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Aim: Investigation of Anti–Asthmatic Potential of *Dillenia Indica* (L.) in Ovalbumin– Induced Bronchial Asthma and Airway Inflammation in Wistar Rats.

Nazmul Islam, Burhan Ali, Dr. Sumit Das, Samsuj Zaman, Shahjalal Alam Piyali Dey, Santa Mandal

Huda Group of Institutions

ABSTRACT

Bronchial asthma is characterized by airflow obstruction, bronchial hyperresponsiveness, and inflammation in bronchial airway. Conventional treatments often produce huge margin of side effects, so it is necessary to switch on to the alternative therapies to minimize the side effects. *Dillenia indica* (L.), a medicinal anti–inflammatory properties possessing plant, was evaluated for its potential anti–asthmatic effects. This study aims to investigate the efficacy of *Dillenia indica* (L.) in mitigating symptoms of bronchial asthma and airway inflammation in an ovalbumin–induced asthma model in Wistar rats. Wistar rats were sensitized and challenged with ovalbumin to induce bronchial asthma. The test rats (groups) were given different concentration of doses of *Dillenia indica* (L.) extract. Parameters to be examined are airway hyperresponsiveness, lung histopathology, inflammatory cell infiltration. Compare the parameters among a control group and a standard anti–asthmatic drug–treated group. *Dillenia indica* (L.) significantly reduced airway hyperresponsiveness and inflammatory cell infiltration in the lungs. Histopathological analysis showed decreased mucus production and epithelial cell damage in treated groups. The effects were dose–dependent and comparable to those observed with the standard anti–asthmatic drug. *Dillenia indica* (L.) exhibits promising anti–asthmatic properties, likely due to its anti–inflammatory and antioxidant activities. This study supports the potential use of *Dillenia indica* (L.) as a complementary therapeutic agent in the management of bronchial asthma. Further research is warranted to isolate the active compounds and understand the underlying mechanisms of action.

Keywords: *Dillenia indica* (L.), Bronchial Asthma, Airway Inflammation, Ovalbumin, Wistar Rats, Anti–Inflammatory

1. Introduction

1. Bronchial asthma is chronic life–threatening airway disease which is linked airway obstruction, airway inflammation, and airway hyper responsiveness [1]. Since asthma affects around 334 million people globally and is the third most common cause of hospital admission, its prevalence has increased in recent decades [2]. The inflammatory response in asthma is thought to be primarily driven by T helper cells, which are activated when an asthma attack progresses. These cells release a variety of cytokines, including TNF–α and interleukins, primarily IL–4, IL–5, and IL–13, as well as activating β–lymphocytes that produce IgE specific to allergens and release the arachidonic acid pathway metabolites, primarily leukotrienes and prostaglandins [3]. In addition to increased airway smooth muscle contraction and releasing histamine from degranulated mast cells, this will result in the well–known symptoms of asthma, such as mucus overproduction from goblet cells, bronchospasm, reversible airway blockage, and airway hyperresponsive with eosinophilic lung [4].

2. Asthma is a heterogeneous illness that manifests in a variety of ways, both immediately and over time. Numerous variables, such as age, sex, socioeconomic level, race and/or ethnicity, and interactions between genes and environment, all have an impact on this heterogeneity. Since there is currently no specific physiologic, immunologic, or histologic feature that can be used to definitively diagnose asthma, the diagnosis is typically made based on clinical factors that are related to the patient's symptoms (such as obstruction of the airways and hyperresponsiveness) and how they respond to treatment (such as partial or complete reversibility) over time [5–7].

3. In addition to mucosal edema, mucus clogging, and increased secretion, bronchial asthma is often characterized by hyperresponsiveness of the tracheobronchial smooth muscle to various stimuli, resulting in air route closure. Symptoms include dyspnea, wheezing, cough and may be limitation of activity. Asthma is now recognized to be a primarily inflammatory condition: inflammation under lying hyperreactivity.

4. The primary goal of the asthma medications currently on the market (glucocorticoids, β_2 adrenergic agonists, leukotriene modifiers, and mast cell stabilizers) is to reduce symptoms without regard to the underlying cause, despite the significant side effects. Asthma is commonly treated with corticosteroids and have been shown to be the most effective nonspecific anti–inflammatory medications. However, they have been

shown to promote systemic immunosuppression, which increases the risk of infection. Therefore, the focus of current research should be on employing natural anti–asthmatic medications with negligible adverse effects [13].

