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Research Article

A Pharmacological Evaluation Of Antihistaminic And Anti-Inflammatory Activity On O. Sanctum Leaves

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ABSTRACT

The objective of the present study to a Pharmacological evaluation of antihistaminic and Anti-Inflammatory activity on O. sanctum leaves. In recent year there has been tremaendous increase in demand for herbal drugs because of its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic compilation sanctum leaves is also considered to be therapeutically important in traditional system of medicine. To study the A Pharmacological evaluation of antihistaminic and anti-Inflammatory activity on study the in vitro anti-inflammatory activity of the extract on sanctum Leaves Extraction of the plant material on O. sanctum Leave Conduction of the preliminary phytochemical screening and finding out the phytoconstituents present in the ethanolic extract of on O. sanctum Leaves Evaluation of the anti-asthmatic effect of O. sanctum Leaves In vivo methods Histamine aerosol induced bronchoconstriction in guinea pigs, Milk induced leukocytosis and eosinophil count, Ex vivo methods, Isolated guinea pig tracheal preparation, Evaluation of the Antihistaminic study of (on O. sanctum Leave In vitro methods Hydrogen peroxide scavenging assay, Reducing power assay Evaluation of the anti-inflammatory study of (on O. sanctum Leaves,In vitro methods Protein denaturation method, the rabbit red blood cell (RRBC) membrane stabilization method.

INTRODUCTION

ABOUT HERBAL DRUGS

The term “medicinal plant” includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word “herb” has been derived from the Latin word, “herba” and an old French word “herbe”. Now days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as

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well as a non- woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaid's and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India.

ASTHMA

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen. In addition, mucus production occurs in the airway and muscles surrounding the airway spasm. Combined, these cause a reduction in air flow. Asthma is characterized by Airway inflammation: The airway lining becomes red, swollen, and narrow.

Airway obstruction: The muscles encircling the airway tighten causing the airway to narrow making it difficult to get air in and out of the lungs.Airway hyper-responsiveness: The muscles encircling the airway respond more quickly and vigorously to small amounts of allergens and irritants. Common signs and symptoms of an acute asthma episode include: Coughing, Wheezing, Breathlessness, Respiratory rate increased, Chest tightness , Chest or abdominal pain, Fatigue, feeling out of breath, Agitation, Increased pulse rate

Causes

- Allergens from nature, typically inhaled, which include waste from common household pests. Indoor air pollution from volatile organic compounds.
- Medications, aspirin,βadrenergic antagonists (beta blockers), and penicillin.
- •Food allergies such as milk, peanuts, and eggs.
- Pathophysiology and pathogenesis of asthma
- Airflow limitation in asthma is recurrent and caused by a variety of changes in the airway.

These include:

Bronchoconstriction

In asthma, the dominant physiological event leading to clinical symptoms is airway narrowing and a subsequent interference with airflow. In acute exacerbations of asthma, bronchial smooth muscle contraction (bronchoconstriction) occurs quickly to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants. Allergen-induced acute bronchoconstriction results from an IgE-

dependent release of mediators from mast cells that includes histamine, tryptase, leukotrienes and prostaglandins that directly contract airway smooth muscle.

Airway edema

- As the disease becomes more persistent and inflammation more progressive, other factors further limit airflow. These include edema, inflammation, mucus hypersecretion and the formation of inspissated mucus plugs, as well as structural changes including hypertrophy and hyperplasia of the airway smooth muscle. These latter changes may not respond to usual treatment.

Airway hyper responsiveness

This is an exaggerated bronchoconstrictor response to a wide variety of stimuli is a major, but not necessarily unique, feature of asthma.

Airway remodelling

In some persons who have asthma, airflow limitation may be only partially reversible. Permanent structural changes can occur in the airway; these are associated with a progressive loss of lung function that is not prevented by or fully reversible by current therapy. Airway remodelling involves an activation of many of the structural cells, with consequent permanent changes in the airway that increase airflow obstruction and airway responsiveness and

MATERIALS

Plant selected

In the present study, 1(on O. sanctum Leaveswas selected because of its traditional uses. The part used was Leave.

Chemicals and reagents used

a. Carboxy methyl cellulose (Spectrum reagents and chemicals pvt. Ltd.)
b. Ascorbic acid (Spectrum reagents and chemicals pvt. Ltd.)
c. Histamine (NICE chemicals pvt.Ltd.)
d. Hydrogen peroxide (Spectrum reagents and chemicals pvt. Ltd.)
e. Glacial acetic acid (Ozone international, Mumbai)
f. Trichloro acetic acid (NICE chemicals pvt. Ltd.)
g. Diclofenac sodium (Rajesh chemicals, Mumbai)

Drugs used

a. Chlorpheniramine maleate (Abbott Laboratories pvt. Ltd.)
b. Dexamethasone (Zydusbiogem, cadila health care Ltd.)

