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Evaluation of Anti-Asthmatic and *In Vitro* Anti-Oxidant Potential of *Tragia involucrata* Linn

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Abstract

The objective is to evaluate the *in vivo* anti-asthmatic and *in vitro* antioxidant potential of Hydroalcoholic Leaf Extract of *Tragia involucrata* (HAETI) on experimental animals. *In vivo* anti-asthmatic activity of HAETI was evaluated by Arachidonic acid-induced Leucocytosis and Eosinophilia in guinea pigs, Arachidonic acid-induced mast cell degranulation in guinea pigs, and Mast cell Degranulation studies. Parameters like hematological analysis, percentage protection against mast cell degranulation, and time of occurrence of Pre-Convulsion Dyspnea (PCD) were calculated as the end point of the study. Further sections of the lung were prepared for histopathology analysis. In addition, *in vitro,* anti-oxidant studies were carried out to determine the percentage of inhibition of HAETI on oxidative stress parameters. After the assigned treatment to the group of animals with HAETI showed normalized hematological parameters, the bronchodilatation effect was confirmed by a significant ($p<0.001$) increase in the latency time of Pre Convulsion Dyspnoea (PCD) and pre-treatment with HAETI in mast cell degranulation study showed significant ($p<0.001$) reduction in degranulation of mesenteric mast cell number. The histopathological analysis of lung sections showed a reduction of total histological score in HAETI-treated guinea pigs compared with the disease control group ($p< 0.0001$). Based on IC_{50} values from *in vitro* assays, the free radical scavenging property of HAETI was confirmed due to the presence of active phytoconstituents. Based on the above findings, it was concluded that *Tragia involucrata* could be effectively used in the treatment of asthma and justified with traditional claims of the plant.

Keywords: Asthma Inflammation, Arachidonic Acid, *Tragia involucrata*

1. Introduction

Asthma is the most prevalent disorder among the worldwide population, which affects the daily routine life and normal survival of a person with dependence to long-term medication. It is characterized by recurrent respiratory symptoms such as wheezing, breathlessness, chest tightness, coughing, and variable airflow obstruction that is reversible spontaneously over a period of time with or without treatment. The prevalence of asthma has increased in recent decades, which accounts to nearly 7%-10% of the world population in each year[1]. The sequence of events in an asthmatic attack starts from bronchoconstriction, mucous production, inflammation of the lungs and

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trachea, and severe wheezing with breathing difficulty. Bronchial hyperactivity with constriction of the airways are the common features involved in the pathogenesis of asthma, characterized by mucus formation and swelling in the airways. In this inflammatory process, several types of mediators are involved and drugs available were used for the management of asthma like narcotics (e.g., Opioids), non-narcotics (e.g., salicylates), and corticosteroids like hydrocortisone[2,3]. These drugs produce symptomatic relief, provide round-the-clock inhibition, and are effective in asthma management but the treatment regimen has to be followed for the long term, the dependency on the medication willbe increased and resistance to the drug will develop over a period of time. Furthermore, prolonged medication may cause adverse effects such as gastrointestinal ulcers and thrombosis leading to discontinuation of treatment[4]. Hence, there is a need to evaluate and standardize a drug or formulation, which is nontoxic and free of any associated adverse effects.

Traditional systems of medicine are indigenous medicines, people are dependent on them for their healthcare needs. Therapeutic claims of most of the herbal drugs and their formulations still have not been validated. The nature of drug action of most of the herbal formulations and individual plants of the traditional system of medicine is not well established and documented. Asthma is triggered by factors such as allergens, and pollen, and by the release of inflammatory mediators called histamine, which belongs to the group Leukotrienes. The antibody involved in the production of allergic reaction is IgE which contains about a thousand tiny granules called histamine. The other factors that trigger asthma include cold air, exercise, and emotional stress.