5. Ovalbumin is a prominent protein found in the albumin of avian species, particularly prevalent in chicken eggs, is a glycoprotein. ovalbumin exhibits a well–defined structure, including a distinctive beta–barrel domain, and undergoes post–translational modifications, such as glycosylation, influencing its biochemical characteristics. It used for investigating immune responses, studying allergic reactions, or examining the effects of ovalbumin in different biological systems [14,15]..
6. The term 'traditional medicine' refers to the entire body of expertise, knowledge, and methods utilized in the preservation of health as well as the avoidance, detection, improvement, or treatment of physical and mental illnesses. These methods might be derived from local beliefs, myths, and experiences. The use of traditional medicinal plants (TMPs) as herbal medicines which have good potential for treating physiological disorders like asthma or as an adjuvant to modern therapy has been extensively reported since ancient times [18].
7. *Dillenia indica* (L.), commonly known as the elephant apple is a tropical evergreen tree belonging to the family *Dilleniaceae*. Native to Southeast Asia, including India, Bangladesh, Sri Lanka, and Malaysia, this tree is often found in moist, deciduous forests and along riverbanks. It is widely found in India and in the state of Nort–Eastern Region especially 'Assam' and it locally referred as 'Ou–tenga'. It is characterized by its large, glossy leaves, fragrant flowers, and distinctive, ellipsoidal fruits that resemble small greenish–yellow apples. Due to its adaptability and ornamental value, *Dillenia indica* (L.) is also cultivated in gardens and parks outside its native range. Its unique features, ecological versatility, and potential medicinal benefits make it a subject of interest for both traditional practices and scientific research [19–22].
8. This study aimed to examine the impact of *Dillenia indica* (L.) methanolic extract on inflammation using several animal models, with the goal of substantiating the traditional use of the plant. The plant's fruit, bark, and leaves are all utilized in the traditional medical system. It controls body temperature and eases pain in the abdomen. The bark and leaves have laxative and astringent properties [23–25].

2. Materials and methods

2.1 Materials

2.1.1. Plant Material

Fresh leaves were collected from Bhella, a district of Barpeta, Assam (India). The leaves were collected and dried under shade at room temperature for 5 days. *Dillenia indica* (L.) as authenticated by Dr. Souravjyoti Borah, Curator of the Department of Botany, Gauhati University, Guwahati, Assam, India. Later, the leaves were grinded into powder coarsely. The powdered sample were stored in a closed container free from pollution and environmental contaminants.

2.1.2 Reagents

Ovalbumin (A5503) and Dexamethasone (D1756) were procured from Sigma–Aldrich (St. Louis, MO, USA), Aluminium hydroxide was purchased from Hi–Media Laboratories, Normal Saline purchased from medical shop in Guwahati, Assam, India. All other chemicals which were utilized in this study were of analytical and chromatography grade.

2.1.3 Preparation of Dillenia indica (L.) Leaf Extracts (DILE)

The sample dipped into methanol till 72 hours. Then the sample were double filtered through cotton fabric cloth. The filtered solvent was completely evaporated using a rotary evaporator (BUCHI, Switzerland which components includes: Interface I–100, Vacuum Pump V–100, Rotavapor R–100, Heating Bath B–100, Recirculating Chiller F–105) under reduced pressure (250 psi) at a controlled temperature (50°C) and obtained dark greenish gummy exudates. This crude extract was used for further evaluation for *in–vitro* & *in–vivo* [26].

2.2 Phytochemical Screening Methods

The crude extract of *Dillenia indica* (L.) leaves were examined chemically for the presence of several phytoconstituents including alkaloids, carbohydrates, glycosides, flavonoids, phenols, triterpenes, proteins, saponins, steroids, tannins, terpenoids etc. [23,26–32].

2.3 In–Vitro Studies

2.3.1 Antioxidant Activity

a) DPPH Radical Scavenging Activity

The DPPH method was used to measure antioxidant activity where methanolic extract was used to measure antioxidant activity. The antioxidant activity was compared to that of ascorbic acid, a standard compound [27,33,34].

2.3.2 Determination of Total Phenolic Content

The total phenolic content of extracts was determined using the Folin–Ciocalteu method where methanolic extracts was used to measure total phenolic content. Total phenolic content was then compared to that of gallic acid as standard compound. The values of TPC were produced as mg of gallic acid equivalents (GAE) per ml of sample [24,34–36].

2.3.3 Determination of Total Flavonoid Content

The flavonoids content was determined by a State Pharmacopeia of USSR (1989) method using rutin as a reference compound. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$X = (A \times m_0) / (A_0 \times m)$

Where, X is the flavonoid content, mg/mg plant extract in RE, A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract, mg and m_0 is the weight of rutin in the solution, mg [24].

