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Evaluation of changes of lungs in asthmatic rats treated with aqueous and non aqueous extract of *clerodendrum serratum and theophylline*

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ABSTRACT

The aim of the present work is help the researchers for the development of an alternative methods rather than inhalers and oral anti asthmatic drugs for the treatment of asthma and COPD which will minimize the complication. Many plants obtained from the natural source play a significant role in the health care system. Literature survey on herbal drugs has shown significant anti asthmatic activity which has not shown any remarkable side effect. The pharmacological mechanism which the phyto constituents producing the anti asthmatic activity are not clearly understood till date. The several herbal formulation have derived from the Ayurveda, traditional system of Indian medicine and its additional system of medicine, yet to be scientifically validated that they have exhibited pharmacological action against Asthmatic. Only less number of scientific data of traditional medicines is available for the treatment of Asthmatic.

INTRODUCTION

Pharmacognosy has been basically evolved as an applied science pertaining to the study of all types of the drugs of natural origin. However, its subject matter is directed towards the modern allopathic medicine. During the course of development many civilizations has raised and perished but the systems of medicines developed by them in various parts of the world are still practised, and are also popular as the alternative system of medicines. These are alternative systems in the sense that modern allopathic system has been globally acclaimed as the principal system of the medicine, and so all the other systems prevent and practised in various parts of the world are supposed to be alternative systems. The philosophy and the basic principles of these so called alternative systems might differ significantly from each other, but the fact cannot be denied that these systems have served the humanity for the treatment and management of disease and also for maintenance of good health [1-5].

About 80 % of the world population still rely and use the medicines of traditional systems. Asthma is a hyper reactive airway disease running

a chronic course; it has worldwide prevalence and is a common cause hospitalization in children. It is estimated that currently 300 million peoples suffer from asthma with a possibility of an additional 100 million likely to suffer from the disease over the next 15-20 yea There are two types of asthma; 1. Allergic or extrinsic 2. Idiosyncratic or intrinsic. Allergic asthma is a result of an antigen/antibody reaction on mast cells in the respiratory tract. This type is often associated with a family history of atopy (allergic diseases) such as eczema. Idiosyncratic asthma or intrinsic asthma is a result of neurological imbalances in the autonomic system in which the system in which the sympathetic and parasympathetic systems are not properly coordinated. [6-10]

PATHOPHYSIOLOGY

Chronic inflammatory reaction is the characteristic features in the pathogenesis of bronchial asthma which results in intermittent airflow obstruction and bronchial hyper responsiveness. The airway obstruction in asthma is due to factors that includes bronchospasm oedema of the airway, increased mucus secretion, cellular infiltration of the airway walls and injury to the airway epithelium. Repetition of the inflammatory events in asthma can cause irreversible functional changes in the airway passages, a process called remodelling. The remodelled airway passages are persistently narrow as the disease progresses and become less and less responsive to drug treatment. The mechanism of inflammation in asthma may be acute, sub acute or chronic. [11-15]

Inflammatory reaction

An attack of asthma begins when the allergens is inhaled. The allergen binds to IgE antibodies that have binding side for the allergens mast cells in the lungs. Binding of allergen to IgE triggers exocytosis of the mast cells with the release of histamine leukotrienes. Leukotrienes cause the smooth muscle cells of the bronchi to contract, narrowing the lumen of the bronchi. This is the early phase. Next, they attract on accumulation of the inflammatory cells, especially eosinophils-leading to production of mucus. This is the late phase which occurs 4-6hrs after the early phase [16-20].

MATERIALS AND METHODS

Collection of plant

A novel approach to plant selection is a computerized selection method or literature information selection technique (LIST) that correlates biological activity, botanical facts and chemotoxanomical information using NAPRALERT database. Based on the literature review., the plant was collected from eastern part of the Ooty. *Clerodendrum serratum* is a small tree belonging to family verbenaceae. The plant was taxonomically identified by the botanist Dr.A.Balasubramanian, ABS Botanical Conservation, Research and Training Centre KAARIPATTI, SALEM -636003 T.N., INDIA.

Extraction of plant

The leaf of *Clerodendrum serratum* were shade dried at room temperature for 10 to 15 days. After shade drying of leaves, which was converted in to coarse powder form by using the mechanical mixer. Powdered leaf material was defatted using petroleum ether. Defatted plant material was extracted in soxhlet apparatus. Further extract with 90% of ethanol and concentrated by using desiccators for the removal of remaining moisture. The final amount of solid residue was 35% w/w.

