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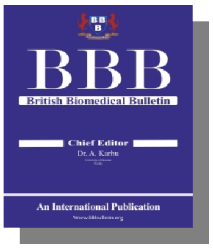

Original

Evaluation of *In vitro* Anti-inflammatory Activity of *Viburnum punctatum* Buch-Ham. Ex D.Don

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A B S T R A C T

The present study was to estimate the preliminary phytochemical screening and *in vitro* anti-oxidant activity of aerial part extracts of *Viburnum punctatum* by using solvents like Petroleum ether, Chloroform, Methanol and Water. Preliminary phytochemical analysis reveals the presence of alkaloids, glycosides, flavonoids, phenolic compounds, proteins, phytosterols and saponins. The chloroform and methanol extract were screened for its anti-inflammatory activity by cyclooxygenase inhibition and lipooxygenase inhibition on Human peripheral lymphocytes cell lines using Aspirin as a standard drug. The chloroform and methanol extracts of the *Viburnum punctatum* was taken at three different concentrations (100µg/ml, 500µg/ml, and 1000µg/ml) and its percentage inhibition was compared with that of the standard Aspirin. The methanolic extract showed good results with that of the standard and hence proved to have Cyclooxygenase and lipo-oxygenase inhibitory activity higher than that of the chloroform extract.

Introduction

Pain, heat, redness and swelling are the classic manifestation of the inflammatory process. Abnormalities of the joints of the spine, associate muscles, tendons, ligaments and bone structural abnormalities can all result in pain and need for neurosurgical consultation[1]. These pro inflammatory cytokines result in chemo attractant for neutrophils and help them to stick to, the endothelial cells for migration. They also stimulate white cell phagocytosis and the production of inflammatory lipid prostaglandin E_2.

Prostaglandins act as short lived localized hormones that can be released by any cell of the body during tissue, chemical, or traumatic injury and can induce fever, inflammation and pain, one they are present in the intercellular space. Thromboxones, which are hormone activators, can regulate blood vessel tore, platelet aggregation and clot formation to increase the inflammatory response[2,3].

The present study was taken up on the medicinal plant namely *Viburnum punctatum* belongs to the family Caprifoliaceae. It is shrubs or small trees, evergreen, to 9mm tall. It belongs to monotypic genus Viburnum, native to India, Indonesia, Bhutan, Cambodia, Nepal, Thailand, Vietnam and China. This species is not originally from North America, Asian Viburnum features dainty lymes of creamy white flower at the ends of the branches form early to mid-spring. It has dark green foliage throughout screen. The red fruits are held in abundance in spectacular clusters in mid-summer, expected to live for 40 years or more[4,5].

The leaves were traditionally used for the treatment for fever, stomach disorder and mentioned to possess antiperiodic effect. The preliminary phytochemical investigation shows presence of flavonoids, alkaloids, glycosides, phenolic compounds, phytosterols and saponins[6,7]. To our knowledge more report on the effect of this plant on experimental explanation. This study was therefore undertaken to evaluate the effect of aerial parts of *Viburnum punctatum* on Anti-inflammatory activity in COX inhibition and lipooxygenase inhibition methods using Aspirin as a standard.

Materials and Methods

Plant Material

Aerial parts of *Viburnum punctatum* were collected from Kalakkad-Mundenthurai, Thirunelveli in the month of June 2009. The plant was authenticated by Prof. V. Chellathurai, Former Professor, Govt. Siddha Medical College, Thirunelveli. A voucher specimen of *Viburnum punctatum* (DVCP/11/09) was deposited in the department of Pharmacognosy in the DVCP, Trivandrum for future reference. The plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 meshes and stored in an air tight and light resistant container for further use.

Preparation of Extract

The coarsely powdered aerial parts of *Viburnum punctatum* was first defatted with Petroleum ether using soxhlet apparatus. The extract was concentrated using rotary evaporator to get solid residue. The marc from the central compartment was removed, dried and successively extracted with a series of solvents of increasing polarity with soxhlet extractor was done. Solvents used with increasing polarity are Chloroform, Methanol and Water[8,9].

Reagents and Chemicals

Tris HCl (pH8), GSH, hemoglobin, arachidonic acid, TCA in HCl, thiobarbituric acid, sodium phosphate buffer, sodium linolente, EDTA, Plant Exratcts, LPS, Penicillin, Streptomycin, FBS, Aspirin.

Lymphocyte Culture Preparation

HPL'S was cultured in RPMI 1640 (HIMEDIA) media, supplemented with 20% heat inactivated FBS, antibiotics (Penicillin and Streptomycin). The culture was filtered using 0.2µm pore sized cellulose acetate filter (Sartorious) in completely aseptic conditions. Fresh plasma was aseptically added to the culture at a concentration of $1x10^6$cells/ml. The culture was then incubated for 72 hours. After 24 hours culture is activated by adding 1µl LPS. Incubation was done for 24 hours[10].