2.4 Experimental Design

All experiments were carried out using Wistar rats (male, weighing about 210–250 g, 8–10 weeks old) obtained from Central Animal Facility of Assam down town University, Guwahati, Assam, India. They were housed in hygienic, air–conditioner rooms by maintaining temperature: 22–25ºC, humidity: 40–70% and 12 hours light–dark cycles and proper ventilation was ensured with 15 to 21 air changing cycles per hour and provided with water *ad libitum*. Prior to experimenting the animals were acclimatized for 7 days to standard laboratory conditions in compliance to the Organization for Economic Cooperation and Development (OECD) guidelines [37,38]. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC), Assam down town University, Guwahati, Assam, India vide registration number 1574/PO/Re/S/11/CCSEA. According to OECD guideline 436: Acute Inhalation Toxicity–Acute Toxic Class Method has described rats to be the species of choice for conducting studies related to inhalation toxicity, therefore Wistar rats have been a model of choice for conducting the experiment [39–41].

Table 1 – Animal groupings.

S. No.	Animal Groupings	No. of animals required
1	Control	6
2	Negative Control	6
3	OVA + STD (Dexamethasone) 30 mg – Oral	6
4	OVA + Treatment [(*Dillenia indica*) (L.)] (Low dose) 200 mg/kg b.w	6
5	OVA + Treatment [(*Dillenia indica*) (L.)] (High dose) 400 mg/kg b.w	6
Total number of animals required		30

*Abbreviation: OVA, Ovalbumin; STD, Standard

2.5 Body Weight, Daily Food Consumption and Water Intake

During the experiment, body weight fluctuations, daily food intake patterns, and water consumption levels to gain comprehensive insights to elucidate the intricate relationships governing nutritional balance and physiological responses [40].

2.6 Induction of Bronchial Inflammation

To induce airway inflammation, rats was sensitized with an intraperitoneal injection of 0.3 mg ovalbumin (OVA) + 20 mg Aluminium hydroxide [$AL(OH)_3$] in 2 ml of normal saline on day 0 to 7. Control rats were received an injection of normal saline. Then, rats were challenged with the exposure of 1% OVA in saline through inhalation chamber (Width 11 inch × Height 5.10 inch × Length 11.2 inch; total volume of the inhalation chamber is 0.01030 m^3 or 10.3 L) which was connected with Comp Air Compressor Nebulizer (OMRON, Model No. NE–C28) with a regulated flow rate for 1 h/day from day 9 to day 11. Rats was orally administered with plant extract from day 13 to day 19. No food and water were given during exposure, and after that the animals were separated, and their behavior was observed [39,42–44].

2.7 Specimen Collection

The rats were euthanized and killed by cervical dislocation after administration of ketamine 0.3 ml via i.p. Lungs tissues were removed and washed with normal saline. Part of the lungs tissue was then fixed in 10% formalin for histopathological investigation [39,40].

2.8 In–Vivo Studies

2.8.1 Lung Function Test

Lung function tests were conducted using the previously mentioned methodology. Using a calibrator and a small animal bias flow generator, the WBP system was used for all tests (DSI System, St. Paul, USA, Model No. 600–2400–001). For data acquisition, the ACQ7700XE system was used (Software: P3 Plus Ponemah, Version–5, DSI Ponemah, 5525 Cloverleaf Pkwy, Valley View, OH). After being calibrated at 100 ml/min, the plethysmograph was conditioned at a bias airflow rate of 2 litre/min. Penh and tidal volume were recorded. Water and food were supplied during the experimentation according to needs [40].

2.8.2 X–Ray Imaging Test

Thoracic radiographic examination and imaging were performed using a laser x–ray imager (Siemens Ltd. L6 Verna, Multimobil 2.5, Model 08633864, Goa, India). The obtained images were then processed by Fujifilm Corporation (FCR Capsula XL II, Model CR–IR 359, Tokyo, Japan) [40]..

2.8.3 Hematology Analysis

Blood samples were collected through orbital sinus vein puncture technique from retro orbital sinus of rats by 75 mm heparinized capillary tube (Hematocrit Capillary, Hi–Media Laboratories Pvt. Ltd., Mumbai, Maharashtra, India). The blood samples were collected in nonvacuum blood collection tubes containing K_3 ethylenediaminetetraacetic acid (EDTA) (Peerless Biotech Pvt. Ltd., Chennai, Tamil Nadu, India) and analyzed within 60 min. The hematological parameters including white blood cell (WBC) count, WBC differential counts that is lymphocyte (Lym), monocyte (Mon), neutrophil (Neu), eosinophil (Eo) and basophil (Ba) counts, red blood cell (RBC) count, mean corpuscular volume (MCV), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb) concentration, and platelet (Pct) were examined by Automated Hematology 3 Part Differential Analyzer (Model No. BC–3000 Plus, Agappe Diagnostics Ltd., Kerala, India) [33,46].

2.8.4 In–Vivo Investigation

After performing all the followed tests, all animals were humanly sacrificed by giving mild anesthesia i.e., Ketamine 0.3 ml via i.p. Both lungs were then extracted and stored for further analysis [40,49,50].

