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## ***In-vitro* antioxidant, *in-vivo* anti-inflammatory and antiarthritic activities of aqueous and ethanolic extract of leaves of *Altingia excelsa* species**

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**ABSTRACT-**With a wide range of medical uses, *Altingia excelsa* is intrinsic to Southeast Asia that can be found as far west as northeastern India. The antioxidant, anti-inflammatory, and antiarthritic properties of *Altingia excelsa* were assessed in this study, along with the amount of flavonoids and total phenolic compounds. Using 2, 2'-Diphenyl-1-picrylhydrazyl hydrate scavenging, the antioxidant activity of the extracts was determined. The carrageenan-induced paw oedema technique and the CFA-induced arthritis model were utilised to examine the anti-inflammatory and antiarthritic capabilities in Wistar rats, respectively. For biological assessment, *A. excelsa* aerial parts extracts in both aqueous and ethanolic forms were utilised. The results revealed significant antioxidant activity and substantial anti-inflammatory effects in ethanolic extract as well as aqueous extract and are similiar to the standard drug Indomethacin. Furthermore, the study investigates the impact of *Altingia excelsa* on hematological parameters and biochemical parameters in rats. The findings contribute valuable insights into the multifaceted nature of anti-inflammatory and antarthritic agents, supporting their potential use in preventive healthcare strategies

This study lays the groundwork for further research on *Altingia excelsa* encouraging exploration of its applications in pharmaceuticals and dietary interventions for managing inflammatory disorders.

**Keywords:** *Altingia excelsa*; Antioxidant Activity; Anti-inflammatory Activity; Antiarthritic activity.

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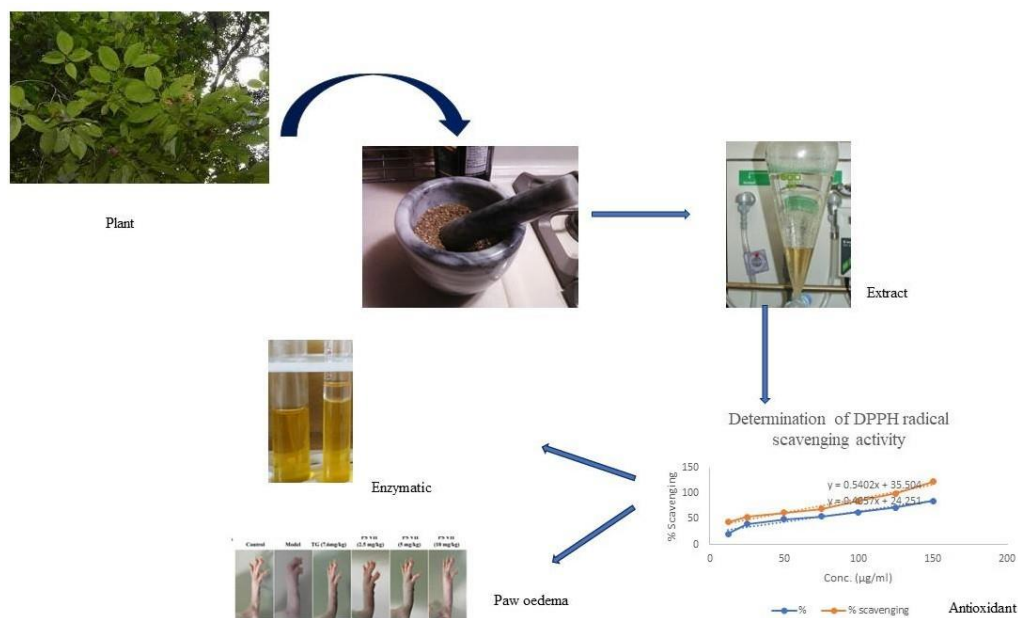
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## GRAPHICAL ABSTRACT



### 1. Introduction

Millions of people suffer from arthritis and the physical abnormalities it causes, which significantly limits their everyday activities [1]. It is commonly used by medical experts to describe the progressive inflammatory reactions that result in limited range of motion and muscular stiffness to one or more joint structures caused by a variety of disorders, from rheumatic to degenerative. In addition, there are more than a hundred varieties of arthritis. Arthritis regularly affects people of all ages, races, sexes, and geographical locations. Its clinical manifestations in individuals who are suffering can differ from individual to individual and range from moderate pain and and distension to severe manifestations such as contractures, muscle atrophy, and full or partial immobility [2]. NSAIDs are given to patients as the primary treatment as part of the drug regimen. But regular use of NSAIDs has been linked to potential side effects, including renal insufficiency and gastroduodenal diseases [3]. These side effects are likely caused by the reticence of cyclo-oxygenase, which lowers prostaglandin content. While there are many different kinds of medications available today, such as DMARDs, corticosteroids, and NSAIDs, they are mostly intended to address symptoms rather than the underlying cause, such as membrane stabilisation, protein denaturation, etc. Additionally,

according to Craig and Cappelli, treatment with the aforementioned modalities may result in severe liver damage and gastrointestinal haemorrhage, death and hospitalisation [4]. Thus, researchers are looking to plants as a potential source of medicine in an effort to overcome all of these problems and discover a safer and just as effective therapeutic option. These plant-based medical systems originally served as the basis for Indian folk or ethnomedical practices. Patients often opt for plant-based drugs over conventional medications due to factors like persistent illness, apprehension about surgery, severe side effects, rising medical expenses, limited response to established drugs, and drawbacks of new medications. Therefore, considering the traditional usage of *A. excelsa* for joint pain, it was deemed worthwhile to scientifically assess its anti-arthritic potential using both *in vitro* and *in vivo* methods, given the lack of scientific data on its effects. [5,6]. *Altingia excelsa*, known for its traditional use in Indonesian herbal medicine for ailments ranging from inflammation to coughs, presents an intriguing avenue for research into its prospective as an anti-inflammatory agent. Conducting *in vivo* studies to evaluate its effectiveness and safety profile could shed light on its therapeutic potential and contribute to the development of safer alternatives to conventional anti-inflammatory drugs [7].

## 2. Experimental

**2.1 Plant Material** *Altingia excelsa* (Rasamala) leaves were collected from plain region of Himachal Pradesh. Voucher specimens of *Altingia excelsa* were deposited. The plant was identified and authenticated by Prof. Vinay. An herbarium specimen No. R.S. – 125 CIN No. U24233PB200BPTC032243 in Herbal Health Research Consortium Pvt. Ltd.