Instruments used for the study

a. UV- Visible spectrophotometer- Jasco international
b. Incubator- Rotek Instruments, B&C Industries, W. Vengola.
c. Centrifuge- Rotek Instruments, B&C Industries, W. Vengola.
d. Histamine chamber- Orchid Scientific Innovations India pvt. Ltd

Animals

Swiss albino mice (25-40 gm) and Guinea pig (400-600 gm) were used to carry out the activities. The animals had free access to standard commercial diet and water. Animals were housed in cages under standard conditions i.e., 12:12 hour light or dark cycle at 25±20 C. The experiments were carried out as per the guideline of CPCSEA, New Delhi, India.

METHODS

Collection and authentication of (on O. sanctum Leaves)

The dried Leaves a were collected.were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Detection of protein: -

Biuret test (General test)

3 ml of test solution add 4% NaOH and few drops of 1% CUSO4 solution. Violet or pink color appear.

Million's test (for proteins)

Mix 2 ml of extract with million's reagent. white precipitate formed. warm precipitate turns brick red or the precipitate dissolves giving red colored solution.

Detection of proteins and amino acids: -

About 100 mg of extract was dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

Biuret test (General test)

3ml of test solution add 4% NaOH and few drops of 1% CUSO4 solution. Violet or pink color appear.

Million's test (for proteins)

Mix 2 ml of extract with millions reagent. White precipitate formed. Warm precipitate turns brick red or the precipitate dissolves giving red colored solution

Ninhydrin test (for amino acid)

About 2 drops of ninhydrin solution were added to 2 ml of test solution. Purple or bluish color appears

Detection for Fats and oils: -

Solubility test

Oils are soluble ether, benzene, chloroform but insoluble in 90% ethanol and in water. Hence filter paper get permanently stained with oils

Detection of steroid: -

Salkowski reaction

To 2 ml of extract, add 2 ml chloroform and 2 ml con H2SO4 shake well. Chloroform layer appear red and acid layer shows greenish yellow fluorescence.

Liebermann – burchard reaction

Mix 2 ml extract with chloroform. Add 1-2 ml of acetic anhydride and 2 drops of con H2SO4 from the side of test tube. First red, then blue finally green color appear.

Detection of glycoside: -

Cardiac glycoside

Legal 's test (for cardiac glycoside)

To aqueous or alcoholic extract add 1 ml pyridine and 1 ml sodium nitroprusside. pink to red color appears.

Test for reducing sugar (for cardiac glycoside)

To 2 ml extract add glacial acetic acid one drop 5% Fecl3 and con H2SO4. Reddish brown color appears at junction of two liquid layer and upper layer appears bluish green.

Anthraquinone glycoside

Bontragers test

To 3 ml extract, dil. H2SO4 was added, boiled and filtered. To cold filtrate, equal volume of benzene or chloroform was added and shaken well. The organic solvent was separated and ammonia was added. The ammonical layer turns pink to red.

Saponin glycoside

Foam test

Shake the drug extract or dry powder vigorously with water. Persistant form observed.

Heamolytic test

Add drug extract or dry powder to one drop of blood placed on glass slide. Heamolytic zone appears

Cyanogenic glycoside

Grignard reaction or sodium picrate test

Soak a filter paper strip first in 10% picric acid, then in 10% sodium carbonate, dry. In conical flask place moistened powdered drug. Cork it, place the above filter paper strip in the slit in cork. Filter paper turn brick red or maroon.

Coumarin glycoside

Coumarin glycoside have aromatic odour

Alcoholic extract when made alkaline, shows blue or green fluorescence

Detection for flavonoids

Shinoda test

To dry powder or extract, add 5ml 95% ethanol, few drops con. Hcl and

0.5 g Magnesium turnings. Pink color observed.

- To small quantity of residue, add lead acetate solution. Yellow color precipitate is formed.

- Addition of increasing amount of NaOH to residue shows yellow coloration, which decolorizes after addition of acid.

Detection of alkaloid

Aqueous alcoholic and chloroform extract was evaporated separately. To residue dilute Hcl was added. Shaken well and filtered. With the filtrate the following test was performed.