Free radicals rotate on the peripheral layer around the nucleus as unpaired electrons and they act as chemical substances causing oxidative stress. ROS are ions, atoms, or molecules that have the ability to oxidize reduced molecules and various forms of activated oxygen such as superoxide anion radicals ($.O_2$) and hydroxyl radicals ($OH\cdot$), as well as non-free radicals (H_2O_2) and singlet oxygen[4]. Reactive Oxygen species are the major sources of primary catalysts that create oxidative stress and cause oxidative damage leading to numerous diseasesand disorders such as cancer cardiovascular disease, neural disorders, Alzheimer's disease, DNA damage, and various lung disorders like asthma, chronic obstructive, pulmonary disorders, acute lung injury and lung cancer[5]. The ROS directly stimulates histamine release from mast cells and mucus secretion from airway epithelial cells resulting in asthma. Plants are important sources of natural antioxidants such as vitamins C and E, carotenoids, flavonoids, and Tannins and potent free radical scavengers. At present synthetic antioxidants are used but cause many side effects such as the risk of liver damage and carcinogenesis[6]. Hence, there is a need to produce natural, more effective, less toxic, and cost-effective antioxidants. *T. involucrata* L. is commonly found in tropical regions of India as a weed and it belongs to the family Euphorbiaceae. The decoction of leaves of this plant is used for many disorders such as scabies, cut wounds, inflammation and skin infection, headache, fever, skin, venereal complaints, and baldness among the tribal communities[7]. The fresh leaves cause burning pain and inflammation because of the leaf hairs that contain acid, hence it can be made into powder form so that the acid effect can vanish. The same can be proved by administration of this drugin powder form to the laboratory animals. To understand this concept and to explore the effectof the plant extract on experimental animals using appropriate screening methods, the safety/toxicity profile and efficacy of the plant material can be established. According to the traditional system of Indian Medicine, the whole plant is widely used for loss of appetite, cough, asthma, and urinary disorders[8]. Although, no scientific study is reported and documented to prove the traditional claim this investigation was conducted to evaluate the anti- asthmatic effect of the Hydroalcoholic leaf extract of *Tragia involucrata*

2. Materials and Methods

2.1 Plant Material

Leaf of *Tragia involucrata* Linn. belongs to the family Euphorbiaceae were collected from Kasampatti, Natham (Tk) Dindigul (Dt) and was authenticated by Dr. Stephen, Professor, Department of Botany, American college, Madurai-20. The herbarium of this specimen was kept in the department for further reference.

2.2 Chemicals

Hydrogen peroxide, Phosphate buffer, Ascorbic acid, Potassium Ferricyanide, Trichloroacetic acid, Ferric chloride, sulphuric acid, Sodium phosphate, ammonium phosphate. All the chemicals and reagents used in this project are of analytical grade.

2.3 Extraction

About 250 g of the dried leaves were crushed into pieces, dried under shade, and made into a coarse powder suitable for extraction. The solvents used for extraction were water and ethanol in the ratio of 30:70 %. Then by using the maceration technique the powder was extracted completely and filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a dark solid residue. The yield of the extract was 5 g. Then it is stored at 4°C for further use. The percentage yield of the extract was found to be 2%.

2.4 Experimental Animals

About 40 guinea pigs weighing 350-400 g of either sex were utilized for anti-asthmatic activity. From this, a total of 20 animals were used for the induction of leucocytosis, eosinophilia, and mast cell degranulation by the inducing agent arachidonic acid. Then 10 animals were used for Arachidonic acid bronchospasm and the remaining 10 animals were used for Acetylcholine-induced bronchospasm. They were obtained from the Animal House facility of Arulmigu Kalasalingam College of Pharmacy, Virudhunagar district, Tamil Nadu. The animals were housed in standard polypropylene confines at 25 ± 2 °C with 12 hrs light and 12 h dark cycles. They were fed with a standard pelleted diet (VRK Nutritional Solutions, Pune) and had free access to water. The experiment was conducted as per CCSEA guidelines and permission to conduct these experiments was obtained after being duly approved by the Institutional Animal Ethics Committee which bears the IAEC approval number: AKCP/IAEC/79/2018-19.

2.5 Experimental Design

2.5.1 Arachidonic Acid-Induced Leucocytosis and Eosinophilia

Guinea pigs of either sex were divided into four groups of 5 animals in each

Group I received distilled water 10 ml/kg, p.o.

Group II received arachidonic acid in a dose of 100mg/kg, (0.1 % i.p)

Group III received arachidonic acid in a dose of 100 mg/kg, i.p and HAETI 200 mg/kg, p.o

Group IV received arachidonic acid in a dose of 100 mg/kg, i.p and HAETI 400 mg/kg, p.o

An acute oral toxicity study was conducted in mice and it was found that the methanolic extract of the leaf of *Tragia involucrata* was safe up to the dose level of 2000 mg/kg b.w[9]. So based on that 1/10 th and 1/5th of the dose i.e., 200 mg/kg, 400 mg/kg b.w was selected for the efficacy study. After the above-assigned treatment to each group, animals were placed in the anesthesia induction system (Somno Suite Anesthesia Chamber, Kent Scientific, USA) and according to the body weight the dose of Isoflurane is administered to the animal for the induction of anesthesia and nose cone is placed for the maintenance of anesthesia. Blood samples were collected from each guinea pig from the medial canthus of the eye under a nictitating membrane at 45° angle in K_2 EDTA tubes using heparinized capillary tubes and analyzed using a Haematology Vet5 analyzer (Diatron, Hungary). Total leukocyte count and eosinophil count were recorded in Group II before drug administration and 24 hrs after arachidonic acid. The same parameters were calculated for Group III, and Group IV after 24hrs of administration of arachidonic acid and drug treatment HAETI 200 mg/kg p.o. and 400 mg/kg p.o. respectively[10]. The results of Group II were compared with the control Group I. The results of Groups III and IV were compared with the results of Group II. Total Leukocyte count and eosinophil count were calculated for all the Groups and the results are given in Table 1