## 2.2 Chemicals and Reagents

Analytical-grade solvents, chemicals, and reagents were sourced from Rankem in Jalandhar. DPPH, carrageenan, and indomethacin were obtained from Sigma-Aldrich Chemical Co., based in St. Louis, MO, USA, and Novartis. Paw volume was estimated using a plethysmograph. Test drugs, reference standard (indomethacin) was suspended in vehicle 1% CMC and administered orally. For Adjuvant induced arthritis, CFA, a product of Sigma Aldrich, St. Louis, MO, USA, was employed, along with methotrexate (MTX) as a standard antirheumatic drug administered orally.

**2.3 Preparation of extracts** *Altingia excelsa* (Rasamala) leaves were washed, cut into small pieces and dried. The leaves dried powder underwent defatting with petroleum ether, then extraction with 70% ethanol and water. The extracts were concentrated under reduced pressure

using a Rota evaporator and stored in airtight containers in a refrigerator at temperatures below 10°C.

## 2.4 Animals:

The anti-inflammatory study used an inbred colony of female Wistar rats weighing between 250 to 300 grams. Within polypropylene cages, they were maintained at  $25 \pm 2^\circ\text{C}$  and 45–55% relative humidity with 12-hour light and dark cycles. A week was spent acclimating each animal to the lab setting before it was used. They were given water on demand and regular animal feed from the Laureate Institute of Pharmacy Kathog Jawalaji, Kangra. The CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines were followed in the execution of all pharmacological activities. The Institutional Animal Ethics Committee (CPCSEA/LIPH/2022/17) of the Laureate Institute of Pharmacy Kathog Jawalaji, Kangra, authorised all of the pharmacological experimental protocols.

## 2.5 Determination of Ash values

### 2.5.1 Total ash

Take around 2-4 grams of the ground material that has been air-dried. Employ a crucible constructed from either platinum or silica. Ignite the crucible beforehand to remove any impurities and tare it (adjust the balance to account for the weight of the crucible). Place the accurately weighed air-dried material into the earlier burnt and tared melting pot. Support the material in a uniform layer to ensure uniform ignition. Gradually increase the heat to 500-600°C. Continue heating until the material turns white, indicating the absence of carbon. This step ensures complete combustion of organic matter. After ignition, cool the crucible in a desiccator to prevent absorption of moisture from the atmosphere. Once cooled, weigh the crucible with the ash residue. If the preceding procedures are unable to produce carbon-free ash, take the following extra actions. Allow the crucible holding the residue to cool. Use two millilitres of water or an ammonium nitrate solution that has been saturated to wet the residue. The mixture is first dried on a hot plate and then on a water bath. Reignite until the weight remains consistent. After allowing the crucible to cool in an appropriate desiccator for half an hour, weigh it right away [8]. Use the following formula to get the amount of total ash in milligrammes per gramme of air-dried material:

$$\text{Total Ash Content (mg/g)} = \frac{\text{Weight of Ash (mg)}}{\text{Weight of Air-Dried Material (g)}}$$

### 2.5.2 Acid-insoluble ash

Use the crucible that held all of the previously collected ash. Transfer 25 millilitres of hydrochloric acid into the crucible that holds all of the ash. To stop spills, place a watch glass over the crucible. For five minutes, slowly boil the mixture. Pour the 5 cc of boiling water that was used to rinse the watch glass into the crucible. Gather the insoluble material that accumulates on ash-free filter paper. Use hot water to wash the gathered insoluble material on the filter paper until the filtrate turns neutral. Replicate the crucible with the filter paper that contained the insoluble material. Light up the crucible with the filter-paper and insoluble matter until a constant weight is achieved. Ensure that the temperature does not exceed the safe range for the material being analyzed. Allow the crucible to cool in a suitable desiccator for 30 minutes to prevent moisture absorption. Weigh the crucible with the residue without any delay [8]. Calculate the content of acid-insoluble ash in mg per gram of air-dried material using the formula:

$$\text{Acid-Insoluble Ash Content (mg/g)} = \frac{\text{Weight of Residue (mg)}}{\text{Weight of Air-Dried Material (g)}}$$

### 2.5.3 Water-soluble ash

Pour 25 millilitres of water into the crucible with all of the ash in it. Heat the blend for five minutes. This step helps dissolve the water-soluble components of the ash. Once the mixture has boiled, filter the insoluble material. To get rid of any soluble materials that might have stuck to the collected mysterious material, give it a thorough wash in hot water. Dry the insoluble material after washing. Ignite the dried insoluble matter in a melting pot for 15 minutes. Ensure that the temperature does not exceed 450°C during ignition. Weigh the crucible containing the residue after ignition. The weight of this residue should be deducted from the weight of all the ash [8]. Using the following formula, determine the amount of water-soluble ash in milligrammes per gramme of air-dried material:

$$\text{Water-Soluble Ash Content (mg/g)} = \frac{\text{Weight of Total Ash (mg)} - \text{Weight of Residue (mg)}}{\text{Weight of Air-Dried Material (g)}}$$

## 2.6 Determination of extractive values

This method is used to determine the extractive values of medicinal plant materials using different solvents, such as alcohol and water. Take 4.0 grammes of the air-dried, coarsely powdered substance. Transfer the precisely weighed substance into a conical flask with a glass cap. Add 100 mL of the solvent (alcohol or water) to the flask. Macerate the mixture by shaking frequently for 6 hours. Allow the mixture to stand for 18 hours to ensure thorough extraction. After the mixture has macerated, quickly filter it. Transfer 25 millilitres of the filtrate into a

tared, flat-bottomed dish. Evaporate the solvent to dryness on a water bath. This step concentrates the extract in the dish. Dry the residue obtained from evaporation at 105°C for 6 hours. Ensure complete drying to constant weight. To stop moisture absorption, cool the dry residue in a desiccator for thirty minutes. Weigh the dish containing the dried residue without any delay [8].

### **2.7 Determination of loss on drying**

The previously dried and tared weighing bottle is filled with 5 grammes of the air-dried material. After that, the sample in the weighing vial is dried in an oven set to between 100°C and 105°C. This temperature range is chosen because it effectively removes moisture without causing significant degradation to the plant material.

The sample undergoes drying until two successive weighings show a discrepancy of no more than 5 milligrams. This indicates that the sample has reached a consistent weight, meaning that most of the moisture has been removed [8].

### **2.8 Conducting an initial qualitative phytochemical screening**

It is a standard procedure used to identify various phytoconstituents present in plant extracts by using standard conventional procedures. The presence and absence of phytochemicals are indicated with signs “+” and “-”, respectively [9].