Dragondorff 's test

To 2-3 ml. Filtrate, add few drops of dragondorff's reagent. Orange brown precipitate

Mayers test

To 2-3 ml of filtrate with few drops of mayer's reagent gives precipitate

Hager's test

2-3 ml filtrate with hager's reagent gives yellow precipitate

Wagner's test

2-3 ml of filtrate with wagner's reagent gives reddish brown precipitate

ACUTE TOXICITY STUDIES

Acute toxicity of O. sanctum Leaves was done as per OECD guidelines 423. The substance was administered in a single dose by gavage using specially designed mice oral tube. Animals were fasted prior to dosing with food but not water withheld overnight. Following the period of fasting, the animals were weighed and the test substance was orally at a dose of 5, 50, 300 and 2000 mg/kg. The animals are observed continuously for first three hours, four any toxic manifestations like increased motor activity, salivation, acute convulsion, coma and death. Changes in the animal behavior should be noted before and after administration for 24hours. Treated animals are to be further observed for 14 days. If the extract does not produce mortality at the highest dose, then the 1/10th or 1/20th of the dose was selected for experiment.46, 47

EVALUATION OF ANTI ASTHMATIC ACTIVITY

In vivo anti-asthmatic activity

Histamine aerosol induced bronchoconstriction in guinea pigs

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received carboxy methyl cellulose. The animals of group 2 and 3 were given, by oral intubation, 100 and 200 mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hours, to determine pre convulsive time (PCT).48, 49, 50

Percentage protection was calculated using the formula.

Percentage protection = $Eta{-}Etb \times 100 \;\; Eta$

Where Eta is the preconvulsion time after administration of drug and Etb is the preconvulsion time before administration of drug.

Milk induced leukocytosis and eosinophilia

Mice were divided into 4 groups with six in each group. Blood samples were collected from retro-orbital plexus. Group 1 served as control and received carboxy methyl cellulose solution, groups

2-3 received plant extract at (100-200 mg/kg) group 4 received dexamethasone at 50 mg/kg i.p. All the groups injected boiled and cooled milk (4 ml/kg, s.c.) 30 min after treatments. Total leukocyte and eosinophile count was done in each group before administration of test compound and 24 hours after milk injection. Difference in total leukocytes and eosinophile count before and after 24-hour drug administration was calculated.51, 52

Ex vivo anti-asthmatic activity

Isolated guinea pig tracheal preparation

Isolated guinea pig tracheal tissue was obtained by, Animals were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred into a dish containing Krebs solution and cut crosswise between the section of the cartilage of the trachea and continuously ventilated and maintained at

37 + 0. 5°C.The adjourned trachea was allowed to make steady for at least

40 minutes. On equilibrium, the bath was supplied with Krebs solution for every

15 minutes Dose response curve of histamine (10 µg/ml) in plane Krebs solution and in 1 mg/ml of plant extract act in Krebs solution was taken. Percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of plant extract.48, 54

IN VITRO ANTIOXIDANT ACTIVITY

1. **Hydrogen peroxide scavenging**

Hydrogen peroxide solution (20 Mm) was prepared with standard phosphate buffer (pH 7.4). Extract samples (25, 50, 100, 200 and 400 µg/ml) in distilled water were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the reference standard. The percentage scavenging of hydrogen peroxide of plant extract was calculated using the formula.55

$$\textbf{\% Scavenged} = Ac{-}As \times \textbf{100}$$

Where, Ac = Absorbance of control

As = Absorbance of sample

2. **Reducing power assay**

The reducing power of the extract was determined by the method. 1 ml of the extract solution (25, 50, 100, 200 and 400 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, Ph 6.6) and 2.5 ml of potassium ferricyanide ([K2 Fe (CN)6] (10g/l)), then the mixture was incubated at 500 C for 20 minutes. A portion (2.5ml) of trichloroacetic acid (TCA) (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5ml ferric chloride (FeCl3. 0.1%) and absorbance was measured at 700 nm in UV- visible spectrophotometer. The experiments were performed in triplicate. Increased absorbance of reaction mixture indicates stronger reducing power.56, 57

IN VITRO ANTI-INFLAMMATORY ACTIVITY

1. **Protein denaturation**

A solution of 0.2% of bovine serum albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of different concentration (25, 50, 100, 200 and 400 µg/ml) was prepared using ethanol as solvent. 50 µl of each test drug was transformed to test tubes using micropipette. 5 ml of 0.2% w/v of BSA was added to the test tubes. The control consists of 5 ml of 0.2% w/v of BSA solution and 5µl alcohol. The test tubes were heated at 720 C for 5 min and then cooled for 10 min. The absorbance of these solution was determined using UV–visible spectrophotometer at 660nm. Diclofenac sodium was used as standard and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated using the following formula.58, 59