2.5.2 Arachidonic Acid-Induced Mast Cell Degranulation in Guinea Pig

Guinea pigs of either sex were divided into four Groups of 5 animals in each

Group I - Distilled water 10 ml/kg b.w orally for 7 days.

Group II - Arachidonic acid 0.1 % w/v (i.p)+Sodium cromoglycate intraperitoneally 0.5 mg/kg for 7 days

Group III - Arachidonic acid 0.1 % w/v (i.p)+HAETI 200 mg/kg b.w. orally for 7 days

Group IV - Arachidonic acid 0.1 % w/v (i.p)+HAETI 400 mg/kg b.w. orally for 7 days

Except for Group I all the other groups were treated intraperitoneally injected with 10 ml/kg b.w. of normal saline into the peritoneal cavity and the abdomen was massaged gently for about 90 seconds. To determine the percentage of protection of the test drug (intact and disrupted), the mast cell suspension will be prepared by the following method.

Table 1. Results of Arachidonic acid-induced Leucocytosis and Eosinophilia in guinea pig

S. No	Groups	Diff in no. of Leukocyte Count (per cu mm)	Diff in no of Eosinophil Count (per cu mm)
1.	Distilled water(10 ml/kg)	82.4 ± 4.8	24.60 ± 4.05
2.	AA (100 mg/kg)	4004 ± 20.80	168 ± 6.09
3.	AA + HAETI (200 mg/kg p.o.)	2980 ± 90.29**	150 ± 6.80**
4.	AA + HAETI (400 mg/kg p.o..)	1820 ± 15.30**	141 ± 6.82**

n = 5, values are mean ± SEM. @ p<0.001. Group II was compared with Group I (Student's 't' test) *p<0.05, **p<0.01, Group III, and Group IV were compared with Group II. (ANOVA followed by Dunnet's Test).

Table 2. Arachidonic acid-induced mast cell degranulation

Mast Cell Percentage				
S.No	Treatment	Intact	Disrupted	% Protection
1	Distilled water (10 ml/kg)	23.7 ± 0.45	86.3 ± 0.45	-
2	Sodium Cromoglycate (0.5 mg/kg)	73.20 ± 6.25**	26.80 ± 6.25**	86.03
3	HAETI (200 mg/kg)	49.02 ± 0.23**	50.98 ± 0.23**	50.71
4	HAETI (400mg/Kg)	40.05 ± 5.01**	59.95 ± 5.01**	59.68

n=5, **p<0.01, Group II, III and IV compared with Group I (ANOVA followed by Dunnet's test)

The peritoneal cavity was located and carefully opened, using an aspirator needle to locate the site, and the contents of the mast cells were collected in a siliconized test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4). The collected sample was centrifuged using a Microcentrifuge (5418 R, Eppendorf) at a very low speed of 400-500 rpm for 5 minutes. After that supernatant was removed using a micropipette and the mast cell pellets were collected and washed three times with 0.9% w/v isotonic saline and it was carefully taken from the medium. Then 0.1% w/v solution of arachidonic acid was added to the mast cell suspension (approximately 1 x 10^6/ml) and incubated at 37°C in a water bath for 10 min. Later they were stained with 1 % Toluidine blue (Dye) and observed under a high-power microscope field (Compound Microscope 40 X). A total of 100 cells were counted from different visual areas and percentage protection against induced mast cell degranulation was calculated[11]. The results are shown in Table 2

% inhibition of mast cell degranulation = 1-(DMC/TMC) x 100

DMC = Degranulated mast cells

TMC = Total Mast cells

2.5.2.1 Histopathology Studies

Animals were sacrificed using an Isoflurane anesthesia chamber with a predetermined flow rate and volume of injection according to the body weight of the animal. The vital organ like the lung was carefully dissected out, trimmed, and perfused with 10% NBF with the help of a syringe. Lung sections were prepared and fixed in 10% neutral buffered formalin, after 24-48 hrs of fixation, tissue was embedded using paraffin wax, cut into 2 µm sections using a semi- automated microtome (Leica Biosystems), and stained with hematoxylin-eosin and observed under compound microscope with 40 X magnification. Inflammatory parameters in lung tissue (peribronchial, perivascular, and parenchymal infiltration of inflammatory cells) of bothcontrol and drug treatment groups were evaluated blindly by a senior lung pathologist. This study was to find out the intact and degranulated mast cells. The results were shown in Figure 1.

