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EVALUATION OF ANTI - ASTHMATIC, ANTIOXIDANT ACTIVITIES OF KAEMPFERIA ROTUNDA FLOWERS

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ABSTRACT
The study includes the phytochemical and pharmacological investigation of the ethanolic extract of *Kaempferia rotunda* flowers. The powdered flowers were extracted by means of soxhlet using the solvent ethanol. Preliminary phytochemical screening revealed the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and phenolic compounds. These constituents may be represented the presence for biological activities of the plant. The acute toxicity study revealed that there is no mortality with the ethanolic extract of *Kaempferia rotunda* flowers up to the dose level of 2000 mg/kg. Histamine contracts the tracheal-bronchial muscle of guinea pig, goat, horse, dog and man. Guinea pig trachea is used for the screening of anti-asthmatic activity. The H1 receptor after stimulation produces well-ordered dose related contraction of isolated guinea pig trachea. In current study *Kaempferia rotunda* significantly inhibited histamine induced contraction of isolated guinea pig trachea preparation indicating itsH1 receptor antagonist activity and supports anti asthmatic property of the plant. The antioxidant property was studied by hydrogen peroxide scavenging assay and reducing power assay. Hydrogen peroxide scavenging ability of ethanolic extract of *Kaempferia rotunda* flowers revealed that the extract scavenges the hydrogen peroxide. However, the hydrogen peroxide scavenging ability was low comparing to standard (ascorbic acid). Reducing power of ethanolic extract of *Kaempferia rotunda* flowers significantly increased with increasing concentration.

KEYWORDS: Ethanolic Extract, *Kaempferia rotunda* flowers, Anti - Asthmatic, Antioxidant.

INTRODUCTION
Asthma is a common inflammatory disease that affects the lungs and people worldwide are affected in millions due to this obstructive lung disease.[1-3] Important advancements have been made in the drugs used for therapy on the care, and its prevalence has not decreased much in recent decades. Although the mortality rates are considerably low, it can be avoided in many cases due to routine care.[4] But it has been projected to be the leading cause of death worldwide due to the incidence of industrialization and rapid change in the weather conditions of the earth that the pollutants and asthma causing agents can travel to far off places. It would cause impairment in the quality of life physiologically.

The major symptoms of asthma include bronchial hyper-responsiveness, increased mucus production and narrowing of airways and its remodelling which are due to the infiltration of the immune cells into the lungs and the subsequent consequences causing lung inflammation. Due to this, the patients may exhibit narrowing of the airways and accumulation of the mucus causing shortness of breath, chest discomfort, wheezing, and cough.[4-6]

In recent year there has been tremendous 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 compilations. *Kaempferia rotunda* is also considered to be therapeutically important in traditional system of medicine. Aim of the study to evaluate the anti-asthmatic, antioxidant and anti-inflammatory activities of ethanolic extract of *Kaempferia rotunda* flowers

Materials
Plant selected in the present study, *Kaempferia rotunda* was selected because of its traditional uses. The part used was flowers.

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 CCSEA, New Delhi, India.

Methods of Collection and Authentication

The dried flowers of the *Kaempferia rotunda* were collected locally from local area of Tirupati. The flowers were cleaned and shade dried and milled into coarse powder by a mechanical grinder. Preparation of plant extract. The powdered flowers were extracted using ethanol by soxhlet extractor. In this process the powdered drug is placed into the extractor with ethanol as solvent. After extraction the extract was concentrated by evaporation then it was kept in a refrigerator for further use. Preliminary phytochemical screening the ethanolic extract of *Kaempferia rotunda* flowers were subjected for the following chemical tests for the identification of various active constituents.

Acute toxicity studies

Acute toxicity of *Kaempferia rotunda* 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 behaviours 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.

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 preconvulsion 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).

***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 15 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.

***In vitro* antioxidant activity**

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.

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.

Statistical analysis

The statistical analysis was carried out by using oneway analysis of variance (ANOVA) followed by Dunnett' s multiple comparison test. The results are expressed as Mean±S.E.M., n=6

RESULTS

- **Collection & Authentification of *kaempferia rotunda* flowers**

The flowers of *Kaempferia rotunda* were collected and authentified.

- **Extraction of plant material**

Kaempferia rotunda flowers was collected, washed and shade dried. Dried flowers 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**

The phytochemical screening of the ethanolic extract of the *Kaempferia rotunda* flowers indicate the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and phenolic compounds.