Percentage of inhibition of denaturation =

$$\frac{Absorbance\ of\ control - Absorbance\ of\ extract}{Absorbance\ of\ control} \times 100$$

2. **The rabbit red blood cell membrane stabilization method**

Preparation of red blood cell suspension (RBCs suspension)

The fresh whole rabbit blood (5 ml) was collected from marginal ear vein to syringes containing sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed 3 times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Membrane stabilization test by hypotonicity induced haemolysis

The reaction mixture consists of 1 ml of test sample of different concentration (25, 50, 100, 200 and 400 µg/ml) in normal saline and 0.5 ml of 10% RBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 370C for 30 minutes and centrifuged at 3000 rpm for 20 minutes and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 n

RESULT AND DISCUSSION

COLLECTION & AUTHENTIFICATION OF ALP

Collection and authentication of (on O. sanctum Leaves)

EXTRACTION OF PLANT MATERIAL

O. sanctum Leaves were collected, washed and shade dried. Dried Leaves were crinkled in to powdered form, weighed out. Extraction of coarse powder was done by soxhlet extraction with ethanol. The percentage yield of the product was found to be 17 % w/w.

PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF ON O. SANCTUM LEAVES)

The phytochemical screening of the ethanolic extract of the O. Sanctum Leaves indicate the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and phenolic compounds.

Table No.3: Preliminary phytochemical analysis

Sr. No	Constituents	Presences/ Absences
1.	Carbohydrate	+
2.	Proteins	+
3.	Amino acids	-
4.	Fats and Oils	-
5.	Steroids	+
6.	Cardiac Glycosides	+
7.	Anthraquinone Glycoside	-
8.	Saponin Glycoside	-
9.	Cyanogenic Glycoside	-
10.	Coumarin Glycoside	-
11.	Flavonoids	+
12.	Alkaloids	-
13.	Tannins	+
14.	Phenol	+

(+: presence, -: absence)

ACUTE TOXICITY STUDIES

Acute toxicity studies of ethanolic extract of on O. sanctum Leaves was performed according to OECD guidelines 423 using swiss albino mice. At the dose 2000 mg/kg, the ethanolic extract were neither produced mortality nor the sign of morbidity. Hence the dose 100 mg/kg (1/20th of 2000 mg/kg) and 200mg/kg (1/10th dose of 2000 mg/kg).

EVALUATION OF ANTI- INFLAMATION ACTIVITY

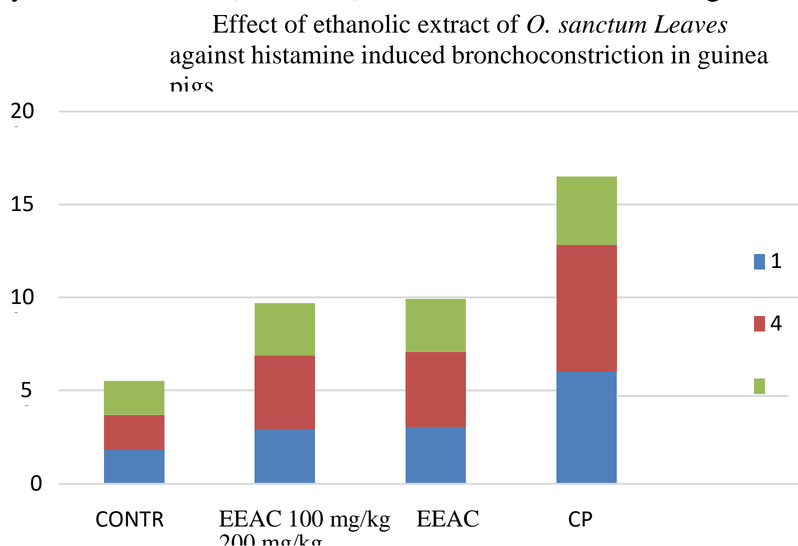
In vivo anti Inflammation activity

Histamine aerosol induced bronchoconstriction in guinea pigs Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of pre convulsive dyspnoea

produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Histamine aerosol induced bronchoconstriction in guinea pigs

Group	Latent period of convulsion			
	Before	1 hour	4 hour	24 hour
Control	16.3±2.23	18.36±0.183	18.63±0.186	18.4±0.12
On O. sanctum Ethanolic extract (100 mg/kg)	16.71±1.31	29.65±.28	39.38±0.05*	28.2±0.23
on O. sanctum Leaves Ethanolic extract (200 mg/kg)	15.71±0.77	30.5±3.08	40.36±1.04*	28.4±.35
Standard (CPM) (1 mg/kg)	18.46±0.89	60.25±0.03*	68.26±1.01**	36.5±0.55

