbonaceae, planta utilizada na medicina Ayurvédica, é erva aquática rizomatosa grande, predominante no sul da Índia. A triagem fitoquímica preliminar das flores brancas e cor-de-rosa de *Nelumbo nucifera* revelou a presença de constituintes fitoquímicos (flavonoides, alcaloides, fenóis etc). Assim, tentou-se a triagem do efeito das flores de *Nelumbo nucifera* de ambos os tipos na agregação plaquetária. A atividade antiplaquetária dos extratos hidroetanólico de ambos os tipos de flores foi estudada, utilizando-se plasma rico em plaquetas em duas diferentes concentrações (100 – 500 µg/mL). Tanto os extratos das flores brancas quanto daquelas de cor-de-rosa mostraram atividade antiplaquetária dose-dependente, com o máximo na concentração de 500 µg/mL. A prevenção da agregação plaquetária foi 50% daquela alcançada com o padrão de ácido acetilsalicílico. Além disso, a atividade antiplaquetária das flores brancas foi, relativamente, alta ($p<0,05$; ANOVA), comparativamente às flores cor-de-rosa. Estas observações sugerem que ambas as variedades de extratos de flores de *Nelumbo nucifera* exercem diferentes níveis de ação inibitória nas plaquetas *in vitro* (supressão da secreção e da agregação plaquetária) devido a diferentes constituintes fitoquímicos (alcaloides, flavonoides, fenóis, taninos, fitoesteróides, glicosídeos e saponinas).

Unitermos: *Nelumbo nucifera* Gaertn./constituintes fitoquímicos. *Nelumbo nucifera* Gaertn./farmacognosia. *Nelumbo nucifera* Gaertn./atividade antiplaquetária. Medicina Ayurvédica. Produtos naturais. Ácido acetilsalicílico.

INTRODUCTION

Platelets play a prominent role in homeostasis and thrombosis. The normal haemostatic system limits blood loss by regulated interactions between components of vessel wall, circulating blood platelets and plasma proteins (Saengkhae *et al.,* 2008). Platelets can adhere to the walls of the blood vessels, release bioreactive compounds and aggregate to each other. These properties increase to a well established level in conditions of arterial thrombosis and atherogenesis (Guyton, 1991).

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Several antiplatelet drugs have been developed to inhibit platelet activity in acute thrombotic situations as well as to prevent adverse events. Antiplatelet therapy is one of the most effective therapies for treatment of atherothrombotic disease. Considering the recently proved role of platelets in atherosclerosis the long-term use of antiplatelet drugs could also be postulated to slow down progression of the disease (Olas *et al.,* 2005). Therefore, the compounds that inhibit platelet function are of great interest, and it has also been shown that some plants, such as garlic and tomato may be beneficial in protecting against cardiovascular diseases as a result of platelet aggregation inhibition. Medicinal plants are rich in bioactive compounds (flavonoids, polyphenolic, vitamins and carotenoids) which inhibit platelet function (Olas *et al.*, 2005).

Nelumbo nucifera Gaertn (Nelumbonaceae), commonly known as Indian lotus, have been widely used in folk medicine for the treatment of various inflammatory and infectious diseases (Liu *et al.,* 2004). Studies using *in vitro* and *in vivo* models have demonstrated that this plant has antidiabetic, antipyretic, anti-inflammatory, anticancerous, antimicrobial, antiviral and anti-obesity properties (Kashiwada *et al.,* 2005). Furthermore, *Nelumbo nucifera* flowers are served as healthy beverages to treat hypertension, cancer, diarrhea, fever, weakness, infection and body heat imbalance (Saengkhae *et al.,* 2008).