Plant profile

Clerodendrum serratum Linn native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia, but some in the tropical Americas and northern Australia, and a few extending north into the temperate zone in eastern Asia. *Clerodendrum serratum* Linn (Verbenaceae).is a slightly woody shrub with blunty stems and branches. These trees are about 2-8 ft high. It is annual or perennial, usually aromatic.

Chemical constituents

Leaf extracts contains: Stigma sterol,spinasterol, Luteolin, Luteolin-7-0 glucuronide, Apigenin, Baicalin, Suctellarin 7-0 glucuronide

Traditional use

Clerodendrum serratum as effective treatment against asthma, bodyache, cholera, eye disorder, ulcers, snake-bite, wound, tuberculosis and epilepsy. It has antibacterial, antihistaminic, hepatoprotective, antipyretic, antinociceptive and anti-inflammatory. Root is pungent, bitter, acrid, dry, heating, anti-inflammatory, digestive, carminative, depurative, expectorant, antispasmodic, stimulant, appetizer and anthelmintic. Leaves are used in fever and hiccough. Its boiled leaves are used in cephalgia and opthalmia where as its boiled seeds in butter milk is used as aperients, in dropsy and in catarrhal affection of lungs (Shah, 2003). *Clerodendrum serratum*,its methnolic extract exhibit significant anticancer activity as compared to aqueous extract. (Zalke et al. 2010).Antibacterial activity of *Clerodendrum serratum .L* (Vidya S.M et al. 2010).

PRELIMINARY PHYTOCHEMICAL STUDIES

The pharmacological and therapeutic action of crude drug is determined by the nature of its constituents. Thus the plant species may be considered as a biosynthetic laboratory not only for the chemical compounds e.g. carbohydrates, proteins and fats that are utilized as a food by humans and animals, but also for a magnitude of compounds including alkaloids, flavonoids, glycosides etc. which exert definite physiological effects.

Tests for carbohydrates and glycosides

A small quantity of the extracts was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to various tests to detect the presence of Carbohydrates.

Molisch's test

1. Filtrate was treated with 2-3 drops of 1% alcoholic _- napthol solutions and 2ml of Con.

Sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

2. Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate I ml of pyridine and few drops of sodium nitropruside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides

Bortanger's test

Hydrolysate was treated with choloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution added. Ammonia layer acquires pink color, showing the presence of glycosides.

TEST FOR ALKALOIDS

- A small portion of the solvent free alcohol and aqueous extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for the
- Presence of alkaloids.
- Mayer's reagent - Cream ppt
- Dragendroff's reagent - Orange brown ppt.
- Harger's reagent - Yellow ppt
- Wagner's reagent - Reddish brown ppt

TEST FOR PHYTOSTEROL

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Libermann burchard test

The residue was dissolved in few drops of dil. Acetic acid; 3 ml of acetic anhydride was added followed by few drops of Con. Sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

TESTS FOR FIXED OILS

Spot test

Small quantities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for gums and mucilages

Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Test for proteins and free amino acids

Small quantities of the extracts were dissolved in few ml of water and treated with following reagents.

- Appearance of red color shows the presence of protein and free amino acid
- Appearance of purple color shows the presence of proteins and free aminoacid
- Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added.
- Appearance of pink or purple color shows the presence of proteins and free amino acids.

Test for phenolic compounds and tannins.

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1. Dil. Ferric Chloride solution (5%) – Violet Color.
2. 1% solution of gelatin containing 10% sodium chloride – White ppt.
3. 10% lead acetate solution – White ppt.

TEST FOR FLVAVONOIDS

With aqueous sodium hydroxide solution

Blue to violet color (anthocyanins) Yellow color (flavones), yellow to orange (flavononones)

With concentrating sulphuric acid

Yellow orange color (anthocyanins) yellow to orange color (flavones) orange to crimson (flavononones)

Shinoda's test

Small quantities of the extract were dissolved in alcohol, to them piece of magnesium followed by Conc. Hydrochloric acid drop wise added and heated. Appearance of magenta color shows the presence of flavonoids.

