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Anti inflammatory activity of the whole plant of *Stachytarpheta cayennensis*

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Abstract

The present study was designed to assess the anti-inflammatory activity of the aqueous and ethanolic extracts of the whole plant of *Stachytarpheta cayennensis*. The studies were carried out by in-vitro HRBC membrane stabilisation method using different concentrations of aqueous (1000, 500, 250 mcg/ml) and ethanol (1000, 500, 250 mcg/ml) extracts. The results showed that both the extracts has got a significant anti-inflammatory activity on human red blood cells when compared with the standard drug diclophenac (50 mcg/ml) and distilled water as control.

Key-Words: *Stachytarpheta cayennensis*, anti-inflammatory, flavonoids, human red blood cells

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Introduction

Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property¹. The relation between inflammation & atherosclerosis, diabetes, cancer, arthritis and alzheimer's disease has been well substantiated².

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars.

On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations³.

Stachytarpheta cayennensis is a weedy distributed herbaceous plant from the Verbenaceae family commonly called Brazilian tea. Two common very similar species of *Stachytarpheta cayennensis* grow in the tropics and are used interchangeably (and share the same common names) in the herbal medicine systems of many countries. Ethnobotanically, *Stachytarpheta cayennensis* is used to treat various ailments such as, pain, fever, hepatic and renal disorder, helminthiasis, constipation, hypertension, stress and diabetes⁴.

Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes^{5,6,7}. It is well known that vitality of cells depends on the integrity of their membranes⁸. Exposure of RBC to injurious substances such as hypotonic medium, methyl salicylate or phenyl hydrazine results in the lysis of membrane accompanied by haemolysis and oxidation of haemoglobin^{9,10}. Drugs like anesthetics tranquilisers and non-steroidal anti-inflammatories stabilize erythrocytes against hypotonic haemolysis at low concentration¹¹. When the RBC is subjected to hypotonic stress the release of hemoglobin (Hb) from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So, the stabilization of HRBC membrane by drugs against hypotonicity induced haemolysis serves as a useful in vitro method

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for assessing the anti-inflammatory activity of various compounds¹².

Material and Methods

Fresh plants of *Stachytarpheta cayennensis* was collected from Pariyaram area (Kannur), during the month of October 2010. Plant was identified by Mr. Madhusaudanan Nambudiripad.T.A (MD), Dept. of Dravyagunavijnana, Govt. Ayurveda College, Pariyaram, Kannur. Voucher specimen (APSC/02/11) is preserved in Department of Pharmacology, ACPS, Pariyaram Medical College, Kannur. The plant extract was prepared after drying the collected plants for several days and the dried plants were pulverized to a coarse powder, sieved through no: 10 sieve¹³.

Methods of extraction

Ethyl alcohol extract

About 500g of shade dried powdered plants were extracted with 95% ethyl alcohol using Soxhlet apparatus. The extract was concentrated in vacuum to be syrupy consistency. The percentage yield of extract was found to be 10.65%.

Aqueous extract

About 200gm of dried powder was kept for maceration with 100ml distilled water for 24hrs. The extract was double filtrated by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation. These were stored in airtight containers in refrigerator below 10°C. Percentage yield was found to be 14.15%.

Anti-inflammatory activity

The HRBC membrane stabilization method is used to study the anti-inflammatory activity¹⁴. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in Table 2), 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclophenac (50mcg/ml) was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixture were incubated at 37 °C for 30min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%. The percentage of haemolysis was calculated.

Results and Discussion

Preliminary phytochemical screening reveals that ethyl alcohol extract contains steroids, alkaloids, terpenoids, flavonoids, tannins and polyphenols and aqueous extract contains carbohydrates, alkaloids, flavonoids, tannins and polyphenols as active secondary metabolites. The ethyl alcohol and aqueous extracts of whole plant of *Stachytarpheta cayennensis* were studied for in-vitro anti-inflammatory activity by HRBC membrane stabilization method. The inflammatory reaction involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair. Since HRBC membrane similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. Ethyl alcohol extract at a concentration of 1000mcg/ml showed 61.2% protection and aqueous extract shows 59.1% protection. Both are compared with diclophenac which shows 73.4% of protection.

All the extracts of the leaves of *Stachytarpheta cayennensis* showed biphasic effects on HRBC membrane stabilization. They increasing a activity at low concentration levels but decreasing activity with high concentration. The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The diclophenac drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane¹⁵.

The extract may inhibit the process which may stimulate or enhance the reflex of the intracellular components.

Table1: Phytochemical constituents of
Stachytarpheta cayennensis

Phytochemical constituents	Ethyl alcohol extract	Aqueous extract
Carbohydrate	+	+
Flavonoids	+	+
Steroids	+	-
Phenolic compounds	+	+
Terpenoids	+	-
Tannins	+	+
Alkaloids	+	+

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(+): Present (-): Absent

Table 2: In vitro anti-inflammatory activity of ethyl alcohol and aqueous extract of *Stachytarpheta cayennensis*

Treatment	Conc (mcg/ml)	Absorbance (540nm)	% inhibition
Control	-----	0.49±0.018	-----
Ethyl alcohol	1000	0.19±0.05	61.2
	500	0.22±0.08	55.1
	250	0.24±0.001	51.0
Aqueous	1000	0.20±0.02	59.1
	500	0.23±0.004	53.0
	250	0.25±0.002	49.9
Diclofenac	50	0.13±0.05	73.4

Values are expressed as mean ±SEM.n=6 animals in each groups

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