The major constituents isolated from the lotus plant are alkaloids (liensinine, neferine, nuciferine, remrefidine and isoliensinine) and flavonoids ((+)-1(R)-coclaurine, (-)-1(S)-norcoclaurine and quercetin 3-O-b-D-glucuronide) (Sridhar and Rajeev, 2007). One of the most potent mechanisms by which flavonoids inhibit platelet aggregation is by mediating increase in platelet cyclic AMP levels by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase activity (Iman *et al.,* 2009). The composition of flavonoids, polyphenols, alkaloids and natural antioxidants present in pink and white flowers is completely different (Iman *et al.,* 2009). Therefore the present study was designed to evaluate the antiplatelet activity of hydroethanolic extract of white and pink *Nelumbo nucifera* flowers (phytochemical composition is different in both varieties) and to elucidate the inhibitory mechanisms of flavonoids in platelet aggregation. Furthermore, it is a well-known fact that many patients taking aspirin continue to have adverse cardiovascular events. One potential explanation for aspirin failure is variable response of individual patients to aspirin with consequent inadequate platelet inhibition. Previous studies have estimated that adequate anti-platelet effect is not achieved in 0.4 - 35% patients taking aspirin, leading to increased occurrence of cardiovascular diseases (Eikelboom *et al.*, 2002). Hence, there is a need to seek medicinal plants and their natural constituents which show minimal side effects. It is in this context the present study is carried out.

MATERIALS AND METHODS

Plant material

The flowers of *Nelumbo nucifera* were collected from different localities of Coimbatore District, Tamil Nadu in September 2008 and authenticated by the Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SC/5/23/09-10/Tech.279 MH) has been deposited at the Herbarium of the Botany department of "Tamil Nadu Agriculture University" for future reference.

Plant extraction

The air-dried and powdered flowers (100g each) were cold macerated with 50% ethanol for 3 days, with occasional stirring. After 3 days, the suspension was filtered through a fine muslin cloth and was evaporated to dryness at low temperature (< 40ºC) under reduced pressure in a rotary evaporator. Dark brown colored crystals of approximately 8g were stored in an air-tight dessicator and used for further analysis.

Phytochemical screening

Qualitative phytochemical screening of both white and pink *Nelumbo nucifera* flowers of different solvent extracts was carried out (Khandelwal, 2002) to test for the presence of secondary metabolites including alkaloids, flavonoids, phenols, tannins, steroids, glycosides and saponins.

Antiplatelet activity

Platelet-rich plasma and Tyrode buffer were used for the antiplatelet activity according to Iman *et al.* (2009). Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 22°C for 6min, at 400g. Platelets were adjusted to 3.0×10^{8} cell/ml with sterile saline. Tyrode buffer was prepared using sodium chloride 149mM, potassium chloride 2.6mM, sodium bicarbonate 9.5mM, glucose 5.5mM, sodium dihydrogen phosphate 0.5mM, magnesium chloride 0.6mM and gelatin 0.25%. The platelet-rich plasma 0.13×10^{-7} for each assay was resuspended in Tyrode buffer (pH adjusted to 7.4 with 0.25 M Hcl). Aggregation of the platelets was induced by

$CaCl_2$ at a final concentration of 2μM. Platelet aggregation was recorded by increasing transmittance value of spectrophotometric measurements. To determine the *in vitro* antiplatelet aggregation property, different concentrations (100, 200, 300, 400 and 500μg/ml) of plant extract were added to the platelet suspension for 1min exposure at 37ºC before treatment with platelet aggregating agents. Aspirin at 500μg/ml was used as a standard.

RESULTS AND DISCUSSION

Platelet dysfunction contributes to the development and progression of many cardiovascular diseases such as arterial hypertension, atherosclerosis and thrombosis. Indeed, it has been reported that patients with hypertension or coronary heart disease tend to have increased platelet reactivity. Therefore, many investigations were carried out toward the prevention of the abnormal hyperactivity of platelets reported in cardiovascular disorders employing different therapies, including use of medicinal plants (Massberg *et al.*, 2005). The objective of the present study was to determine whether both white and pink *Nelumbo nucifera* flower extracts would affect platelet function and blood coagulation.