TLC METHOD

Aluminium sheets and glass backed TLC plates were used for the isolation of compounds. The plates were divided in to size of 10 cm x 1.5 cm. A light pencil was drawn 1cm from the bottom and top edge of the chromatographic plate. 6mg of column fraction sample was dissolved in 60 ml of absolute methanol and placed as preparatory on two TLC plates (10x1.5 cm) using a 10 micro litre of capillary which delivers approximately 10 microgram spot on to the place until each plate contains 150 microgram and subsequently placed in the eluting solvent. (Chloroform: methanol: water (5:4:1)) in a TLC tank which was fill to a depth of 0.5 cm the solvent migrated upwards on to the TLC plate until the pencil line drawn across the top edge (solvent front) was reached. The plates were removed from the chamber and air dried. A portion of the plates (1 cm) was cut off using a glass cutter and sprayed with a detecting reagent (diphenyl ethantamine (glycosides)) in order to visualize the constituents on the eluted plates after heating for three minutes at 110o c in on oven. The plates were also visualized under UV light at 360 nm and 254 nm and the fluorescence (360 nm) or quenching (254 nm). Compounds were marked and the spot (layer) were out lined with the pencil.

Sample 1: 5/7 = 0.7142
Sample 2: 4.7/7 = 0.6714.

Report

The RF value of sample 1 and sample 2 were coinciding with standard values.
RF value for sample 1 = 0.7142
RF value for sample 2 = 0.6714.

ACUTE ORAL TOXICITY STUDIES

Procedure

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.

Preparation of doses

In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture to be tested however, the use of the undiluted test substances, i.e. at a constant concentration, may be more relevant to the subsequent risk assessment of the substances, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of the liquid that can be administrated at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1ml/100 gm of body weight. However in the case of aqueous solution 2ml/100 gm body weight can be considered. With respect to the formulation the dosing preparation, by use of an aqueous solution/ suspension/ emulsion is recommended wherever possible, followed in order of preference by solution/suspension/emulsion in oil ex. (Cornoil) possible solution in other vehicle. For vehicles other then water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

Administration of the dose

The test substance is administered in a single dose by gavages by using a stomach tube or suitable incubation cannula. In the unusual circumstance that a single dose is not possible, the dose may be given in small fraction over period not exceeding 24 hours. Animal should be fasted prior to dosing (e.g. with rat, food but not water should be withheld over night, with the mouse food but not water should be withheld for 3-4 hours) following the period of fasting, the animal should be weighed and the test substances is administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered infraction over a period it may be necessary to provide the animals with food and water depending on the length of period.

Number of the Animal and Dose Levels

Three animals are used for each step. The dose level to be used as the starting dose is selected from one hour of four fixed levels, 5, 50, 300, 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal Exceptionally, and only when one justified by specific regularity needs the use of additional upper dose level 5000 mg/kg body weight may be considered. For reason of animal welfare concern, testing of animals in GHS category 5 ranges (2000 – 5000 mg/kg) is discouraged and should only be considered and when there is a strong likelihood that results of such a test have direct relevance for protecting human or animal health or the environment. welfare reasons it recommended using the starting dose of 300 mg/kg body weight. The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals.

SCREENING METHODS FOR ANTI ASTHMATICS

Animals

Adult male albino rats, weighing 150 – 200 g were used for the present study. Animals were acclimated for 15 days in our disease free animal house prior to the start of the experiment. The animals were kept in clean and dry plastic cages, with 12 h light: 12 h dark cycle at 25+20 c temperature and 45 – 60% relative humidity. Animals were given free access to standard feed and water and *libitum*. For experimental purpose the animals were kept on overnight fasting but allowed free access to water. The research conducted under the guidelines of CPCSEA and approved by Institutional Animal Ethics Committee in ref. No. M.Pharma/2013/09.

Invivo method

Overnight fasted Rats were divided into six groups

1. Inducer control (IC) = Acetylcholine + Citric acid (0.2% spray),
2. STD received Theophylline (200 mg/kg)
3. AECS (100mg/kg) (200mg/kg),
4. NAECS (200mg/kg) (400mg/kg),p.o. Bronchospasm was induced in rats by exposing them to Acetylcholine & Citric acid (0.2%

spray) produced by an ultra sound nebulizer in an aerosol chamber (24*14*24 cm) made of Perspex glass.