### **2.9 Determination of total phenolic content**

The Singleton et al. [10] method measures the total phenolic content of plant extracts using the Folin-Ciocalteu test; the results are expressed as gallic acid equivalents (GAE) per gramme of the plant material. Use 1.5 millilitres of distilled water to dilute the test sample 0.5 ml (1:10 v/v). To the test sample that has been diluted, add 1.5 ml of the Folin-Ciocalteu reagent. The mixture should be left to stand at 22°C for five minutes. Add 2.0 ml of the 7.5% sodium carbonate solution to the mixture after 5 minutes. The mixtures should be shaken occasionally while being in the dark for ninety minutes. Watch the combinations as a blue hue begins to take shape. Calculate the blue's absorbances. Using distilled water as a blank, measure the absorbances of the blue colour in samples at 765 nm using a UV/VIS spectrophotometer. To calculate the phenolic content in terms of gallic acid equivalents (GAE), use a typical gallic acid curve. Utilising the formula, determine the total phenolic content. Total phenolic content Percentage weight/weight (% w/w)=  $\frac{GAE \times V \times D \times 10^{-6}}{W} \times 100$

GAE-Gallic acid equivalent ( $\mu\text{g/ml}$ ), V-Total volume of sample (ml), D-Dilution factor,  
W-Sample weight (gm)

### 2.10 Determination of Flavonoid content

The total flavonoid content was calculated using Park et al.'s methodology [11]. Take the plant extract 0.3 ml. In 10 ml test tube, mix it with 3.4 ml of 30% methanol. To the test tube, add 0.15 ml of the 0.5 M sodium nitrite ( $\text{NaNO}_2$ ) solution. Fill the test tube with 0.15 ml of the 0.3 M aluminium chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ) solution. To allow a colour reaction to develop, let the mixture stand for five minutes. Add 1 ml of 1 M sodium hydroxide ( $\text{NaOH}$ ) solution to the mixture after 5 minutes. With a spectrophotometer, find the solution's absorbance at 506 nm. For calibration, use a reagent blank that has all of the reagents except the plant extract. Using the same process, create a standard curve for total flavonoids using the quercetin standard solution. The standard curve should relate known concentrations of quercetin to their corresponding absorbances at 506 nm. Determine the concentration of total flavonoids in the plant extract using the standard curve.

### 2.11 DPPH radical scavenging activity

The DPPH assay is used to calculate the radical scavenging activity (RSA), which quantifies an antioxidant's capacity to scavenge free radicals [12]. Get a 0.2 mM DPPH solution ready. Combine 2.7 mL of the made 0.2 mM DPPH solution with 0.3 mL of the extract solution at different concentrations. To guarantee adequate mixing, give the mixture a good shake.

Let the mixture sit for one hour at room temperature. Using a spectrophotometer, determine the reaction mixture's absorbance at 517 nm after an hour of incubation. Apply the following formula to determine RSA as a percentage of DPPH discoloration:

radical scavenging activity of DPPH discoloration using the following formula:

$$\% \text{ RSA} = [(A_0 - A_s) / A_0] \times 100 \dots\dots\dots (3)$$

Where  $A_0$  = absorbance of the control (containing all reagents, except the test compound) and  $A_s$  = absorbance of test sample.

### 2.12 Acute toxicity studies

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines 425 [13].

### 2.13 *In-vivo* Anti-Inflammatory Evaluation by Carrageenan-induced rat paws edema method

Carrageenan-induced paw edema test to examine the anti-inflammatory properties of extracts from *Altingia excelsa* in wistar rats [14]. The experimental groups, each consisting of 6 rats, received oral administrations of the extracts at doses of 200-500 mg/kg, with Indomethacin

serving as the positive control. The untreated control group received only 0.5% carboxy methylcellulose (CMC). After carrageenan injection, paw volumes were measured at 0, 1, 2, 3, 4, and 5 h intervals using a plethysmograph. The percent inhibition of paw edema was calculated, demonstrating the extracts' potential anti-inflammatory effects compared to the control.

#### **2.14 Adjuvant-Induced Arthritis In Rats:**

Adult Wistar rats weighing between 200 and 250 grams were randomly assigned to four groups, each containing six rats:

Group I: Normal Control (NC) Group

Group II: CFA-Induced Arthritic (DC) Group

Group III: Reference Methotrexate (dose: 10 mg/kg)

Group IV: Extract Treated Group (dose: 250 mg/kg)

Arthritis was induced in the rats by injecting 0.2 ml of Complete Freund's Adjuvant (CFA), which contains 10 mg/ml of heat-killed *M. tuberculosis*, into the left hind paw, except for Group I. One hour before the CFA injection, animals in Groups III and IV received standard methotrexate and *Altingia excelsa* leaf extract, respectively, starting from day 0 and continuing until day 28. In contrast, normal control and disease control animals were administered only 0.1 ml of the vehicle [15,16].

To assess joint stiffness, rats were held from the back, and bending and extension of limbs were performed within the limits of motion. Scores were assigned based on the extent of restriction of ankle movement:

Score 2: Restriction of both bending and extension movement

Score 1: Restriction of either bending or extension movement

Score 0: No restriction of ankle movement

On day 28, mobility was evaluated based on criteria including paw positioning and movement:

Score 6: Normal walking

Score 5: Full contact of the ipsilateral hind paw with the floor

Score 4: Only toe contact of the ipsilateral hind paw with the floor

Score 3: Full contact of the contralateral hind paw with the floor

Score 2: Only toe contact of the contralateral hind paw with the floor

Score 1: Crawling with forelimbs

Score 0: No movement

At the study's conclusion, a gait test was conducted on a tabletop, with scores reflecting the rat's movement:

Score 2: Creeping behavior (using forelimbs to move, dragging hind limbs)

Score 1: Minimal paw usage for support

Score 0: Normal paw usage. Gait score for adjuvant-treated paws was excluded due to observed swelling and abscess formation in some animals. Adult Wistar rats weighing between 200 and 250 grams were randomly assigned to four groups, each consisting of six rats.

## **2.15 Arthritic index**

Ears, nose, tail, forepaws, and hind paws were examined by two observers who were blinded to the study for the presence of inflammation and/or nodules. The Bartlett and Schleyerbach, 1985 [17] scoring system was used to calculate the arthritis index.