The preliminary phytochemical screening is shown in Tables I and II. From the results it was evident that all the solvent extracts were found to contain selected analysed phytochemicals. When compared with different solvent extracts, hydroethanolic extract was found to contain major active phytoconstituents such as alkaloids, flavonoids, phenols, tannins, steroids, glycosides and saponins screened in both white and pink flowers of the plant. In white flowers, relatively higher amounts of phytochemicals were present (Tables 1 and II). In hydroethanolic extract (used in the present study) the phytochemicals are present in relatively higher amounts in white flowers. (Tables I and II). These results are in accordance with Babayi *et al.* (2004) who reported that the phytochemical analysis of the crude extracts of *Eucalyptus* revealed the presence of saponin, glycosides, steroid, tannins and phenols.

From Figures 1 and 2 it is evident that both white and pink *Nelumbo nucifera* flower extracts showed effective antiplatelet activity in a dose-dependent manner with maximum activity at 500μg/ml concentration. Furthermore, the antiplatelet activity of white flowers was relatively high ($p<0.05$; ANOVA) compared to the pink flowers. However, prevention of platelet aggregation was lower compared to standard aspirin at 500μg/ml concentration. The flavonoids present in hydroethanolic extract might have prevented the adhesion and aggregation of platelets besides release of cytoplasmic calcium that stimulates the release of ADP. These results are in accordance with those of other studies demonstrating that flavonoid compounds isolated from many plants, including the *Solidago* species, inhibit platelet aggregation (Koleckar *et al*., 2008).

There are numerous pathways leading to platelet aggregation. Prostaglandins (PG) and serotonin (5-HT) are considered the major chemical mediators of platelet aggregation (Ogawa *et al.,* 1998). The clinical study of platelet aggregation reveals that both lotus flower extracts

TABLE I - Phytochemical screening of white *Nelumbo nucifera* flowers in different solvent extracts

S.No	Extract	Alkaloids	Flavonoids	Phenols	Tannins	Steroids	Glycosides	Saponins
1.	50%Ethanol	++	++	++	+	+	++	–
2.	Benzene	+	++	+	+	–	+	–
3.	Chloroform	+	–	+	++	+	++	+
4.	Petroleum ether	++	+	–	+	+	–	+
5.	Water	+	+	–	–	+	–	+

TABLE II - Phytochemical screening of pink *Nelumbo nucifera* flowers in different solvent extracts

S.No	Extract	Alkaloids	Flavonoids	Phenols	Tannins	Steroids	Glycosides	Saponins
1.	50%Ethanol	+	+	+	+	+	+	–
2.	Benzene	+	–	+	+	–	+	–
3.	Chloroform	–	+	–	–	+	+	+
4.	Petroleum ether	–	+	–	–	+	–	–
5.	Water	+	+	–	–	–	–	–