The time required for appearance of pre convulsive dyspnoea caused by the Acetylcholine & Citric acid (0.2% spray) was recorded for each animal. Prior to drug treatment, each animal was placed on Histamine chamber and exposed to Acetylcholine + Citric acid 0.2% aerosol. The preconvulsive time (PCT), i.e the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the preconvulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover as basal value. Rats were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs, the animals of STD received Theophylline (200 mg/kg), AECS (100mg/kg) (200mg/kg), NAECS (200mg/kg) (400mg/kg). These animals were again subjected to Acetylcholine + Citric acid 0.2% aerosol later at an interval of 1hr, 4hrs, and 24 hrs to determine preconvulsion time (PCT). The protection offered by the treatment was calculated by using the following formula Percentage protection = (1 – T1/T2) * 100 Where, T1 = the mean of PCT before administration of test drugs, and T2 = the mean of PCT after administration of test drugs at 1 hr, 4 hrs and 24 hrs.

Statistical analysis

All the values were expressed as mean +
- SEM. The results were analyzed for statistical significance by using one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered significant.

INVITRO METHOD

Parameters for investigation

The other major type of blood cells are the white blood cells (WBC's), which are also referred to as leukocytes. There are many more RBC's than there are WBC's. For every leukocyte present in a sample there will normally be 600 to 700 RBC's. The major role of the white blood cells is to defend the body against invading organisms such as bacteria, viruses, and fungi. There are different types of leukocytes, and a white blood count (WBC) is a total of all the various kinds. The normal range for a WBC count in the dog would be between 6,000 and 17,000 per microliter, and in the cat, 4,900-20,000/µ l. The number of WBC's is typically elevated when the body is fighting a severe infection or stressed by metabolic toxins (a patient that was in kidney failure with waste products building up in its body would normally have an elevated WBC). In addition, when extremely excited (if we overly excite or frighten the animal when drawing the blood sample) white blood cells will be released into the blood and the levels will rise. The WBC count will be lower than normal, if an animal has been weakened from a prolonged, debilitating disease and in some viral infections. WBC's are divided into two groups depending on how they react to the stains that are used to better observe them under a microscope. There are granulocytes (they are WBC's with granules that absorb the stain) and the agranulocytes (those that do not absorb the stain). The granulocytes include the neutrophils, eosinophils, and the basophils, while the agranulocytes are the lymphocytes and monocytes Histopathological examination After 14 days experimental period and the last blood sampling, the whole Lungs were removed after sacrificing the animal and were fixed in 10% formalin for histopathological examination. Sections were cut and stained by hematoxylin and eosin (H&E) for histological examination.

Statistical analysis

All the values were expressed as mean +
- SEM. The results were analyzed for statistical significance by using one-way ANOVA followed by Dunnett's test.
$P<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

Asthma is common respiratory disease. The morbidity and the mortality of the disease is increasing and making a global concern. The syndrome of bronchial asthma is characterized by wide spread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical mediators such as Ach and Citric acid. In the presence study *c.serratum*..... Significantly inhibited the Ach and Citric acid induced anti asthmatic properties of the plant. Ach and Citric acid induced Bronchoconstriction is the traditional

immunological model of the antigen induced air way obstruction. Ach and Citric acid when inhaled causes hypoxia and leads to convulsion in rats and causes very strong smooth muscle contraction, profound hypotension, capillary dilation in cardio vascular system a prominent effect caused by histamine leads to severe Bronchoconstriction in rats that causes asphyxia and death. Bronchodilator can delay the occurrence of these symptoms. The results of the study confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.

Drugs effective in the asthma are mostly steroidal and also flavonoids in nature. Phytochemical profile of the plant reveals the presence flavonoids (Apigenin), steroidal nucleus (_-spinasterol) in the form of triterpenoids. The anti asthmatic activity showed by leaves of the plant extract (Apigenin and _-spinasterol) may be because of the chemical moieties. However this claims demands for further research and studies are in fact underway to isolate and characterized the active principles responsible for the anti asthmatic activity.

Table:1

PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT EXTRACTS OF LEAVES OF *CLERODENDRUM SERRATUM*

PhytoChemical Constituents	Aqueous extract	Non aqueous extract
Carbohydrates	-	-
Glycosides	-	-
Alkaloids	-	-
Flavonoids	+	+
Phenols	+	+
Fixed oils	-	-
Steroids	+	+
Saponins	-	-
Gums & mucilage	-	-
Proteins & free amino acids	-	-

(+) - Positive
(-) – Negative

Mortality result of sighting study starting dose in main study is decided and carried out with six animals per dose level (1000mg/kg).Based on the mortality result on 14h day of observation, the doses for *in vivo* study were selected.