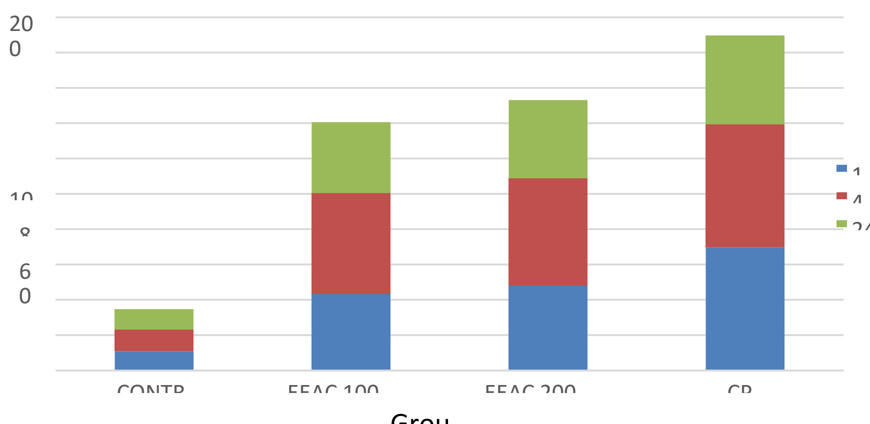
Values are Mean± S.E.M., where n=6 in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Figure Effect of ethanolic extract of O. sanctum Leaves against histamine induced bronchoconstriction in guinea pig



% Protection of the plant O. sanctum Leaves against histamine induced bronchoconstriction in guinea pig

Group	% Protection		
	1 hour	**2 hours**	**24 hours**
Control (carboxy methyl cellulose)	10.9	12.3	11.4
O. sanctum Leaves ethanolic extract (100 mg/kg)	43.2	57.2	40.2
O. sanctum Leaves ethanolic extract (200 mg/kg)	48	60.79	44.3
Standard(CPM)	69.76	78.3	50.1

% Protection of the plant O. sanctum Leaves against histamine induced bronchoconstriction in guinea pigs



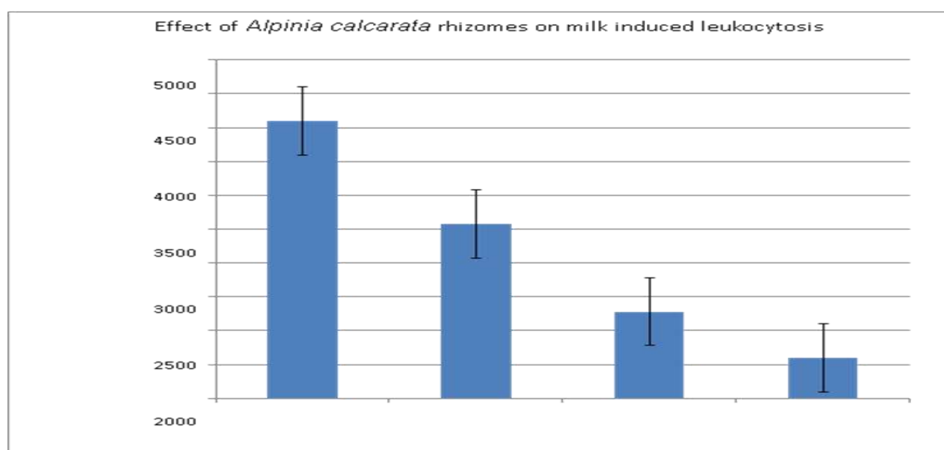
Milk induced leucocytosis and eosinophilia

Milk induced leucocytosis

In the milk induced leukocytes the maximum increase in difference of leukocytes count was observed in control group (4100 ± 9) 24 hour after administration of milk. Groups of mice pretreated with ethanolic extract (200 mg/kg) showed significant activity. The ethanolic extract of plant O. sanctum Leaves (200 mg/kg) showed decrease in number of leukocytes (1280 ± 12) as compared to control. The standard drug possesses significant activity (600±10) and the plant extract (100 mg/kg) showed less significant activity as compared to control Effect of ethanolic extract of O. sanctum Leaves on milk induced leucocytosis