### **Scoring system for arthritic index**

Nodules absent from the ears | 0 Nodules present in the ears | 1 Nodules absent from the nose | 0 Nodules present in the nose | 1 Tail | Nodules absent from the tail | 0 Nodules present in the tail | 1

The Fore Paws show no inflammation at all (0), one joint shows inflammation (4), the Middle Paws show no inflammation at all (1), the Left Paws show moderate inflammation (2), and the Right Paw shows marked inflammation (3).

## **3. RESULTS & DISCUSSION**

### **3.1 Physiochemical parameters**

#### **3.1.1 Ash Values**

Physiochemical characteristics are essential for assessing the potency and quality of plant-based medications. Ash values provide information on the number of inorganic substances, earthy materials, and other contaminants in the medication. Because they serve as markers for

adulteration, replacement, and contamination, they are essential in assessing the drug's purity. Usually, silicates, phosphate, and carbonate make up the total ash. Complete ash and acid-insoluble ash analyses were carried out and documented.

After analysis, the total ash content of the leaves of *Altingia excelsa* was found to be 11.50% w/w. It was discovered that the water-soluble ash content was 39.50% w/w and the acid insoluble ash content, which is produced from total ash, was 1.95% w/w.

### 3.1.2 Extractive Values

After calculation, the water-soluble extractive value came out to be 19.63% w/w. It was discovered that the alcohol-soluble extractive value in ethanol was 28.55% w/w.

### 3.1.3 Loss on Drying

Loss on drying analyses were conducted on the plant material, recording results of 8.55% w/w. These comprehensive physiochemical analyses provide valuable information regarding the quality and purity of *Altingia excelsa* leaves, aiding in their pharmaceutical and medicinal applications.

### 3.1.4 Preparation of Extracts

The ethanolic and aqueous extracts were prepared by continuous hot percolation method using Soxhlet apparatus and rotary evaporator. Both the extracts were semisolid in nature and greenish in colour with percentage yield of 5.75% w/w of ethanolic extract while the aqueous extract was found to be 3.42% w/w.

### 3.1.5 Qualitative Estimation of Phytoconstituents

Following conventional protocols, the extracts were submitted to a qualitative phytochemical examination to determine the various phytoconstituents present. The outcomes are displayed in **Table 1**.

**Table 1: Preliminary phytochemical analysis of extracts**

Chemical constituents	Ethanolic extract	Aqueous extract
Steroids	-	-
Glycosides	+	+
Saponins	-	+
Flavonoids	+	+

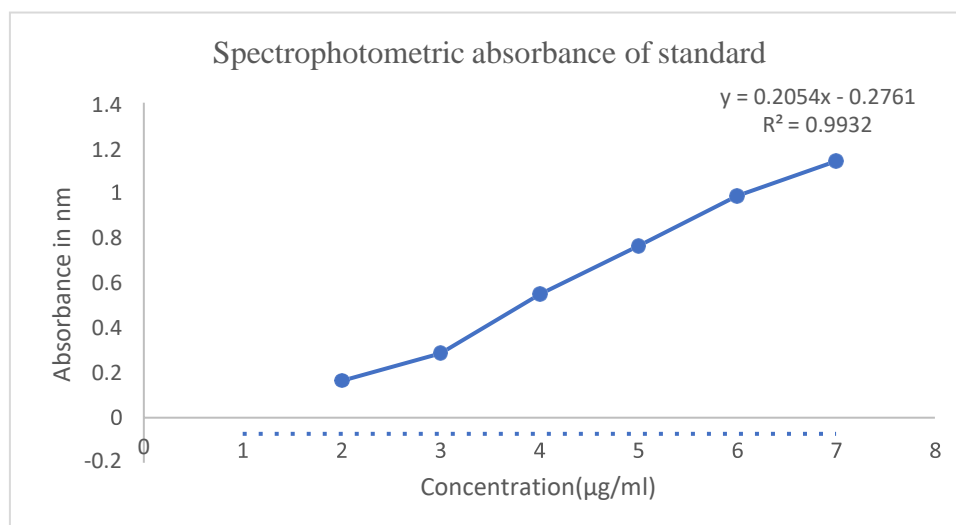
Tannins	+	+
Proteins	-	-
Alkaloids	+	-
Carbohydrates	+	+
Terpenoids	+	+
Fats and oils	-	-
Vitamins	+	+
Minerals	+	+

\*+ indicates presence, - indicates absence

### 3.1.6 Total Flavonoid Content of *Altingia excelsa*

#### Standard Calibration Curve for Quercetin

The total flavonoid content of plant extracts was quantified in terms of milligrams of quercetin equivalents per gram of extract, as depicted in **Figure 1**. The analysis was conducted in triplicate, and Table 2 provides the detailed analytical data for both the ethanolic and aqueous extracts of the plant. The absorbance was plotted versus concentration in the calibration curve, which was created by serially dilution of the quercetin Standard stock solution (20-100 µg/ml).



**Figure 1: Standard Calibration Curve for Quercetin**

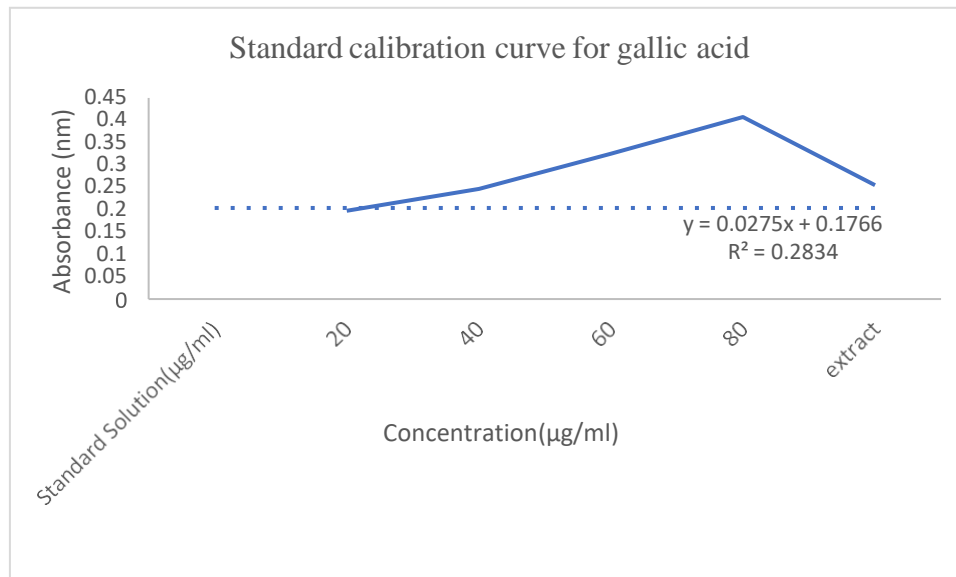
**Table 2: Percentage yield of total flavonoids of ethanolic and aqueous extract of leaves of plant *Atingia excelsa***