2.5.3 Arachidonic Acid and Acetylcholine Induced Bronchospasm

Based on the body weight, the animals were randomized by stratified randomization method and divided into two groups of five animals in each. To determine the time of occurrence of Pre Convulsion Dyspnea (PCD), animals were administered intraperitoneally with 0.1% w/v of Arachidonic acid and placed in a closed chamber (M/s Inco, Ambala, India), to observe signs of dyspnoea. The preconvulsion time was measured i.e., from the time of

Table 3. Arachidonic acid and Acetylcholine induced bronchospasm

Treatment Groups	Preconvulsive Dyspnea time (PCD time in Sec)					
	Arachidonic Acid-Induced Bronchospasm			Acetylcholine Induced Bronchospasm		
HAETI	Before Treatment	After Treatment	% increase	Before Treatment	After Treatment	% increase
200 mg/kg	105.6 ± 2.30	282.42 ± 3.11*	62.6	145.06 ± 1.12	202.20 ± 2.06*	28.25
400 mg/kg	115.01 ± 1.88	530.01 ± 4.52*	78.3	136.02 ± 0.20	252.02 ± 3.06*	46.06

value are expressed as mean ± SEM, n = 5, * p<0.001, as compared to control group

drug administration to the induction of the difficulty/ struggle for breathing. This was noted as Pre Convulsion Dyspnea Time (PCT) of day 0 value. After the day 0 induction, the animals were exposed to fresh air and proceeded for further study.

Group I – On day 0 induction (0.1 % Arachidonic acid i.p) + HAETI 200 mg/kg b.w. for 7 days + On the 7th day 2 h after the last dose of HAETI (0.1 % w/v of Arachidonic acid i.p)

Group II – On day 0 induction (0.1 % Arachidonic acid i.p) + HAETI 400 mg/kg b.w. + On the 7th day 2 h after the last dose of HAETI (0.1 % w/v of Arachidonic acid i.p)

The test drug HAETI 200 mg/kg b.w. and 400 mg/kg b.w. was administered orally to both of the treatment groups respectively and observed for 7 days. On the 7th day 2 hours after the last dose of HAETI, the animals were administered with the inducing agent (0.1% Arachidonic acid i.p.) and tested for the same parameters as discussed above. The percentage increase in the latency of occurrence of PCD time in seconds was calculated using the following formula.

Percentage increase in time of PCD = (1-T1/T2) × 100, where T_1 = time of occurrence of Pre Convulsive Dyspnea in seconds on day 0

T_2 = Time of occurrence of Pre Conculsive Dyspnea in seconds on day 7. The results were obtained in Table 3

The same procedure was followed in another set of animals (n = 5) for acetylcholine to inducebronchospasm using 0.5% acetylcholine chloride, the parameters as discussed above was measured[12].

2.6 *In vitro* Antioxidant Studies

2.6.1 Determination of Scavenging Activity Against Hydrogen peroxide

The Scavenging activity against Hydrogen peroxide was determined using different concentrations of test samples, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then

0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min using a Cary 60 UV-Visible Spectrophotometer (Agilent Technologies). The reaction mixture without a sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula[13]

% inhibition = [(Control–Test) / Control] × 100

The concentration of the sample required for a 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis. The results were showed in Figure 2.

2.6.2 Determination of Reducing Power Assay

The reducing power ability of the test extract was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned by Oyaizu *et al.*, (1986). 0.1 to 0.5 mL of plant extract solution at the concentration of 1 mg/mL was prepared and mixed with 0.75 mL of phosphate buffer and 0.75 mL of 1% potassium ferricyanide [K3Fe (CN6)] and incubated at 50°C for 20 min. About 0.75 mL of 10 % trichloroacetic acid was added to the mixture and allowed to stand for 10 min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally, 1.5 mL of the supernatant was removed and mixed with the same volume of distilled water and 0.1mL of 0.1 % ferric chloride solution, and the absorbance was measured at 700 nm in Cary 60 UV-Visible Spectrophotometer (Agilent Technologies). Ascorbic acid was used as standard and phosphate buffer was used as blank solution[14]. The results are shown in Figure 3.