- **Acute toxicity studies**

Acute toxicity studies were 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 asthmatic activity**

***In vivo* anti-asthmatic activity Histamine aerosol induced bronchoconstriction in guinea pigs**

The present study deals with the screening of anti-asthmatic activity of ethanolic extract of *Kaempferia rotunda* flowers by histamine induced bronchoconstriction in guinea pigs. The ethanolic extract of the plant expressively extended the latent period of convulsion followed by exposing to histamine at the dose 200 mg/kg at time 4 hours as compared to standard drug. The % protection was calculated from the latent period of convulsion. The maximum % protection of ethanolic extract of the plant was calculated as 60.79% at 200 mg/kg. The standard drug used was chlorpheniramine maleate which showed significant % protection at time 1 hour and 4 hours. The plant extract at 100 mg/kg showed 43.2% protection at time 1 hour and the 100 mg/kg plant extract also showed40.2% protection at time 24 hour and also showed 57.2% protection at time 4 hour. The control (Carboxy methyl cellulose) produced 10.9 % protection at time 1 hour and 12.3% protection at time 4 hour and 11.4% protection at time 24 hour. The plant extract at 200 mg/kg showed 48% at time 1 hour and 60.79% at time 4 hour and 44.3% at time 4 hour and 44.3% at 24 hours. The standard drug chlorpheniramine maleate possess 69.76% protection at time 1 hour and 78.3% at time 4 hour and 50.1% at time 24 hour

***In vivo* anti-asthmatic activity Histamine aerosol induced bronchoconstriction**

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***Ex vivo* anti-asthmatic study**

Isolated guinea pig tracheal preparation Histamine (10 µg/ml) produced dose dependent contraction of guinea pig tracheal preparation. Pre-treatment with the ethanolic extract of *Kaempferia rotunda* flowers (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.

***In vitro* antioxidant activity**

The hydrogen peroxide scavenging activity of ethanolic extract of *Kaempferia rotunda* flowers 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 *Kaempferia rotunda* flowers was found to be 53.3 % at 400 µg/ml. The hydrogen peroxide scavenging effect of ethanolic extract was shown in table number 7.

Reducing power assay

Increase in absorbance of the extract indicates the reducing power of the test sample. Reducing power of ethanolic extract of *Kaempferia rotunda* flowers increased with increasing concentration. Results are expressed below.

DISCUSSION

The study includes the phytochemical and pharmacological investigation of the ethanolic extract of *Kaempferia rotunda* flowers. The powdered flowers were extracted by means of soxhlet using the solvent ethanol.

Preliminary phytochemical screening revealed the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and phenolic compounds. These constituents may be represented the presence for biological activities of the plant.

The acute toxicity study revealed that there is no mortality with the ethanolic extract of *Kaempferia rotunda* flowers up to the dose level of 2000 mg/kg.

Asthma is an allergic disease with the utmost clinical and economic effect is an allergic and inflammatory outward sign of respiratory disorders. Asthma is a respiratory disease. The symptoms of bronchial asthma are characterized by wide blowout narrowing of the bronchial tube due to contraction of smooth muscle in replay to stimuli subsequently in the release of histamine.

Bronchoconstriction induced by histamine is an immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to spasm in guinea pigs and causes very strong smooth muscle contraction and capillary dilation in cardiovascular system. Bronchodilators can delay the occurrence of these symptoms. The study revealed the H_1 receptor antagonistic activity and support the plant by anti-asthmatic property.

Herbal formulations used in the treatment of asthma include some anti-stress herbs to enable adoption to stress since excessive stress or nervous debility may aggravate symptoms of asthma.

Histamine contracts the tracheal-bronchial muscle of guinea pig, goat, horse, dog and man. Guinea pig trachea is used for the screening of anti-asthmatic activity. The H1 receptor after stimulation produces well-ordered dose related contraction of isolated guinea pig trachea.

In current study *Kaempferia rotunda* significantly inhibited histamine induced contraction of isolated guinea pig trachea preparation indicating its H_1 receptor antagonist activity and supports anti asthmatic property of the plant. The antioxidant property was studied by hydrogen peroxide scavenging assay and reducing power assay.

Hydrogen peroxide scavenging ability of ethanolic extract of *Kaempferia rotunda* flowers revealed that the extract scavenges the hydrogen peroxide. However, the hydrogen peroxide scavenging ability was low comparing to standard (ascorbic acid).

Reducing power of ethanolic extract of *Kaempferia rotunda* flowers significantly increased with increasing concentration.

CONCLUSION

The result of the investigation showed that the ethanolic extract of *Kaempferia rotunda* flowers possess anti-asthmatic activity.

The antioxidant property of the plant also supports its anti-asthmatic property. Drugs effective in asthma are mostly steroidal in nature. Phytochemical analysis showed presence of flavonoid and steroids. The anti-asthmatic property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for anti-asthmatic activity.

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