S.No	Extract	Flavonoid content mg/g Quercetin equivalents (QE)
1.	<i>Ethanolic</i>	3.67
2	<i>Aqueous</i>	1.95

### 3.1.7 Total Phenolic Content of *Atingia excelsa*

#### Galic acid Standard Calibration Curve

To assess the phenolic compound's accuracy, plot the standard absorbance that was acquired using spectrophotometry. The absorbance was plotted versus concentration in the calibration curve, which was created by serially dilution of the Gallic acid Standard stock solution (20-80  $\mu\text{g/ml}$ ) as shown in **Figure 2**. The analytical data regarding the phenolic content of the plant's ethanolic and aqueous extract is shown in **Table 3**.



**Figure 2: Standard Calibration Curve for Gallic acid**

The dilution factor was used to quantify the concentration of phenolic content present in 1g of the extract based on the triplicate absorbance value obtained from spectrophotometry. Data unequivocally demonstrate that the plant's ethanolic extract has a sizable proportion of total phenolic content.

**Table 3: Percentage of phenolic content present in the extract**

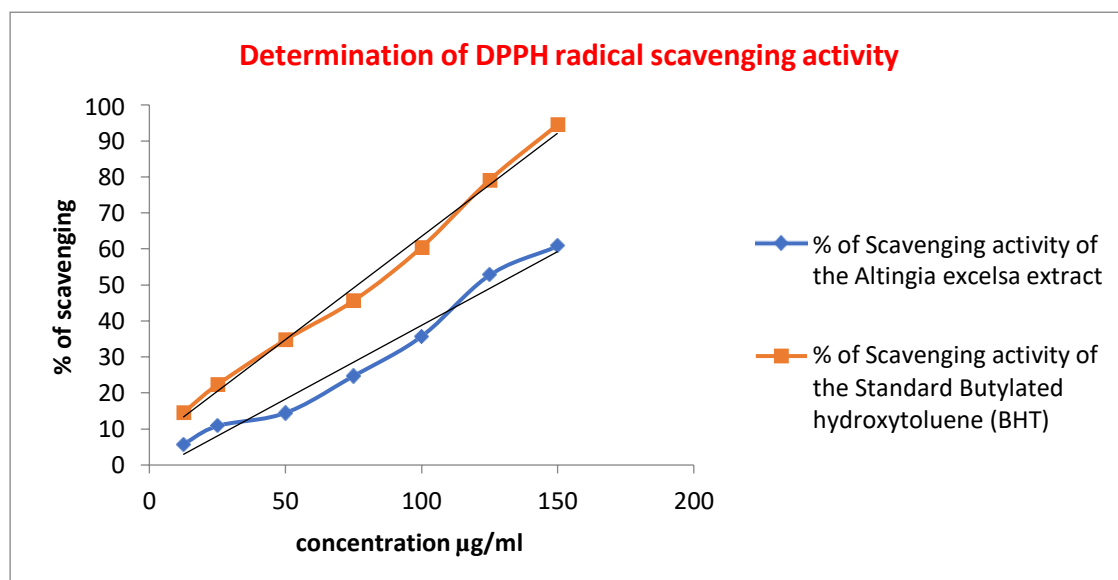
S.NO	Extract	Phenolic content mg/g gallic acid equivalents (GAE)
1.	<b>Ethanolic</b>	18.90
2.	<b>Aqueous</b>	11.65

### 3.2 Antioxidant assay

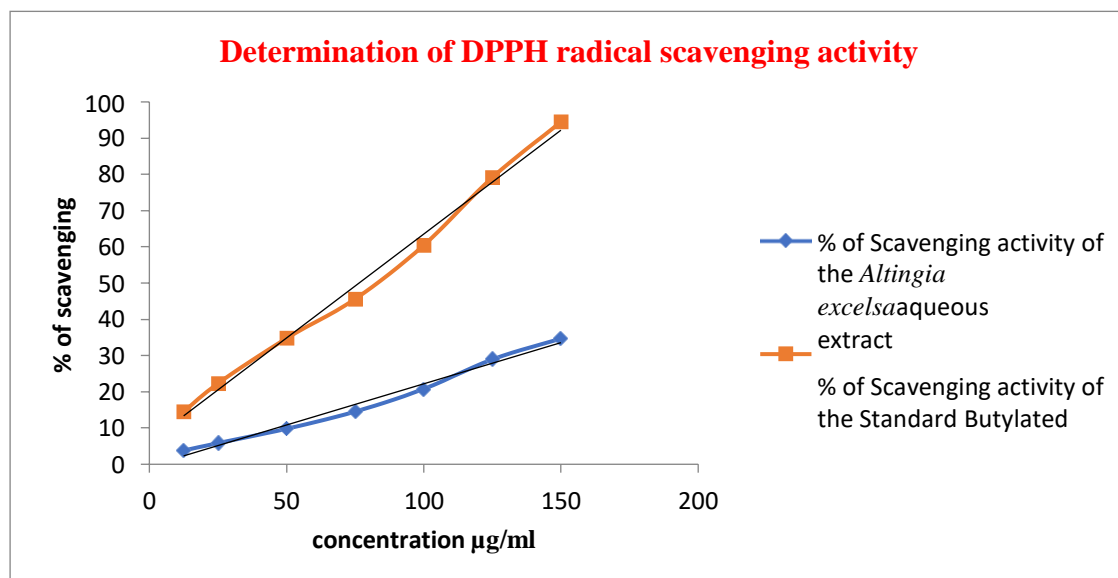
The antioxidant activity of both plant extracts was investigated in the DPPH model at numerous doses between 12.50 and 150 µg/ml. The greatest antioxidant potential of ethanolic extract has been observed, and its antioxidant activity is comparable to that of Butylated Hydroxy toluene standard. **Figures 3 and 4** display the % inhibition of both extracts in the DPPH scavenging test. **Table 4** displays the IC<sub>50</sub> values for the standard and both extracts in the DPPH scavenging experiment.