Table:2 **PRE CONVULSION DYSNOPEA**

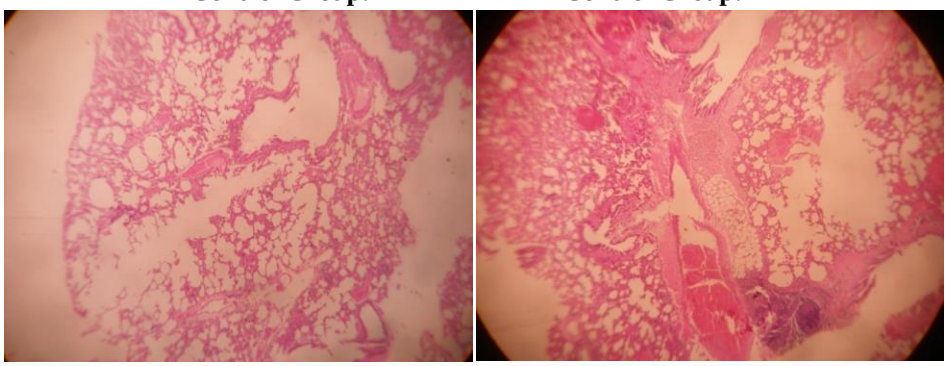
TREATMENT	DOSE	BEFORE	1 Hr	4 Hrs	24 Hrs
Negative control	Ach + Citric acid	18.2 + 2.2739	18.2 +0.1250	17.4 +0.091	17.2+0.110
Positive control	Ach + Citric acid + Theophylline	18.2 +0.1250	55.8 +1.315	64.3 +1.548**	34 +1.472**
AECS	100 mg/kg	17.5 + 0.1371	41 +1.291	43 +1.291	22.8+0.478
AECS	200 mg/kg	17.5 +0.2955	57.8 +1.750	59.8 +1.750**	33 +1.225**
NAECS	200	18.0 +0.4328	38.3 +1.250	40 +1.250	21.8+0.478

	mg/kg				
NAECS	400 mg/kg	17.5 +0.5282	40 +1.291	41.5 +1.258	27.1+0.314

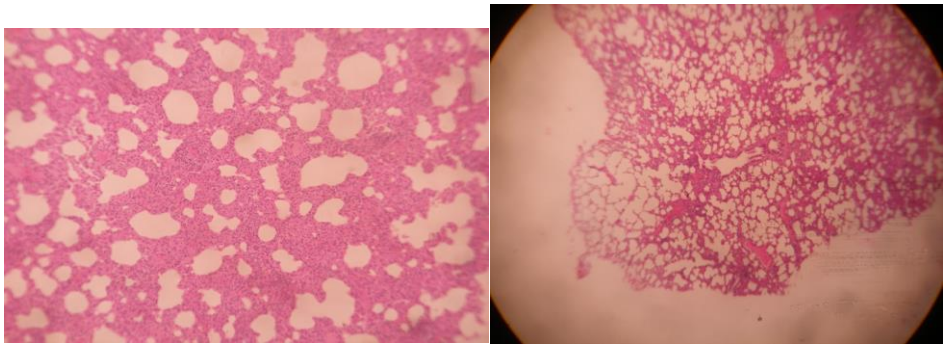
Values are represented as mean ± S.E.M (n=6)
One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001)

