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INVITRO ANTI- INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF APOROSA LINDLEYANA LEAVES

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ABSTRACT

Aporosa lindleyana is a plant belonging to the family Euphorbiaceae, grows as small middle sized tree up to 15 m height, found throughout India. In the indigenous system of medicine the plant Aporosa lindleyana leaves have been claimed to be diuretic, roots to be used in insanity, head ache, fever, diabetes, loss of semen and liver diseases.[1] Traditionally, it is widely used in the treatment of inflammation and related disorders. However there is no scientific report available on its anti-inflammatory activity. Hence, the present study was designed to investigate the anti-inflammatory activity of ethanolic extracts of Aporosa lindleyana leaves by in vitro method. The preliminary phytochemical analysis of ethanolic extract showed the presence of many biologically active phytochemicals such as flavonoids, alkaloids, phenolic compounds, saponins, steroids and tannins and these compounds might be responsible for the anti-inflammatory properties. The possible anti-inflammatory mechanism of the Aporosa lindleyana leaf extract may be through its free radical scavenging activity, its hydrogen donating ability and increase antioxidants levels. Thereby, ethanolic leaf extract of Aporosa lindleyana preventing the spread of inflammation.

KEYWORDS: Aporosa *lindleyana,* alsevers solutions, DPPH assay.

INTRODUCTION

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances with fever usually presenting as one of its sequel. Inflammation underlies almost all disease conditions and it is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of cell injury (for example microbes and toxins) and the consequences of such injuries. Various medicinal plants provide relief from symptoms comparable to that obtained from

allopathic medicines. The majority of clinically important medicines belong to steroidal or non steroidal anti-inflammatory drugs. Though these drugs have potent activity, they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Hence, there is a need to develop safe and new anti-inflammatory agents with minimum side effects. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach.[2]

MATERIALS AND METHODS

Collection of Plant Material

Plant material of Aporosa lindleyana leaves were collected from surroundings fields of Pathanamthitta, Adoor taluk with the help of local farmers of the area. The plant was identified, confirmed and authenticated by comparing with an authentic specimen. Herbarium sheet was prepared and specimen was kept at library museum.

Preparation of plant extracts[3,4]

The collected plant was washed with distilled water to remove the dust and then was dried under shade. The shade-dried material was powdered by means of mechanical grinder and the powder was allowed to pass through sieve no. 60 for powder microscopy. The coarse powder was used for extraction. The suitably crushed plant material of Aporosa lindleyana leaves were placed in a closed vessel and ethanol i.e, (1:10) ratio were added. The system is allowed to stand for seven days, with occasional shaking. The liquid is then strained off and the solid residue is pressed to recover as much occluded solution as possible. The extract was filtered and used for the anti-inflammatory activity.

Chemicals and reagents

All chemicals and solvents were of analytical grade and were obtained from Merck chemicals.

Photochemical Evaluation

The total ethanolic extract of Aporosa lindleyana leaves was subjected to qualitative chemical test for identification of various organic constituents present in it.

INVITRO- ANTIOXIDANT ACTIVITY BY DPPH ASSAY METHOD[5]

- DPPH in methanol (0.1Mm) was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration. Thirty minutes later, the absorbance was measured at 517nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.
- The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{Percentage reduction} = \frac{[\text{control absorbance-test absorbance}] \times 100}{\text{control absorbance}}$$

Study Of Anti-Inflammatory Effects By Membrane Stabilizing Property.[6,7,8]

Procedure

Alsever solution prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water, and then the solution was sterilized. Blood was collected from median cubital vein of healthy volunteers. The collected blood was mixed with equal volume of sterilized alsever solution. The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline and a suspension in 10% (V/V) isosaline was made. Various concentrations of the ethanolic extract of Aporosa lindleyana were prepared in a mixture of 1ml phosphate buffer, 2ml hyposaline and 0.5ml HRBC suspension. Diclofenac sodium was used as the reference drug. Instead of hyposaline, 2ml of distilled water was used in control. The assay mixtures were incubated at 37ºC for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using UV analysis at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the equation.

Percentage inhibition of Haemolysis = 100 X OD1-OD2 /OD1

Where OD1 and OD2 are absorbance of diclofenac and ethanolic extract of Aporosa lindleyana respectively.

RESULT AND DISCUSSION

The results of the preliminary phytochemical screening of the ethanolic extract of Aporosa lindleyana leaves revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, carbohydrates, saponins, tannins and glycosides.

Table 1: Phytochemical screening.