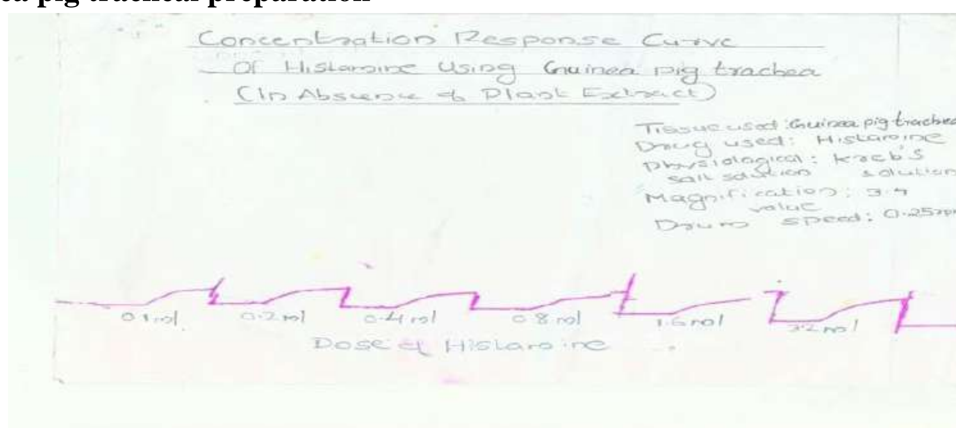
Groups	Difference in no of leukocytes before and after treatment(Cu.mm)
Control (Carboxy methyl cellulose)	4100±9
O. sanctum Leaves ethanolic extract (100 mg/kg)	2580±8*
O. sanctum Leaves ethanolic extract (200 mg/kg)	1280±12**
Standard (Dexamethasone (50 mg/kg))	600±10**

Effect of O. sanctum Leaves on milk induced leukocytosis



Ex vivo anti-asthmatic study

Isolated guinea pig tracheal preparation



Histamine (10 µg/ml) produced dose dependent contraction of guinea pig tracheal preparation. Pretreatment with the ethanolic extract of O. sanctum Leaves (1mg/ml) significantly inhibited the contractile effect of histamine.

Concentration Response Curve of guinea pig tracheal preparation before and after administration of plant extract are shown below

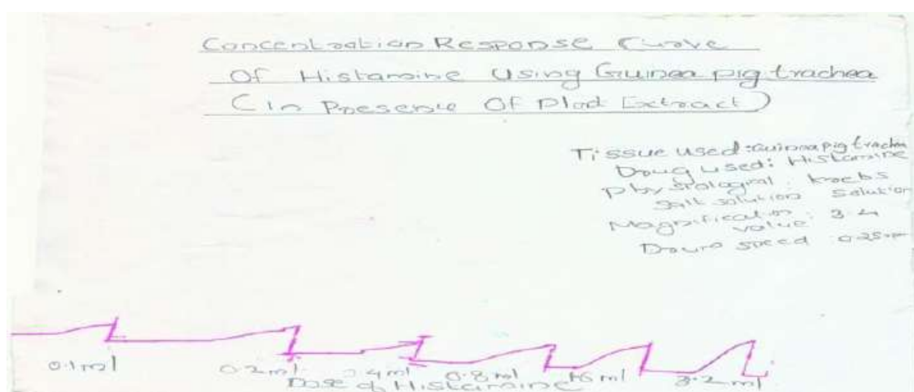


Figure No.7: Concentration Response Curve of Histamine using Guinea pig tracheal preparation (In Absence of Plant Extract).

The results were expressed in table number 8 and the effect of O. sanctum Leaves on histamine induced contraction on isolated guinea pig tracheal is shown in figure number 9

Table Effect of ethanolic extract of O. sanctum Leaves on histamine induced contraction on isolated guinea pig tracheal preparation

Sr. No	Dose of histamine (10µg/ml) in ml	Control (Histamine 10 µg/ml) % maximum contraction	Test Histamine(10µg/ml)+EEAC(1mg/ml) % maximum contraction
1	0.1	38.46 ± 1.58	30.76 ± 1.32**
2	0.2	53.48 ± 4.23	46.15 ± 2.91**
3	0.4	61.5 ± 3.89	53.48 ± 3.31**
4	0.8	73.07 ± 2.32	65.3 ± 1.76**
5	1.6	84.6 ± 2.13	69.2 ± 1.09**
6	3.2	100 ± 1.07	76.92 ± 2.11*

Values are Mean± S.E.M., where n=6 in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test