**Table 4: IC<sub>50</sub> values of free radical scavenging effect by DPPH method of both extracts of *Altingia excelsa* and standard**

S.NO	Sample	IC <sub>50</sub> value (µg/ml)
1.	<b>Ethanolic extract</b>	63.46
2.	<b>Aqueous extract</b>	95.5
3	<b>Butylated Hydroxy toluene</b>	26.83



**Figure 3: Free radical scavenging activity ethanolic extract of *Altingia excelsa* and standard**



**Figure 4: Free radical scavenging activity aqueous extract of *Altingia excelsa* and standard**

**3.3 Acute oral toxicity test** Alcoholic extract of the plant *Altingia excelsa*. was found to be safe at the dose of 5000 mg/kg according to OECD guidelines 425.

### 3.4 *In vivo* Anti-inflammatory Activity

Using the carrageenan-induced paw edema technique, the study sought to assess the *in-vivo* anti-inflammatory potential of extracts from *Altingia excelsa*. The extracts were given at 200–500 mg/kg dosages, with carboxymethyl cellulose (CMC) serving as the control and

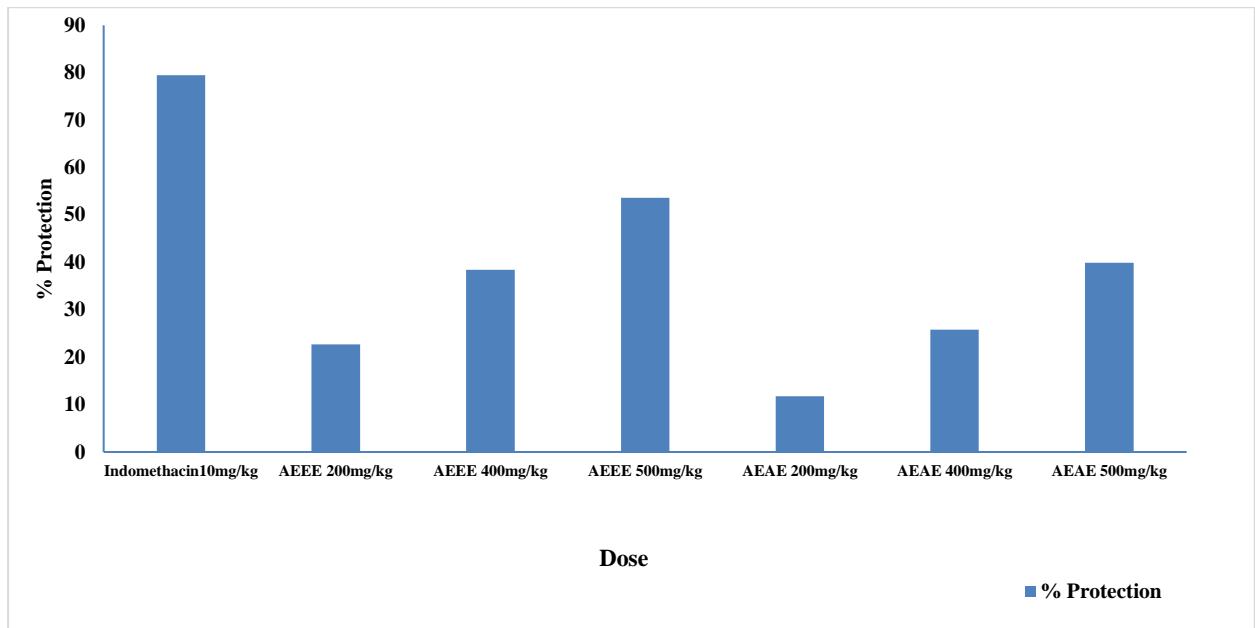
indomethacin serving as the reference standard. After administering carrageenan, the anti-inflammatory effects were evaluated by evaluating the decrease in oedema.

The results indicated that *Altingia excelsa* ethanolic and aqueous extracts exhibited dose-dependent edema reduction, ranging from 22.70% to 53.60% and 11.75% to 39.94% respectively, compared to the standard Indomethacin, which demonstrated an edema reduction of approximately 79.60%. as shown in **Table 5 and Figure 5**. Statistical analysis using Dunnett's test revealed that both extracts significantly exerted anti-inflammatory activity compared to the control group. Based on the significant reduction in carrageenan-induced paw oedema, the study suggests that both ethanolic and aqueous extracts of *Altingia excelsa* contain strong *in-vivo* anti-inflammatory action. These results highlight the potential value of these plant extracts in the development of anti-inflammatory medicines and add to the increasing body of research demonstrating their anti-inflammatory properties.

**Table 5: *In-vivo* anti-inflammatory activity of the ethanolic and aqueous extract of *Altingia excelsa* using carrageenan-induced paw edema model.**

S.No.	Treatment Group	Dose	% Protection
1	Indomethacin	10mg/kg	79.50±2.15***
2	<i>Altingia excelsa</i> Ethanolic extract	200mg/kg	22.70±2.45***
		400mg/kg	38.45±1.90***
		500mg/kg	53.60±1.40***
3	<i>Altingia excelsa</i> Aqueous extract	200mg/kg	11.75±1.90***
		400mg/kg	25.80±2.00***
		500mg/kg	39.94±1.10***

The mean values from three separate experiments  $\pm$  SEM are used to express the results. Dunnett's test (n=3) was used to evaluate the data;  $P \leq 0.0001$  is equal to (\*\*\*)



**Figure 5: *In-vivo* anti-inflammatory activity of the ethanolic and aqueous extract of *Altingia excelsa* using carrageenan-induced paw edema model.**

### 3.5 Antiarthritic Activity:

#### 3.5.1 Freund's complete adjuvant induced arthritis in rats

**3.5.2 Impact of ethanolic extract of *Altingia excelsa* on the functional parameters:** Following FCA administration, the arthritic index increased significantly. Rats treated with methotrexate and rats treated with an ethanolic extract of *Altingia excelsa* had a considerably decreased arthritic index in comparison to the CFA-induced arthritic rats seen in **Figure 6 and Table 6**.