2.6.3 Determination of Total Anti-Oxidant Activity

To the small aliquot of test extract of about 0.3 mL of different concentrations was treated with 2.7 mL of

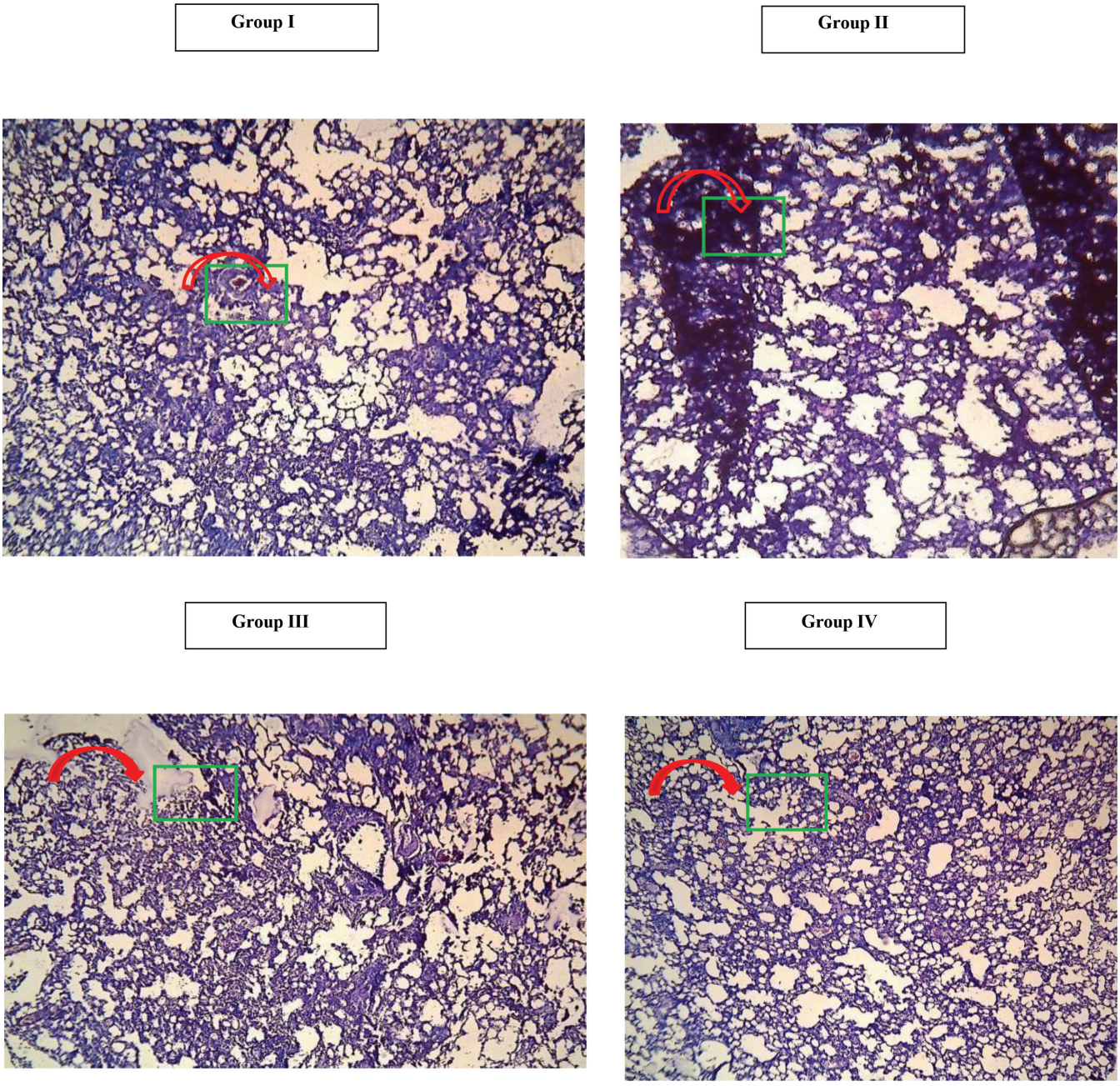


Figure 1 Histopathology of lung tissue under 40X magnification.

the reagent containing H_2SO_4, sodium phosphate, and ammonium molybdate. In the case of blank, 0.3 mL of methanol was used in place of the sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature; the absorbance of the aqueous solution of each concentration was measured at 695 nm using a Cary 60 UV-Visible Spectrophotometer (Agilent Technologies) against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/mL)[15]. The results were shown in Figure 4.

2.7 Statistical Analysis

The results were interpreted as mean ± SEM. Statistical analyses were made using an ANOVA table followed by Fisher's post-hoc test to determine statistical significance between groups (significant result $p < 0.05$).

