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Research Article

Antioxidant and Anti-inflammatory studies on the flowers of *Premna serratifolia* Linn.

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Abstract

Phytochemical, Antioxidant and in vitro anti-inflammatory activity of the methanolic extract of the flowers of Premna serratifolia were evaluated. The anti inflammatory activity of the flowers of Premna serratifolia was evaluated by HRBC membrane stabilization. Flower extract of different concentrations ranging from 5 µg/ml to 1000 µg/ml were subjected for the study. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity and the anti-inflammatory activity of the methanolic extract was comparable to that of the standard drug Indomethacin.The flowers of the plant showed potent antiinflammatory effect due to the presence of flavonoids. The flower extract also showed potent antioxidant activity.

Key words: Premna serratifolia, HRBC Membrane stabilisation, Indomethacin.

INTRODUCTION

Premna serratifolia Linn. (Verbanaceae) also known as Premna integrifolia is a small to medium sized tree and is one of the most widely used plant in the Ayurvedic system of medicine9. The Flowers are very small in size and greenish in colour. The plant is widely distributed along the coasts and islands of tropical and subtropical Asia, Africa, Australia and the Pacific. Premna serratifolia Linn, has cardiotonic [12], anticoagulant[13], anti hyperglycaemic[14], anti-parasitic[15], antioxidant[16] and antimicrobial[17] properties. Most of the plant parts of Premna serratifolia Linn., have been used in the traditional system of medicine in India to treat various infectious diseases.[11]

MATERIALS AND METHODS

Plant material

The Fresh flowers of *Premna serratifolia* were collected from the coastal area of Malpe beach of Udupi district of Karnataka state in January 2013. The plant material was identified and and a voucher specimen (PSERA-F) was deposited in the herbarium of the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram,Kanuur District, Kerala State.

Preparation of Methanolic Extract

The flowers were dried under shade and powdered. The powder was transfered to soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off and the extract was concentrated on a water bath to a dry residue [2].

ANTI-OXIDANT ACTIVITY

Nitric Oxide Scavenging Activity

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates NO_2, N_2O_4 and N_3O_4. It is estimated by

using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm.[3,10]

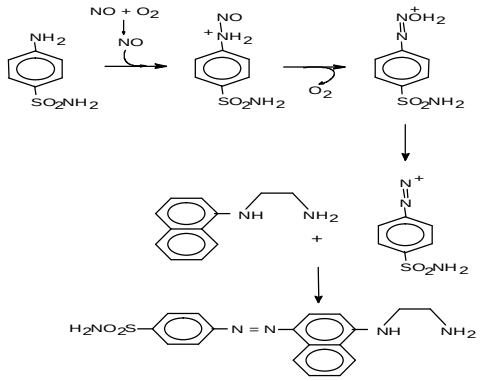

Fig 1 Griess reaction

Sodium nitroprusside 5Mm was prepared in phosphate buffer pH 7.4. to 1 ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25°C for 5hours. After that 0.5ml of Griess reagent was added. The absorbances were measured at 546 nm.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

ANTI-INFLAMMATORY ACTIVITY

The HRBC membrane stabilization has been used as a method to study the anti inflammatory activity. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution The blood was centrifuged at 4000 rpm and packed cells were washed with isosaline and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration ,1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacine was used as the reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37°c for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%.[1,8] The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

Percentage Protection = 100 – (Optical Density of the sample /Optical Density of the Control) x 10

RESULTS & DISCUSSIONS

Inflammation is a local response of living vasculaized tissues to endogenous and exogenous stimuli. The methanolic extract of the flowers of *Premna serratifolia* were subjected to erythrocyte membrane stabilization induced haemolysis by hypotonic solution. The inhibition of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity[8]. The anti inflammatory activity of the extract was found to be concentration dependent. It was observed from the table (3) that the methanolic extract shows significant anti inflammatory activity at the concentration of 300 mg/ml which is comparable to the standard drug Indomethacin. The lysosomal membrane and erytrocyte has many similarities and such the erythrocyte could be extrapolated to the stabilization of the lysosomal membrane.[4] The methanolic extract of the flowers also shows significant anti-oxidant activity. The phytochemical screening of the extract of the flowers revealed the presence of flavonoids, alkaloids, tannins and saponins. Flavonoids are well documented to have strong antioxidant activity and antiinflamatory activity [6,7]. The anti-inflammatory effect of the methanolic extract may be due to the presence of flavonoids and saponins.

CONCLUSION

The flower extract of the plant shows potent antiinflammatory and antioxidant effect. Both he antiinflammatory and antioxidant activity could be due the flavonoid content of the plant. Further studies on the isolation of individual phytoconstituents particularly flavonoids in methanolic fraction followed by its detailed studies on its pharmacologic profile is necessary.

Table 1
Phytochemical screening of the methanolic extract of the flowers of *Premna serratifolia*

Sl No	Active Ingradients	Methanolic Extract of the flowers of *Premna serratifolia*
1	Flavonoids	Present
2	Tannins (Phenolic Principles)	Present
3	Alkaloids	Present
4	Saponins	Present

Table 2
Effect of methanolic extract of the flowers of *Premna serratifolia* on Nitricoxide scavenging

Sl.No.	Conc. µg/ml	Methanolic Extract of the flowers of *Premna serratifolia*		Ascorbic acid (Reference Standard)	
		Absorbance	Percentage Scavenging.	Absorbance	Percentage Scavenging.
1	10	0.936	6.91	0.696	12.31
2	15	0.815	9.50	0.540	16.25
3	20	0.739	13.31	0.502	24.40
4	25	0.701	16.54	0.439	29.67
5	50	0.656	19.95	0.411	34.35
6	100	0.614	24.30	0.307	65.27
7	250	0.590	37.10	0.296	75.80
8	500	0.509	45.60	0.199	86.42
9	1000	0.312	59.05	0.102	98.33
10	Control	0.733		0.699	

Table 3
Anti inflammatory activity of the methanolic extract of the flowers of *Premna serratifolia* at various concentrations

Sl. No	Concentration mg/ml	Anti-inflammatory activity of the methanolic extract of the flowers of *Premna serratifolia*	
		Methanolic Extract of the flowers of *Premna serratifolia*	Indomethacin (STANDARD DRUG USED)
1	Control	-----	-----
2	100	69.41± 0.12	37.78± 0.05
3	150	72.35 ± 0.37	49.79 ± 0.29
4	200	78.56 ± 0.22	63.41 ± 0.01
5	250	89.65 ± 0.45	79.29 ± 0.11
6	300	97.30± 0.59	99.38± 0.19

(Values are expressed as SEM of 3 readings)

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