HISTOPATHOLOGICAL REPORT

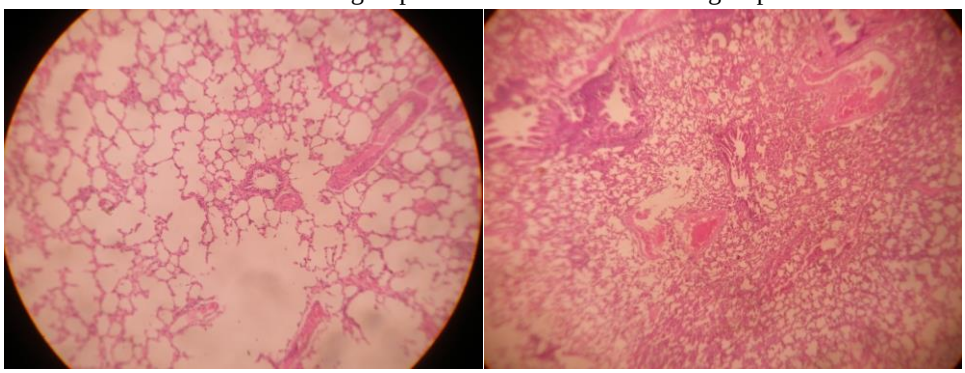
Control Group:1 **Control Group:2**



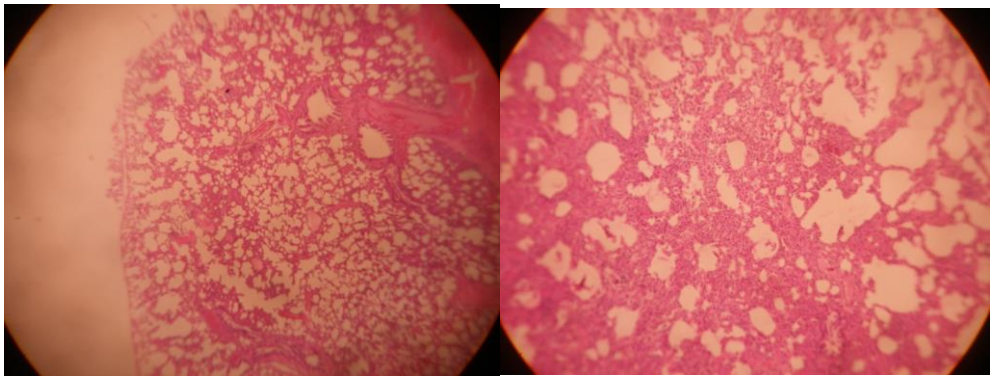
Negative control group:1 Negative group:2



Positive control group:1 Positive control group:2

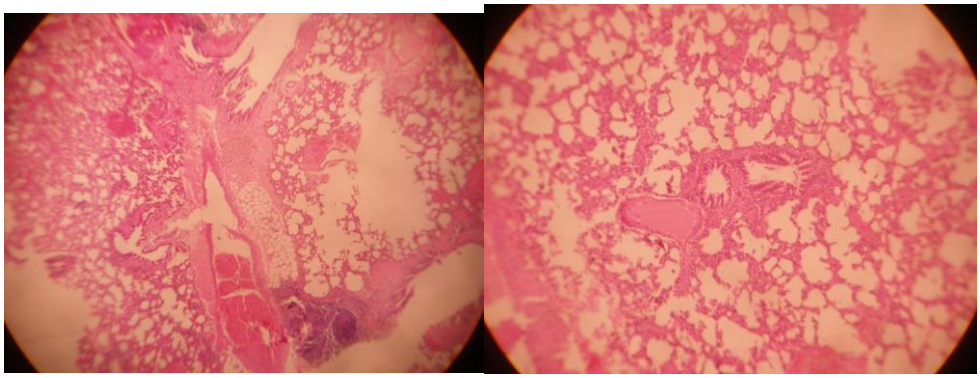


Aq.Extract 100 mg/kg Treated group Aq.Extract 200 mg/kg Treated group



Non Aq.Extract 200 mg/kg Treated group

Non Aq.Extract 400 mg/kg Treated group



CONCLUSION

On the basis of the result in these experiments, this may be stated that the aqueous extract of *Clerodendrum serratum* 200mg/kg has a beneficial effect in asthmatic patients. It will reduce the **alveolar thickness** and **Eosinophils** counts in blood further studies are required to purify the active principle and to study the molecular mechanism of the exact pathway. Non aqueous extract of *Clerodendrum serratum* (400mg/kg) having fewer amounts of steroids and flavonoids because it will filter the all compounds and reduce the quantity of chemical moieties. But aqueous extract of *Clerodendrum serratum* (200mg/kg) having higher amount of chemicals when compare to Non aqueous extract of *Clerodendrum serratum* (400mg/kg) so it will produce anti asthmatic at equal to standard drug.