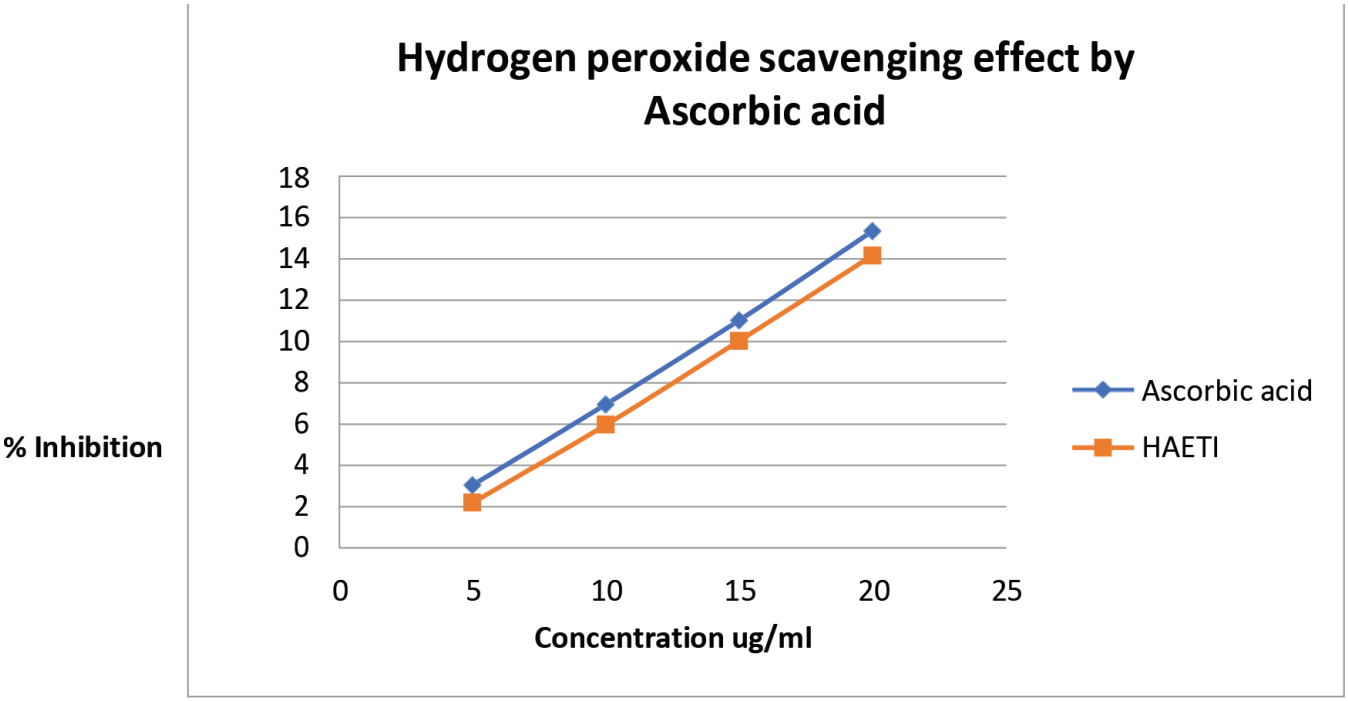


Figure 2. Determination of Hydrogen peroxide scavenging activity of *Tragia involucrata*.