2.8.5 Histopathological Examination

Tissues were fixed by 10% formalin solution for 24 hours. After fixation overs, the tissues were then dehydrated with a sequence of ethanol solution, then embedded in paraffin wax and then 5 µm thick sections were sliced by rotary microtome (Leica Biosystems, Model No. RM2125RTS, Germany), followed by staining with Hematoxylin–Eosin (Sigma Chemical Co., St. Louis, MO, USA) before subjecting to photo microscopic assessment. Photographs of 10x were taken with the help of trinocular microscope (Motic®, Model No. BA210LED) and camera (Moticam 1080) [40].

2.9 Statistical Analysis

The data is displayed as follows: means ± standard deviation (S.D). All statistical analysis were performed using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA). One way ANOVA followed by Dunnett's post–hoc tests were used to analyze the difference among different dosage groups against the OVA group. All value expressed as mean ± S.D. A $p<0.05$ value was considered as statistically significant [40].

3. Results and discussion

3.1 Phytochemical Screening Test

The crude extract of *Dillenia indica* (L.) leaves were examined chemically for the presence of several phytoconstituents including alkaloids, carbohydrates, glycosides, flavonoids, phenols, triterpenes, proteins, saponins, steroids, tannins, terpenoids etc.

Table 2 – Qualitative phytochemical analysis of methanolic extract of *Dillenia indica* (L.).

Phytochemicals	Test Methods	Observation (Color/Precipitation)	Result

Alkaloids	Mayer's Test	Creamy color precipitate	+
Terpenoids	Salkowski Reaction Test	A reddish-brown coloration of the interface was formed	+
Flavonoids	Alkaline Reagent Test	A yellow solution that turns colorless	+
Tannins	Ferric Chloride Test	The solution was observed for deep blue–black color formation	+
Phenols	Ferric Chloride Test	The solution was observed for deep blue – black color formation	+
Saponins	Foam Test	The solution was observed for persistent foam formation	+
Glycosides	Keller–Killiani Test	The solution was observed for reddish brown color at junction of two liquid layers and the upper layer bluish green	+
Carbohydrates	Molisch's Test	Violet ring at the junction of two liquids	-
Triterpenes	-	Red color formation	+
Steroids	Salkowski Reaction Test	The chloroform layer was observed for red color and acid layer was observed for greenish yellow	+
Phytosterols	Liebermann Test	Formation of brown ppt	+

*(+ve), present; (-ve), absent

3.2 In–Vitro Studies

3.2.1 Antioxidant Activity

a) DPPH Radical Scavenging Activity

A comparison between the methanolic extract of *Dillenia indica* (L.) and ascorbic acid's antioxidant activity.

Table 3 – Scavenging of free radical by methanolic extract of *Dillenia indica* (L.) and ascorbic acid in DPPH method.

Sample	DPPH Method IC_{50} (µg/ml)
***Dillenia indica* (L.) leaf extract**	80.36
Ascorbic acid	61.33

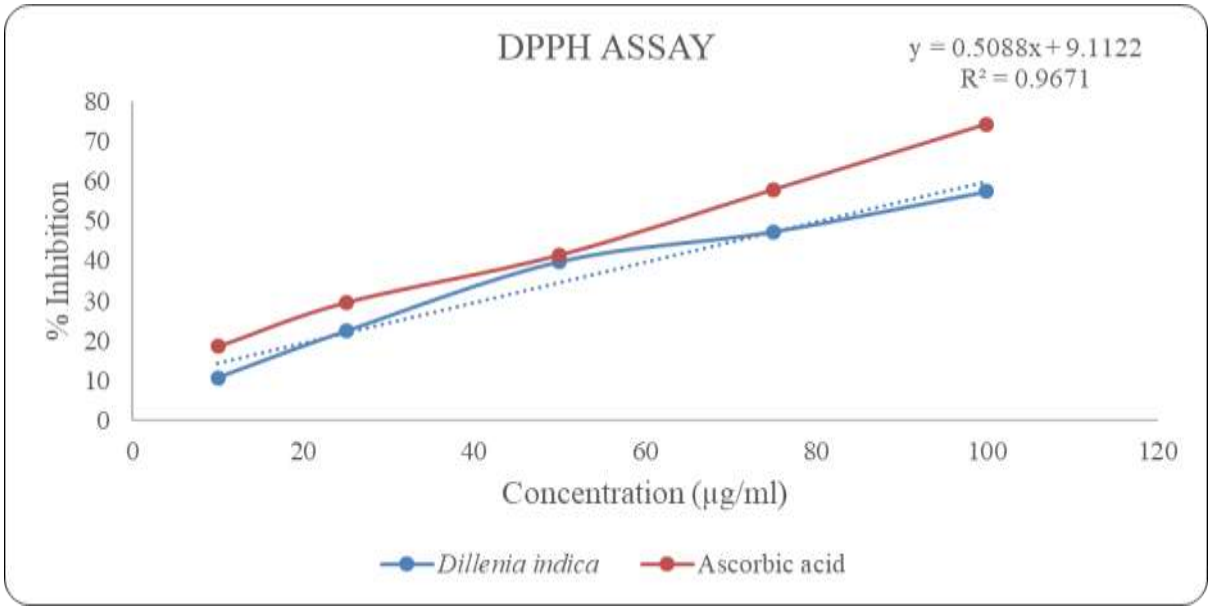

Fig. 4 – DPPH scavenging activity of *Dillenia indica* (L.) leaf extract compared with ascorbic acid.