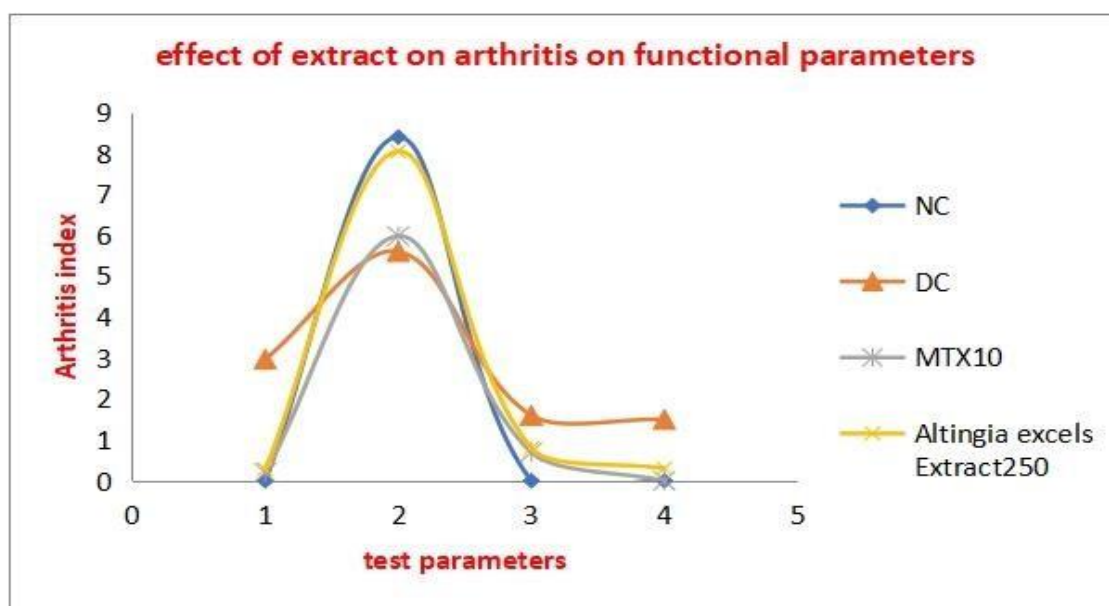
The data presents behavioral and functional assessment of different groups in terms of mobility, joint stiffness and weight bearing ability. Although it might be marginally less effective than MTX10 in some areas, particularly joint stiffness and full restoration of weight-bearing ability, the *Altingia excelsa* ethanolic extract greatly increases mobility and decreases gait abnormalities, joint stiffness, and weight-bearing difficulties.

*A. excelsa* exhibits a preventive or remedial impact on joint function and mobility, indicating promise as a therapy for ailments linked to inflammation of the joints or reduced mobility.

**Table 6: Effect of *Altingia excelsa* ethanolic extract on functional parameters of CFA-induced arthritic rats**

Groups	Gait test	Mobility test	Joint stiffness	Visual weight bearing test
NC	0±0.00	8.65±0.10	0±0.00	0±0.00
DC	2.54±0.65	4.10±0.75	1.25±1.40	1.45±0.30
MTX10	0.18±0.02**	5.65±2.32*	0.40±0.35*	0.00±0.01**
<i>Altingia excelsa</i> ethanolic extract 250	0.28±0.10**	8.05±1.50*	0.80±0.05*	0.30±0.10**

Data represented as mean SEM; \*p < 0.05 compared to NC group and \*\*p < 0.01 compared \*\*\*p < 0.001 to standard group (one-way ANOVA followed by Tukey's multiple comparison test)



**Figure 6: Effect of *Altingia excelsa* ethanolic extract on arthritis on functional parameters**

### 3.2.1 Effect of the aqueous extract of *Altingia excelsa* on functional parameters in arthritic rats

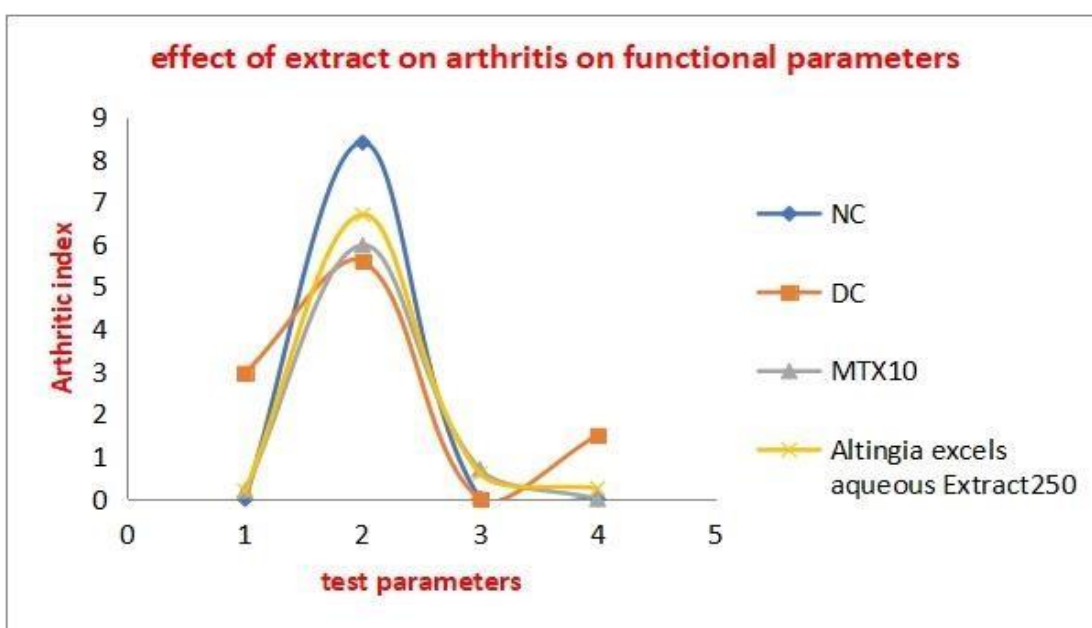
Following FCA administration, the arthritic index increased significantly. The *Altingia excelsa* aqueous extract shows significant benefits in improving gait, movement, joint stiffness, and weight-bearing capacity compared to the disease control group.

It is as effective as MTX10 in improving gait and movement but slightly less effective in reducing joint stiffness and fully restoring weight-bearing capacity.

Overall, the aqueous extract of *A. excelsa* seems to be a promising treatment for conditions involving impaired mobility and joint stiffness, with effects comparable to the standard treatment (MTX10). Rats treated with methotrexate and rats treated with an aqueous extract of *Altingia excelsa* had a considerably decreased arthritic index in comparison to the FCA-induced arthritic rats depicted in **Table 7** and **Figure 7**.