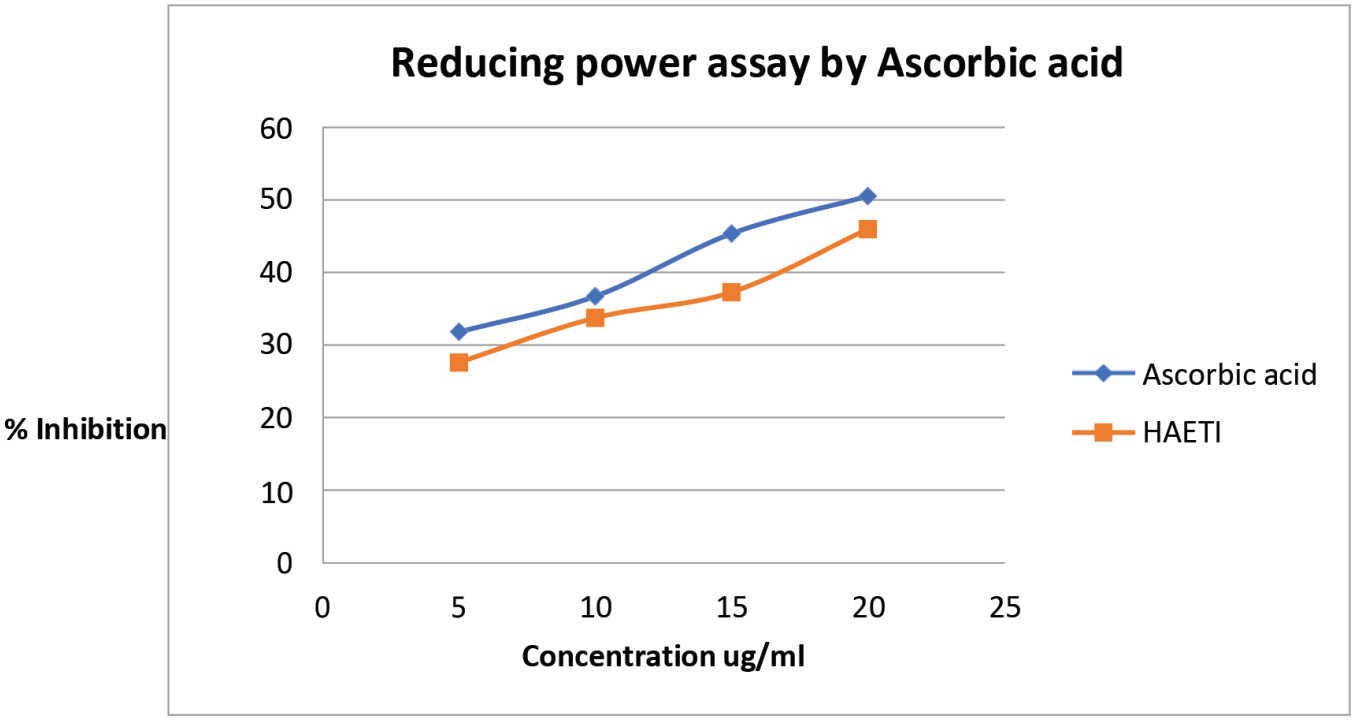


Figure 3. Determination of reducing power assay of *Tragia involucrata*.

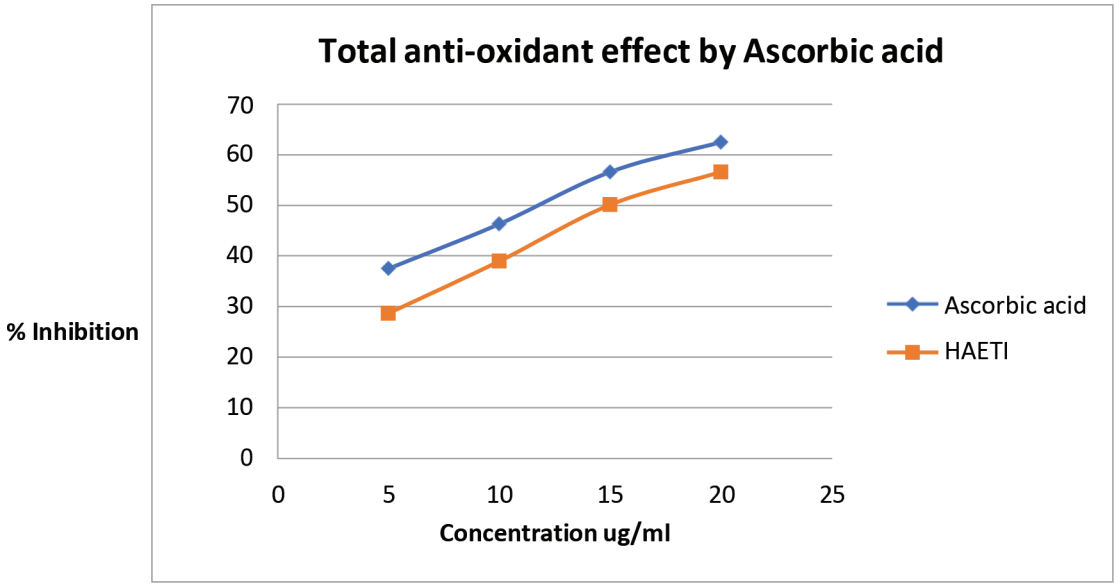


Figure 4. Determination of Total anti oxidant effect by Ascorbic acid of *Tragia involucrata*.

3. Results and Discussion

In the present study, we have observed that Group I animals after intra-peritoneal administration of arachidonic acid showed a significant increase in leukocyte count, whereas the drug-treated group, showed a normal leukocyte count (Table 1). This indicates that HAETI has normalized the differential blood counts which was drastically increased by the arachidonic acid a triggering factor for inducing inflammatory mediators. An increase or decrease in leukocyte count after a triggering event causes a series of changes in the immune system of the human body[18]. To have a healthy immune development and a protective role against pathogens, the influence of blood parameters plays a vital role.