3.2.2 Total Phenolics and Flavonoid Content

Gallic acid and rutin equivalents were used to express the total phenolic and flavonoid content of the *Dillenia indica* (L.) extract, respectively, and the results are shown in the below fig. 4.2 & 4.3. The amount of phenolic content was determined from regression equation of calibration curve (y = 0.0084x + 0.0229). The total phenolic content showed a positive connection, growing as the extract concentration increased. The amount of flavonoids and total phenolics in the *Dillenia indica* (L.) extract was expressed as rutin equivalents and gallic acid equivalents, respectively. The increase in extract concentration also led to a rise in total flavonoids and phenolic content. Using the formula from the text, total flavonoids were calculated and expressed in rutin equivalents.

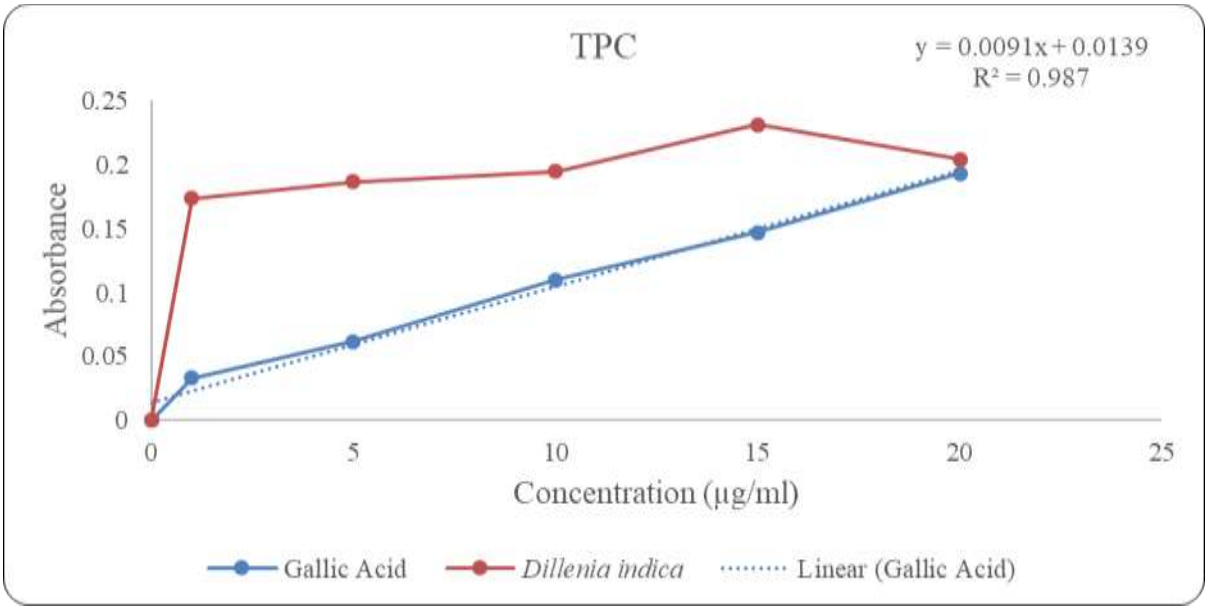

Fig. 5 – Total phenolics content of between *Dillenia indica* (L.) and gallic acid equivalents (GAE).