Sl no.	Chemical constituents	Results
1	Carbohydrates	+
2	Proteins	-
3	Alkaloids	+
4	Glycosides	-
5	Tannins	+
6	Flavonoids	+
7	Steroids & triterpenes	+
8	Saponins	+

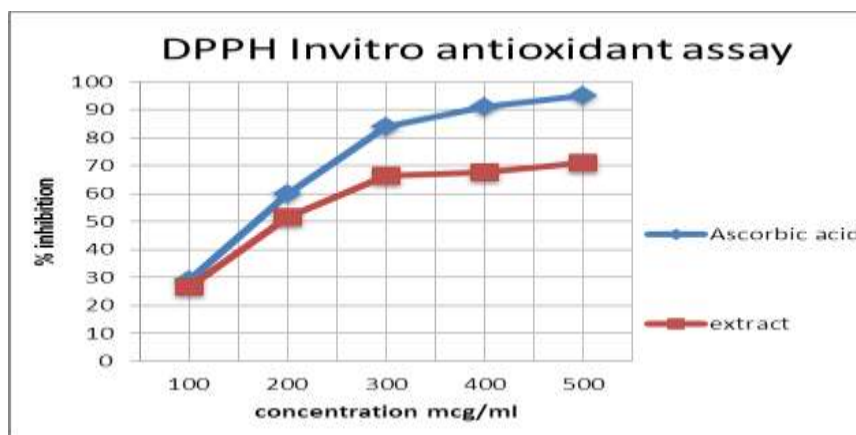

Figure 1: In vitro anti-oxidant activity.

The evaluation of antioxidant activity of plant extracts is considered as important step in the identification of their ability to scavenge the free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Results show that the ethanolic extracts of Aporosa lindleyana has antiradical activity by inhibiting DPPH radical with the IC_{50} value of 198mcg/ml which was comparable with ascorbic acid standard. IC_{50} value is the effective concentration at which the antioxidant activity is 50%. DPPH is usually used as a substrate to evaluate anti-oxidative activity of antioxidants. This method is based on the reduction of methanol DPPH solution in the presence of hydrogen donating antioxidant due to formation of the non radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to yellow colored diphenyl picryl hydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly hydroxy aromatic compounds (hydroquinone, pyrogallol, gallic acid etc) reduce and decolorize DPPH by their hydrogen donating ability. It appears that extracts of Aporosa lindleyana leaves possess hydrogen donating abilities to act as antioxidant.

Table 2: In vitro anti- inflammatory activity.

Sl no.	Drugs	% Prevention of lysis
1	25 mcg/ml	13.2 %
2	50 mcg/ml	15.8 %
3	75 mcg/ml	25.8 %
4	100 mcg/ml	35.9 %
5	Diclofenanc Sodium 100 mcg/ml	43.7 %

The main action of anti-inflammatory agents is the inhibition of cycloxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG). The main action of enzyme is conversion of prostaglandin G_2 (PGG_2) to PGH_2 along with peroxidation which is associated with formation of long channels in membranes. The channel opening occurs due to release of chemical mediatiors and so arachidonic acid is released from membrane and converted to prostaglandin. The extracellular activity of these enzymes is said to be related to acute and chronic inflammation. Non steroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (Cycloxygenase) or by stabilizing the lysosomal membrane. The extract at concentration range of 25-100 mcg /ml protects the human erythrocyte membrane against lysis induced by hypotonic solution. At concentration of 100 mcg/ml, the extract produced 35.9 % inhibition of RBC haemolysis as compared with 43.7 % produced by diclofenac sodium (Table 2). Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The results obtained demonstrate that ethanolic extract of Aporosa lindleyana leaves can significantly and dose-dependently inhibits RBC haemolysis (Table 2). It is well known that vitality of cells depends on the integrity of their membranes. 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. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to RBC membrane will render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This action is consistent with the observation that breakdown of bimolecules leads to the formation of free radicals which in turn enhance cellular damage. Extract with membrane-stabilizing properties are well known for their interfering activity with the early phase of the inflammatory mediators release, namely the prevention of phospholipases release that trigger the formation of inflammatory mediators.

CONCLUSION

In conclusion, the results of the present study indicate that ethanolic extract of Aporosa lindleyana leaves scavenging free radicals due to its polyphenolic compositions and inhibit RBC haemolysis by membrane stabilizing property. Present study validates the traditional use of Aporosa lindleyana leaves and shows significant anti- inflammatory activity. Further investigations are required to find active component of the extract and to confirm the mechanism of action.

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