In response to the allergens and non-allergens in most cases of asthmatic condition induced by those agents i.e., allergic inflammatory mediators and non-allergic inflammatory mediators, including the cases of mild asthma triggered in the series of the inflammatory cascade and those who have bronchial eosinophilia and it is observed from our end that there is a significant association between eosinophil activation and asthma severity a contributing factor for bronchial hyper responsiveness[19]. The increase in eosinophil count by the cascade of events due to the release of inflammatory mediators shows the severity of infection in bronchial asthma. In the present study, it has been found that administration of arachidonic acid (100 mg/kg i.p) to the Group I animals showed a significant increase in the eosinophil count after 24 hours, whereas, the drug-treated groups i.e., HAETI significantly inhibited arachidonic acid- induced eosinophilia in the guinea pig. (Table 1). So based on this inference, we can conclude that HAETI has some inhibitory responses over the blood parameters like eosinophils which are meagre in number but are responsible for causing allergic inflammatory reactions. The defense mechanism which is contributed by our white blood cells should go in parallel line with the homeostatic mechanism of our body to fight against the allergic responses.

In the sequence of the initiation of inflammatory responses, following exposure to allergens mast cell degranulation will be notified from the tissue storage site. From the tissue storage site, massive release of histamine occurs in which granulated ones turn into degranulated mast cells due to the inflammatory changes which may include submucosal infiltration with activation of mast cells, epithelial changes, and basement membrane thicknening[20]. An attempt was made to find out whether HAETI has any effect on the rate of disruption of mast cells following exposure to arachidonic acid, an agent that causes histamine release. It was done in the same manner as discussed above by the inducing agent arachidonic acid (0.1% w/v, 100 mg/kg b.w) and the parameters observed were by cytological (100 number of cells), histological (by lung sections). The total number of degranulated cells and total mast cells can be calculated. In the histological

examination of lung tissue under 40 X magnification we can see the intact and degranulated mast cell. Based on the results it is clear that HAETI offered significant protection against arachidonic acid-induced mast cell degranulation by stabilizing it, which is responsible for decreasing airway inflammation by preventing the release of various inflammatory mediators. The broncho-dilating effect of HAETI was evaluated by observing its effects on the time of occurrence of PCD. In our study, we found that the time of occurrence of PCD was significantly increased suggestive of bronchodilating activity of HAETI against arachidonic acid (Table 3). The Pre convulsion dyspnea time measured gives valuable information about the nature of the mechanism of action of drugs i.e., the drug that increases the latency in the occurrence of dyspnea.

Histopathology analysis of lung specimens of Group II animals showed a marked peribronchial, perivascular, and parenchymal inflammation of inflammatory cells in the arachidonic acid group compared with Group I animals. Guinea pigs treated with 200 and 400 mg/kg b.w. HAETI showed decreased tissue inflammation by mononuclear cells and eosinophils compared with non-treated guinea pigs showing that the treatment with HAETI has some tendency to decrease tissue inflammation by inflammatory cells, but results were not significantly different from those of non-treated guinea pigs (Figure 1). These results were confirmed by a reduction of the total histological score in HAETI-treated guinea pigs compared with non-treated guinea pig. ($P< 0.0001$, n = 5 guinea pig per group)

Hydro-alcoholic extract was further subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, total antioxidant capacity, and reducing power assay. The inhibitory concentration (IC_{50}) of *Tragia involucrata* against hydrogen peroxide scavenging effect is found to be 64.87 µg/ml in comparison with standard ascorbic acid 62.39 µg/ml as shown in Figure 2. Hydrogen peroxide is decomposed into hydroxyl radical which in turn initiates lipid peroxidation and causes DNA damage. The free radical scavenging effect i.e., hydrogen peroxide scavenging is confirmed by the presence of phenolic compounds in the hydroalcoholic extract. The phenolic compound could donate electrons to hydrogen peroxide, thereby neutralizing it into water[21]. The inhibitory concentration (IC_{50}) of *Tragia involucrata* against reducing power assay depends on the presence of reductants[22] by breaking free radical chain, donating a hydrogen atom and was found to be 24.35 µg/ml in comparison with standard ascorbic acid 19.37 µg/ml as shown in Figure 3. The inhibitory concentration (IC_{50}) of *Tragia involucrate* (leaf) against total antioxidant assay was found to be 15.89 µg/ml in comparison with standard ascorbic acid 12.07 µg/ml as shown in Figure 4. The concentration of the test drug and ascorbic acid is almost the same and the test drug also possesses maximum anti-oxidant effect as that of standard ascorbic acid in scavenging the free radicals.

4. Conclusion

From all the above results we can clearly understand that the hydroalcoholic extract of *Tragia involucrata* was found to possess anti-asthmatic activity by blocking the histamine (H_1) mediated bronchoconstriction and possess anti-oxidant potential due to the presence of naturally occurring phenolic compound flavonoids in the hydroalcoholic extract of *Tragia involucrata.*

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