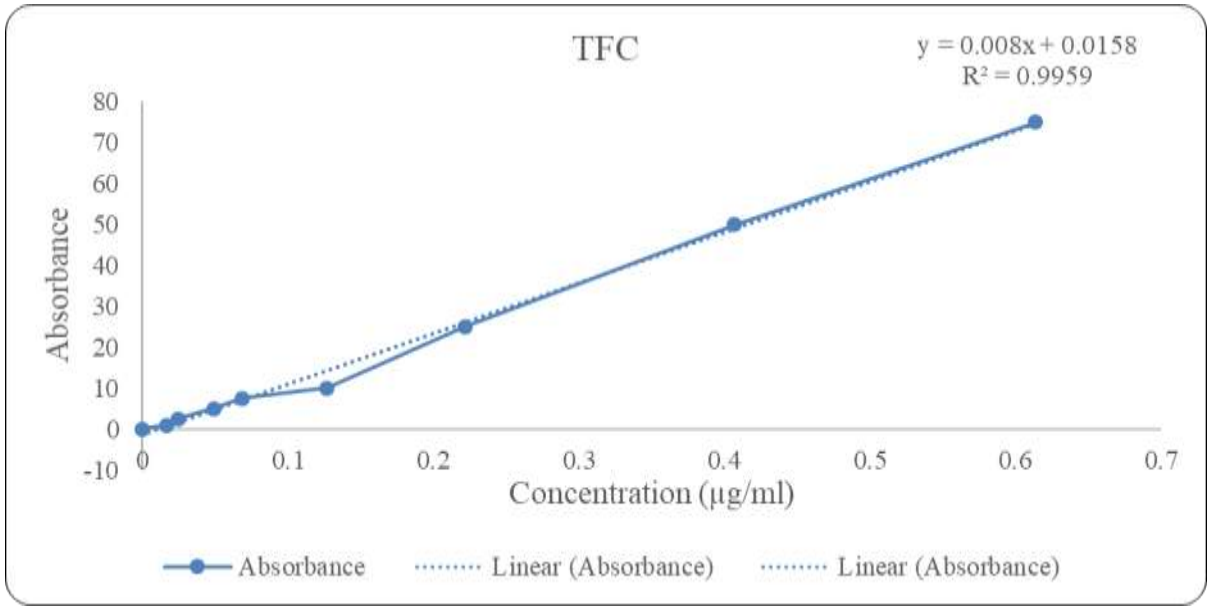

Fig. 6 – Total flavonoid content of rutin equivalents (RE).

3.3 Body Weight

Table 4 – Effect of all groups including the treatment group i.e., *Dillenia indica* (L.) on weight parameters of Wistar rats (n=6).

Days	Control	Negative Control	STD Group	Treatment Group (200 mg/kg b.w)	Treatment Group (400 mg/kg b.w)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Day 1	226.333 ± 8.603	225.167 ± 10.145	227.667 ± 10.112	226.667 ± 10.907	225.833 ± 10.437
Day 7	230.000 ± 7.473	221.167 ± 8.348	225.333 ± 10.537	222.833 ± 10.400	219.000 ± 7.569
Day 11	232.167 ± 7.447	218.000 ± 10.490	225.667 ± 8.135	224.333 ± 10.049	218.333 ± 8.330
Day 19	236.833 ± 8.010	215.333 ± 9.136	221.333 ± 10.137	220.667 ± 9.744	215.167 ± 10.152
Day 21	238.333 ± 5.891	211.667 ± 10.426	222.167 ± 9.079	219.333 ± 8.386	216.333 ± 10.596

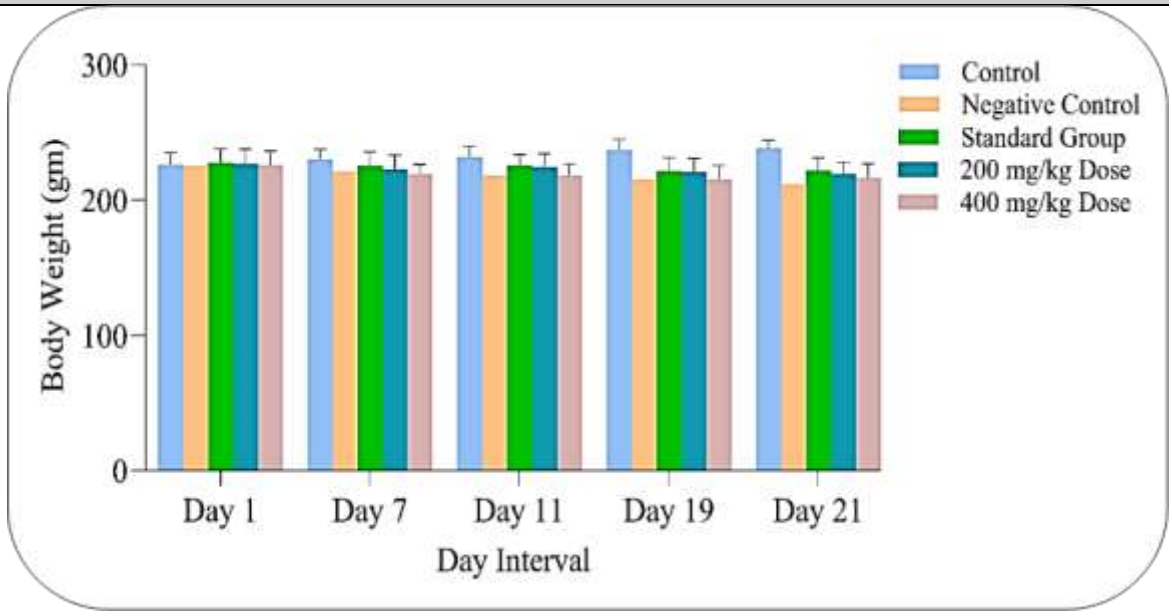

Fig. 7 – Cumulative body weight was measured on the day interval of day 1, 7, 11, 19 & 21 compared with the control group. Data is expressed as means ± S.D. for all groups, n=6 and analyzed using one–way ANOVA followed by Dunnett's post–hoc tests.