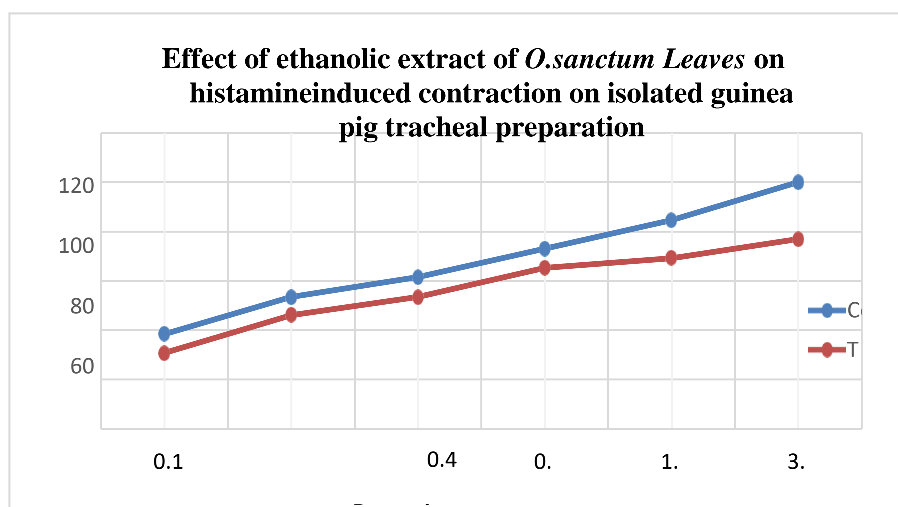


Figure No.9: Effect of ethanolic extract of O.sanctum Leaves on histamine induced contraction on isolated guinea pig tracheal preparation

IN VITRO ANTIOXIDANT ACTIVITY

Hydrogen peroxide scavenging

The hydrogen peroxide scavenging activity of ethanolic extract of O. sanctum Leaves was determined. The percentage hydrogen peroxide scavenging ability of the test extract increased in a dose dependent manner and the reference standard; ascorbic acid (100 µg/ml) exhibited 60.23% hydrogen peroxide scavenging activity. The maximum hydrogen peroxide scavenging activity shown by ethanolic extract of Alpinia calcarata rhizomes was found to be 53.3 % at 400 µg/ml.

Hydrogen peroxide scavenging activity of ethanolic extract of O. sanctum Leaves

Sr. No	Concentration(µg/ml)	Absorbance [A]	% inhibition
1	25	0.632±0.0005	17.16
2	50	0.539±0.0052	29.5
3	100	0.474±0.0056	38.04
4	200	0.414±0.0005	46
5	400	0.357±0.0032	53.3

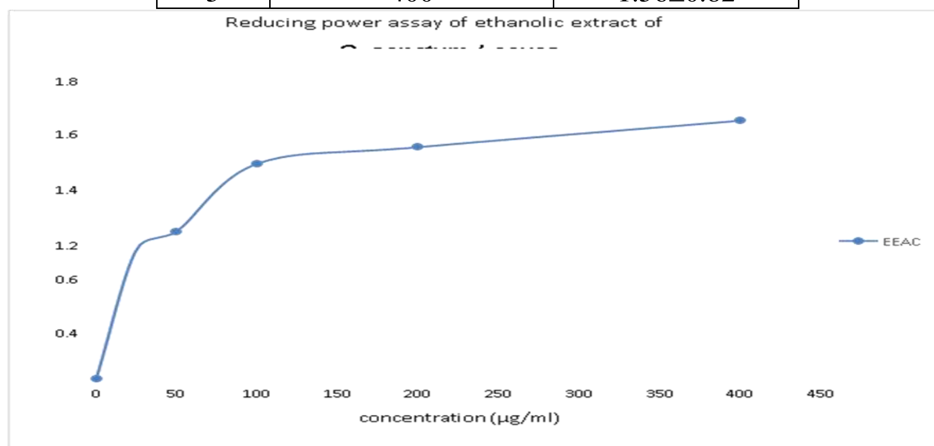
6	Ascorbic acid (100 µg/ml)	0.256±0.056	60.23

Reducing power assay

Increase in absorbance of the extract indicates the reducing power of the test sample. Reducing power of ethanolic extract of O.sanctum Leaves increased with increasing concentration. Results are expressed below

Table Reducing power activity of ethanolic extract of Leaves

Sr. No	Concentration (µg/ml)	Absorbance [A]
1	25	0.782±0.32
2	50	0.891±0.21
3	100	1.3±0.35
4	200	1.4±0.42
5	400	1.56±0.82