In vitro Anti-inflammatory Activity

Assay of Cyclooxygenase inhibition

A standard drug was added in the concentration of 100µg/ml from a stock of 100mg/ml and the sample was added in the concentration of 100µg/ml, 500µg/ml and 1000µg/ml from a stock of 100mg/ml incubated for 24 hours[12]. The isolation was done by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200ul of cell lysis buffer (1MTris-HCl, 0.25M EDTA, 2M Nacl, 0.5% Triton) was added. The incubation was done for 30 minutes at4C and anti-inflammatory assay was done in pellet suspended in a small amount of supernatant[11]. The assay mixture contained Tris-HCL buffer, glutathione, hemoglobin & enzyme. The Reaction was started by the addition of arachidonic acid and terminated after 20 min incubation at 37°C by addition of 0.2ml of 10% TCA in 1N HCL, mixed and 0.2ml of TBA was added and contents heated in a boiling water bath for 20 min, cooled and centrifuged at 1000rpm for 3 min. The supernatant was measured at 632nm for COX activity. The Percentage inhibition was calculated by using following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control-Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Assay of Lipoxygenase inhibition

A standard drug was added in the concentration of 100µg/ml from a stock of 100mg/ml and the sample was added in the concentration of 100µg/ml, 500µg/ml and 1000µg/ml from a stock of 100mg/ml incubated for 24 hours. The isolation was done by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200ul of cell lysis buffer (1MTris-HCl, 0.25M EDTA, 2M Nacl, 0.5% Triton) was added. The incubation was done for 30 minutes at4C and anti-inflammatory assay was done in pellet suspended in a small amount of supernatant.
70mg of linoleic acid and equal weight of tween 20 was dissolved in 4ml of oxygen free water and mixed back and forth with the a pipette avoiding air bubbles (Anthon E., 2001). Sufficient amount of 0.5N NaOH was added to yield a clear solution and then made up to 25ml using oxygen free water. This was divided into 0.5ml portions and flushed with nitrogen gas before closing and kept frozen until needed. The reaction was carried out in a quartz cuvette at 25°C with 1cm light path. The assay mixture contain 2.75ml tris buffer of pH 7.4, 0.2ml of sodium linoleate and 50µl of the enzyme. The increase in OD was measured at 234nm. The percentage inhibition was calculated by using following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control-Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

The data are expressed as mean ± SEM (n=3). Statistical significance was determined by one-way ANOVA followed by Dunnet's t test. At 95% confidence interval, p values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The extract obtained from chloroform as well as methanol was taken in various concentrations (100µg/ml, 500µg/ml, and 1000µg/ml) and its percentage inhibition was compared with standard drug aspirin which

was 93.51 as shown in Table 8.1. The percentage inhibition was closer to that of the standard than chloroform extract. Thus the methanolic extract was the appropriate one that showed anti-inflammatory cyclooxygenase activity[13].

As given in the Table 8.2 the chloroform and methanol extracts of the *Viburnum punctatum* was taken at three different concentrations (100µg/ml, 500µg/ml, 1000µg/ml) and its percentage inhibition was compared with that of the standard aspirin whose percentage inhibition was 76.74 and the methanolic extract showed to be the closest with that of the standard and hence proved to have lipo-oxygenase inhibitory activity higher than that of the chloroform extract[14].

CONCLUSION

The results of the anti-inflammatory activity studies showed methanolic extract to be exhibiting better anti-inflammatory activity than chloroform extract in both cyclooxygenase inhibition assay as well as lipooxygenase inhibition assay methods. So the methanolic extract can be subjected to further isolation to identify the potent phytochemical constituent responsible for exhibiting marked anti-inflammatory activity.

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Table 1. Effect of CEVP and MEVP on Cyclooxygenase Inhibition

Concentration (µg/ml)		Absorbance (at 632nm)	Percentage Inhibition
Control		0.185	---
Standard		0.012	93.51±1.12
Chloroform Extract	100	0.158	14.59±2.13
	500	0.143	22.70±1.21*
	1000	0.118	36.31±2.67
Methanol Extract	100	0.057	69.18±1.45**
	500	0.055	70.27±1.15*
	1000	0.030	83.78±2.19**

Statistical significance was determined by one-way ANOVA followed by Dunnet's t test. Values are mean ± SEM expressed as (n=3) p*<0.05, **<0.01, ***<0.001; as compared with control.

Table 2. Effect of CEVP and MEVP on Lipooxygenase Inhibition

Concentration (µg/ml)		Absorbance (at 234nm)	Percentage Inhibition
Control		0.043	---
Standard		0.010	76.74±1.09
Chloroform Extract	100	0.033	23.25±2.01
	500	0.029	32.55±2.79**
	1000	0.017	60.43±2.34*
Methanol Extract	100	0.030	30.23±0.98
	500	0.026	39.53±2.14*
	1000	0.015	65.11±2.09**