3.4 In–Vivo Studies

3.4.1 Lung Function Test

Table 5 – The results of different lung parameters of Wistar rats (n=6).

Parameters	Control	Negative Control	STD Group	Treatment Group (200 mg/kg b.w)	Treatment Group (400 mg/kg b.w)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Penh (Enhanced Pause)	0.683 ± 0.147	1.433 ± 0.250	0.667 ± 0.121	1.267 ± 0.333	0.583 ± 0.098
Tidal Volume	1.312 ± 0.040	1.062 ± 0.087	1.150 ± 0.046	1.128 ± 0.058	1.070 ± 0.030
BPM (Breaths Per Minute)	156.000 ± 20.159	184.667 ± 9.852	161.500 ± 18.897	160.167 ± 19.783	157.167 ± 8.931

Fig. 8 – (A) Penh, (B) Tidal Volume, (C) Breaths Per Minute. Data is expressed as means ± S.D. for all groups, n=6. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ and ^{ns} not significant when compared with control group, analyzed using one–way ANOVA followed by Dunnett's post–hoc test

3.4.2 X–Ray Imaging Test

It was found that ovalbumin exposure in x–ray imaging caused major negative consequences, as seen in Fig. 9. There was noticeable opacification in some places of both of the lung lobes of negative control groups. In contrast, there was no change in the pleural gaps in the negative control group. The control group, on the other hand, showed no opacification in any of the lung lobes, and all fields were found to be uniform, with no evidence of consolidation, collapse, or effusion. Both the treatment group and the standard group experienced opacification to a lower extent.

3.4.3 Biochemical Analysis

Table 6 – Mean and normal range of measured biochemical parameters in 8–10 weeks Wistar rats (n=6) each group.

Parameters	Normal Range	Control	Negative Control	STD Group	Treatment Group (200 mg/kg b.w)	Treatment Group (400 mg/kg b.w)
		Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Glucose (mg/dL)	70 – 208	70.583 ± 9.687	71.667 ± 10.113	71.533 ± 7.440	71.167 ± 8.208	71.350 ± 4.618
Urea (mg/ml)	12.3 – 24.6	12.398 ± 1.378	13.183 ± 3.088	11 ± 1.633	10.018 ± 1.063	17.717 ± 4.096
Total Protein (g/dL)	5.2 – 7.1	6.108 ± 0.832	7.133 ± 0.852	7.100 ± 0.910	6.450 ± 0.521	7.12 ± 0.401
Uric Acid (mg/dL)	2.5 – 5	3.100 ± 0.654	3.783 ± 0.671	3.167 ± 0.561	3.117 ± 0.412	3.411 ± 0.132
Triglyceride (mg/dL)	20 – 114	67.260 ± 8.805	69.567 ± 9.296	71.167 ± 7.885	61.417 ± 27.883	65.683 ± 3.764
Cholesterol (mg/dL)	37 – 85	84.573 ± 7.012	88.767 ± 6.034	74.683 ± 14.656	72.683 ± 16.754	51.700 ± 4.860
Creatinine (mg/dL)	0.2 – 0.5	0.425 ± 0.127	0.428 ± 0.122	0.470 ± 0.235	0.443 ± 0.174	0.443 ± 0.065
SGOT (AST) (U/L)	74 – 143	70.147 ± 11.301	72.750 ± 11.678	75.500 ± 14.237	74.767 ± 14.871	75.733 ± 7.542
SGPT (ALT) (U/L)	18 – 45	26.305 ± 2.957	28.550 ± 2.652	24.417 ± 2.769	26.183 ± 3.198	28.433 ± 3.552

3.4.4 Hematology Analysis

Table 7 – Mean and normal range of measured hematological parameters in 8–10 weeks Wistar rats (n=6) each group.