In lungs alveolar thickness reduced by the AECS 200mg/kg as equal to standard drug and also it's same as normal lungs. So aqueous extract of *Clerodendrum serratum* having good beneficial effect for the asthmatic patients. This information's will be useful for the development of alternative method rather than anti asthmatic agents (Inhalers, Tablets, Injections, Nasal sprays, Respules) for the treatment of Asthmatic patients. This will minimize the wheezing and or asthmatic symptoms.

REFFERENCE

[1]. The U.S. Food and Drug Administration, Center for food safety and Applied Nutrition. Economic Characterization of the Dietary Supplement Industry Final Report. 1999

[2]. Barz, W. and Ellis, E. Potential of plant cell cultures for pharmaceutical production. In *Natural products as medicinal agents*. (eds.) Beal, J.L. and Reinhard, E. Suppl. to Planta Medica, Hippokrates Verlag, Stuttgart. pp 1981, 471-508.

[3]. Cassady, J.M., Chang, C.-J. and McLaughlin, J.E. Recent advances in the isolation and structural elucidation of antineoplastic agents in higher plants. In *Natural products as medicinal agents*. (eds.) Beal, J.L. and Reinhard, E. Suppl. Planta Medica. Hippokrates Verlag, Stuttgart. 1981, 93-124.

[4]. Compadre, C.M., Pezzuto, J.M., Kinghorn, A.D. and Kalmath, S.K.. Hernandulcin: An intensely sweet compound discovered by review of ancient literature. Science, 227, 1985, 417-419
[5]. Wall, M.E. and Wani, M.C. Structure activity relationship of plant antitumour agents related to camptothecin and the quassinoids. In *Natural products as medicinal agents*. (eds.) Beal, J.L. and Reinhard, E. Suppl. Plants Medica. Hippokrates Verlag, Stuttgart. 1981, 125-149.
[6]. Wermuth, C.-G. Modulation of natural products in order to improve their pharmacokinetic properties. In *Natural products as medicinal agents*. (eds.) Beal, J.L. and Reinhard, E. Suppl. Planta Medica. Hippokrates Verlag, Stuttgart. 1981, 185-215
[7]. Singh Amrit pal, Promising Pytochemicals from Indian medicinal plants, Ethanobotanical leaf lets 9, 2005, 15-23.
[8]. Naranjo P., The urgent need for the study of medicinal plants. In Ethnobotany: Evolution of a discipline. (Eds.) Schultes, R.E., and Reis, S.von., Chapman & Hall, London., 1995, 362-368
[9]. Pearce D, The economic value of biodiversity, Earthscan Publication, London, 1994, 101-102
[10]. Barz, W., Ellis, E.Potential of plant cell cultures for pharmaceutical production. In Natural products as medicinal agents. Beal, J.L., and Reinhard, E., Suppl. To Planta Medica, Hippokrates Verlag, Stuttgart., 19801, 471-508
[11]. Kameswara Rao, C., Sangeetaa, W. Alternate sweeteners. Vatika, No.4, Winter, 1993, 193
[12]. Ralph W. Moss, Ph.D Galen on Cancer- How Ancient Physicians Viewed Malignant Disease 1989 Speech.
[13]. Jemal A. Murray T, Ward E, Samuels A, Tiwari R C, Ghafoor A, Feuer EJ, Thun MJ. "Cancer statistics, 2005". CA Cancer J Clin 55(1), 2005, 10-30.
[14]. The Basic Science of Oncology. Tannock IF, Hill RP et al 4, 2005. McGraw- Hill
[15]. Matoba S, Kang J, Patino W, Wragg A. Boehm M, Gavrilova O, Hurley P, Bunz F, Hwang P. "p53 regulates mitochondrial respiration". Science 312(5780), 2006, 1650-3
[16]. Harsha Mohan, Neoplasia, Text book of pathophysiology, 10, 706
[17]. Lanier AP, Cancer and stilberstrol: a follow- up of 1179 persons exposed in utero and bron 1943-1959. Mayo Clinic Proceeding,; 48, 1973, 793-799
[18]. Principles of Cancer Biology. Kleinsmith, LJ. Pearson Benjamin Cummings 2006.
[19]. Goodman and Gillman, The Pharmacological Basis of Life, 10th edition, 2001, 1381-1385
[20]. Harper et al, "Efficacy of a bivalent L1 virus like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: arandomized controlled trial". Lancet 364(9447), 1757-65.

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