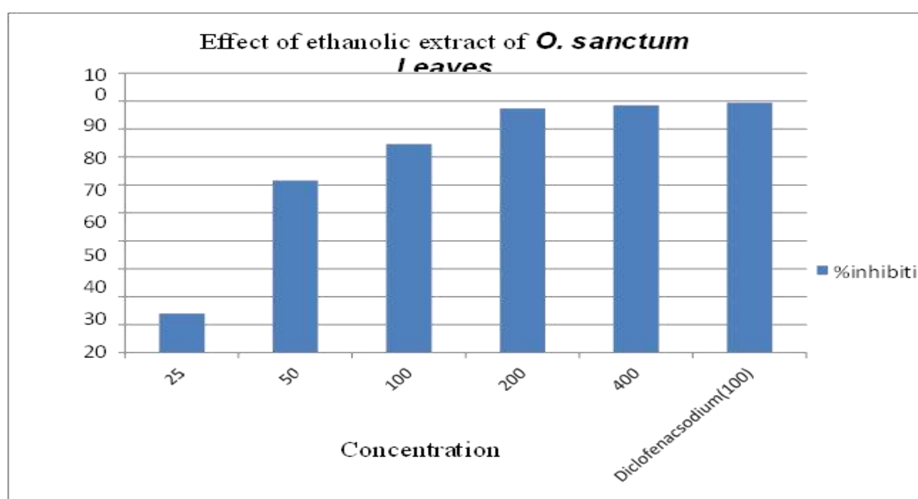


IN VITRO ANTI-INFLAMMATORY ACTIVITY

Protein denaturation

As part of the evaluation of anti-inflammatory activity, ability of plant extract on protein denaturation was studied. It was effective in inhibiting heat induced protein denaturation. Diclofenac sodium a standard anti-inflammatory agent possesses maximum % inhibition. The ethanolic extract of the plant Alpinia calcarata rhizome possess significant % inhibition activity at concentration 200 µg/ml and 400 µg/mlEffect of ethanolic extract of O.sanctum Leaves on protein denaturation

Sr. No	Concentration (µg/ml)	Absorbance [A]	% inhibition
1	25	1.28±0.05	14
2	50	0.578±0.03	61.6
3	100	0.382±0.002	74.63
4	200	0.189±0.01	87.4
5	400	0.172±0.002	88.57
6	Diclofenac sodium (100µg/ml)	0.165±0.005	89.43

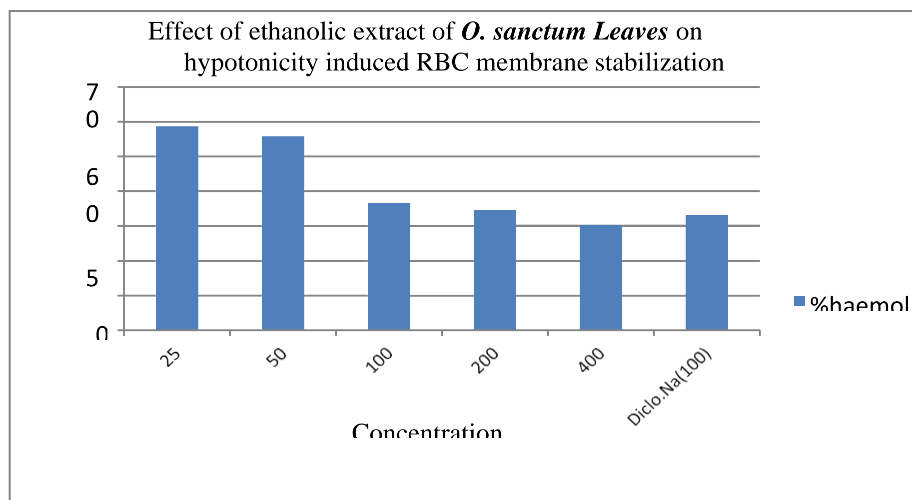


Rabbit red blood cell membrane stabilization method

Effect of ethanolic extract of O. sanctum Leaves on hypo tonicity induced RBC membrane stabilization

Sr. No	Concentration (µg/ml)	Absorbance[A]	% Protection	% Haemolysis
1	25	0.61±0.03	41.4	58.6
2	50	0.58±0.002	44.3	55.7
3	100	0.382±0.004	63.3	36.7
4	200	0.36±0.009	65.3	34.7
5	400	0.32±0.007	69.82	30.18
6	Diclofenac sodium	0.34±0.008	66.75	33.25

Effect of ethanolic extract of O. sanctum Leaves on hypo tonicity induced RBC membrane stabilization



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