Parameters	Normal Range	Control	Negative Control	STD Group	Treatment Group (200 mg/kg b.w)	Treatment Group (400 mg/kg b.w)
		Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
WBC (m/mm^3)	2.45 – 9.55	7.483 ± 0.431	12.370 ± 1.309	7.617 ± 0.694	8.380 ± 0.994	9.808 ± 2.676
Lymphocytes (%)	52 – 92	86.365 ± 3.179	92.150 ± 1.555	87 ± 6.387	77.817 ± 6.444	73.467 ± 12.362
Monocytes (%)	0.21 – 5.99	5.157 ± 0.366	3.448 ± 0.699	5.111 ± 0.797	5.417 ± 0.717	5.717 ± 1.104
RBC (m/mm^3)	5.8 – 9.6	7.630 ± 0.790	6.932 ± 0.804	6.950 ± 0.055	6.032 ± 0.907	5.605 ± 1.183
MCV (fL)	51 – 68	52.917 ± 2.080	46.783 ± 0.703	52.333 ± 1.633	50.500 ± 4.183	47.983 ± 1.561
Hct (%)	32.6 – 49.38	45.983 ± 1.533	33.383 ± 4.249	30.950 ± 0.122	30.117 ± 0.778	31.867 ± 1.610
MCH (pg)	17.7 – 22.03	18.787 ± 1.183	16.417 ± 0.223	14.550 ± 0.838	13.783 ± 1.739	15.767 ± 0.907
MCHC (g/dl)	29 – 34	31.000 ± 1.265	34.700 ± 0.420	30.250 ± 0.612	30.300 ± 1.400	32.000 ± 2.138
Hb (g/dl)	11.8 – 17.6	14.233 ± 0.824	11.620 ± 1.497	11.483 ± 0.813	11.667 ± 0.909	11.200 ± 1.964
Thrombocytes/Platelets (m/mm^3)	463 – 1177	732.667 ± 37.399	677.667 ± 111.905	473.833 ± 24.653	468.333 ± 23.149	469.000 ± 54.743
MPV (fL)	6.2 – 9.4	7.717 ± 0.682	7.317 ± 0.578	7.617 ± 0.845	6.533 ± 0.761	7.000 ± 1.175
PDW (%)	6.75 – 9.2	7.785 ± 0.635	9.028 ± 0.156	7.292 ± 0.163	7.948 ± 0.804	8.367 ± 0.463
RDW (%)	10.1 – 15.2	14.100 ± 0.867	14.650 ± 0.602	13.017 ± 1.805	14.217 ± 1.871	15.100 ± 1.947

3.4.5 Histopathological Examination

Fig. 10. shows the histopathology results using Hematoxylin and Eosin (H&E) stains. In the negative control group, there was evidence of massive neutrophil infiltration along with hemorrhagic alterations and alveolar collapse. The control group did not experience any changes as compared to the negative control group. The groups treated with standard and treatment with low and high dose of *Dillenia indica* (L.) were unable to fully reverse these modifications.

Fig. 10 – (A) Control, (B) Negative Control, (C) Standard, (D) Treatment Group (200 mg/kg b.w), (E) Treatment Group (400 mg/kg b.w).

3.5 Conclusion

One of the most prevalent long–term inflammatory respiratory conditions, asthma is typified by hyperresponsive airways brought on by inflammatory cells infiltrating the airways and excessive mucus production [51]. Animals exposed to ovalbumin through their airways acquire a pattern of inflammation in their airways that resembles human asthma in terms of cellular and pathophysiological characteristics [52]. In this study, OVA–induced bronchial asthma in Wistar rats was studied in relation to the effects of two consecutive doses of *Dillenia indica* (L.) leaf extract. It has been reported that *Dillenia indica* (L.) reduces the symptoms of ovalbumin–induced bronchial asthma when administered against it; however, no research on *Dillenia indica* (L.) has taken two doses in consecutive has been documented yet. This investigation showed that the low dose of *Dillenia indica* (L.) leaf extract is substantially more effective than the high dose. *Dillenia indica* (L.) leaf extract was used in this investigation at a low dosage of 200 mg/kg body weight. The high dosage; however, was 200 + 200 = 400 mg/kg of body weight.

This study demonstrates that *Dillenia indica* (L.) has significant anti–asthmatic potential in an ovalbumin–induced bronchial asthma model in Wistar rats. The administration of *Dillenia indica* (L.) extract significantly reduced airway hyperresponsiveness, decreased inflammatory cell infiltration which indicating its efficacy in mitigating asthma symptoms. The presence of bioactive compounds such as flavonoids and triterpenoids likely contribute to its anti–inflammatory and immunomodulatory effects. These findings support the traditional use of *Dillenia indica* (L.) in treating respiratory disorders and suggest its potential as a natural therapeutic agent for asthma. X–Ray and Histopathological analyses showed decreased inflammatory cell infiltration, reduced epithelial damage, and less mucus secretion in the lungs of treated rats. These beneficial effects are likely due to the antioxidant properties of *Dillenia indica* (L.), which help mitigate oxidative stress, a key factor in asthma. However, more investigation is required to clarify the exact molecular pathways and validate its safety and effectiveness in human patients via clinical trials. This study provides a foundation for future investigations and highlights the potential of *Dillenia indica* (L.) as a promising alternative for asthma management.

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