+ Presence; – Absence

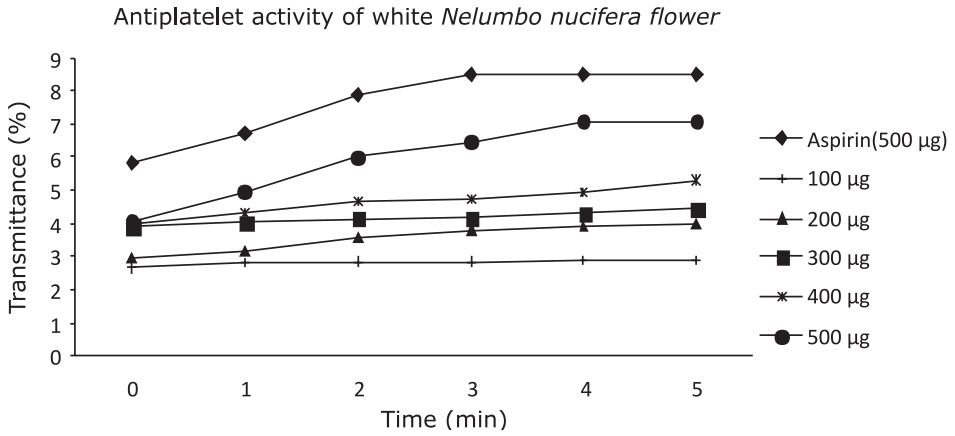

FIGURE 1 - Antiplatelet activity of hydroethanolic extract of white *Nelumbo nucifera* flower extract (♦) antiplatelet activity of Aspirin at 500 µg/mL; (+) antiplatelet activity of hydroethanolic extract of white flower extract at 100 µg/mL; (▲) antiplatelet activity of hydroethanolic extract of white flower extract at 200 µg/mL; (■) antiplatelet activity of hydroethanolic extract of white flower extract at 300 µg/mL; (∗) antiplatelet activity of hydroethanolic extract of white flower extract at 400 µg/mL; (●) antiplatelet activity of hydroethanolic extract of white flower extract at 500 µg/mL.

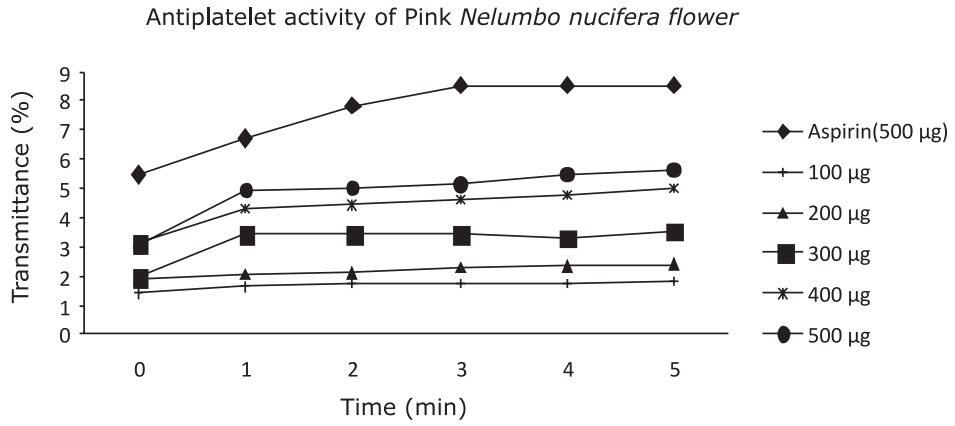

FIGURE 2 - Antiplatelet activity of hydroethanolic extract of pink *Nelumbo nucifera* flower extract (♦) antiplatelet activity of Aspirin at 500 µg/mL; (+) antiplatelet activity of hydroethanolic extract of pink flower extract at 100 µg/mL; (▲) antiplatelet activity of hydroethanolic extract of pink flower extract at 200 µg/mL; (■) antiplatelet activity of hydroethanolic extract of pink flower extract at 300 µg/mL; (∗) antiplatelet activity of hydroethanolic extract of pink flower extract at 400 µg/mL; (●) antiplatelet activity of hydroethanolic extract of pink flower extract at 500 µg/mL.

can either inhibit the PG synthesis pathway of 5-HT release. In addition, other studies have also demonstrated that saponins are effective in inhibiting platelet aggregation induced by ADP, by blocking the receptor-dependent Ca^{2+} influx of human platelets and/or inhibiting the production of leukotriene (Zeng *et al.,* 2004).

CONCLUSION

The obtained data indicated that the hydroethanolic extracts of both white and pink *Nelumbo nucifera* flowers possess potent antiplatelet activity limited to primary haemostasis in human blood. In addition, the antiplatelet activity of white flowers is relatively high compared to the pink flowers due to different composition of phytochemicals. However, further studies are needed to confirm the mode of action and efficacy of both *Nelumbo nucifera* flower extracts in platelet aggregation.

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