**Table 7: Effect of *Altingia excelsa* aqueous extract on functional parameters of FCA-induced arthritic rats**

Groups	Gait test	Movement test	Joint stiffness	Visual weight bearing test
NC	0±0.00	8.65±0.10	0±0.00	0±0.00
DC	2.54±0.65	4.10±0.75	1.25±1.40	1.45±0.30
MTX10	0.18±0.02**	5.65±2.32*	0.40±0.35*	0.00±0.01**
<i>Altingia excelsa</i> aqueous Extract250	0.19±0.70*	6.70±1.05*	0.60±0.05*	0.25±0.40**



**Figure 7: Effect of *Altingia excelsa* aqueous extract on arthritis on functional parameters**

**Statistical Analysis:** The statistical analysis, as detailed in the study, involved employing one-factor analysis of variance (ANOVA) followed by Tukey's multiple comparison test to assess the significance of differences between various groups. Additionally, two-way ANOVA and Bonferroni's post hoc test were utilized to evaluate results related to body weight and paw edema. The significance threshold was set at  $p < 0.05$ , ensuring that observed differences were considered statistically significant.

**3.2.1 Effect of *Altingia excelsa* ethanolic extract on Paw Volume in arthritic rats:** The investigation into the impact of the extract on paw volume provides crucial insights into the physiological responses and potential therapeutic effects. Paw volume serves as a sensitive indicator of the anti-inflammatory efficacy of various drugs. In the context of this study, a continuous and linear increase in paw volume was noted in rats induced with Freund's complete Adjuvant (FCA), indicating the development and progression of arthritis. This observation aligns with expectations, as increased paw volume is a characteristic feature of arthritic conditions.

However, intervention with the extract, particularly in the *Altingia excelsa* ethanolic extract-treated group, demonstrated a significant reduction in paw volume compared to the FCA-induced arthritic rats. This reduction suggests a potential anti-inflammatory effect of the extract, contributing to the mitigation of paw swelling associated with arthritis. Notably, the effect was comparable to that observed in the methotrexate-treated group, a standard reference in arthritis treatment. The minimal difference in paw volume between the methotrexate-treated and *Altingia excelsa* ethanolic extract-treated rats further emphasizes the potential efficacy of the extract in managing arthritic conditions. This similarity in outcomes suggests that the extract could offer benefits comparable to the conventional treatment.

Induction of FCA caused increases in paw volume of injected left hind paw and maximum increase in paw volume was noted on day 28 which was recorded as change in paw volume  $2.80 \pm 0.72$  ml. The left hind paw volume was measured weekly using plethysmometer to all groups. Increase in the paw volume of all FCA injected rats was evident on day 1. The increase in paw volume progressed till day 28 in normal group. Treatment of methotrexate 10 mg/kg showed significant decrease in the paw volume of left hind paw at the day 21 and 28th with the % inhibition 42.33 and 72.14% respectively. *Altingia excelsa* ethanolic extract 250 mg/kg significantly ( $p < 0.001$ ) lower the paw volume from day 14 onwards as compared to control group with maximum inhibition of 71.79% respectively on day 28

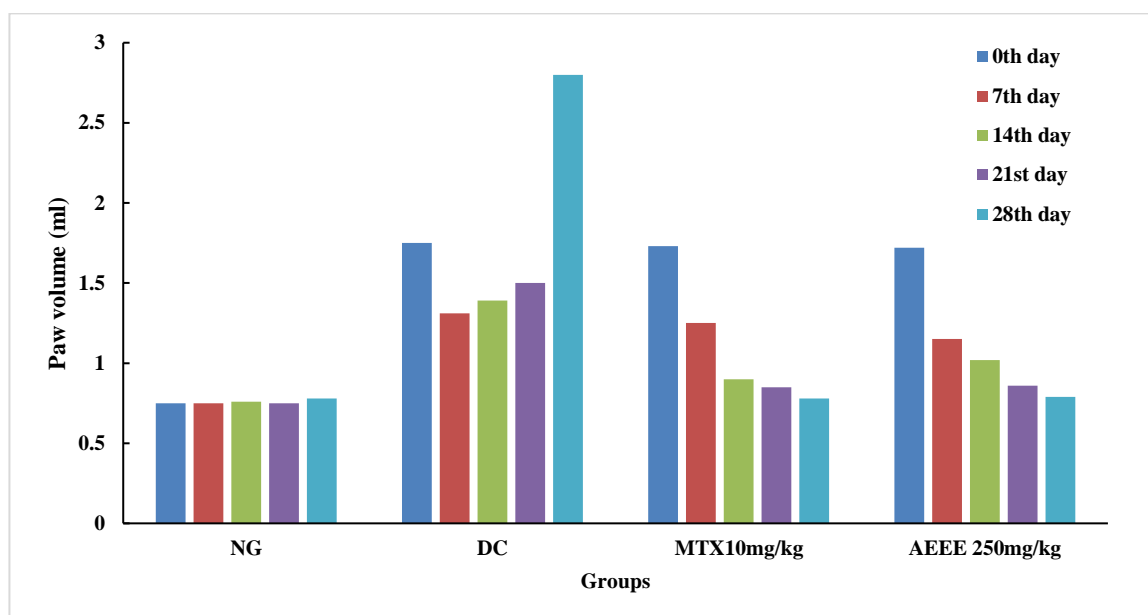
Both methotrexate-treated and *Altingia excelsa* ethanolic extract treated group showed significantly lower paw volume compared to FCA-induced arthritic rats. Moreover, the paw volume was not that much differed in methotrexate-treated rats and *Altingia excelsa* extract-treated rats.

These findings, detailed in **Table 8** and **figure 8** underscore the promising anti-inflammatory effects of the *Altingia excelsa* ethanolic extract on hind paw volume in arthritic rat.

**Table 8: Effect of *Altingia excelsa* ethanolic extract on hind paw volume in arthritic rats**

Groups	Paw volume (ml)				
	0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
NG	0.75±0.66	0.75±0.70	0.76±0.67	0.75±0.38	0.78±0.63
DC	1.75±0.83	1.31±0.42	1.39±0.62	1.50±0.61	2.80±0.72
MTX10mg/kg	1.73±0.74	1.25±0.71	0.90±0.29*	0.85±0.18**	0.78±0.01**
AEEE 250mg/kg	1.72±0.82	1.15±0.64	1.02±0.53	0.86±0.19**	0.79±0.01**

Data represents as SEM Indicates statistical significance compared to the normal group at  $p < 0.05$ . \*\* Indicates higher statistical significance compared to the normal group at  $p < 0.01$ . \*\*\* Indicates even higher statistical significance compared to the normal group at  $p < 0.001$ .

**Figure 8: Effect of ethanol extract of *Altingia excelsa* on hind paw volume in arthritic rats**

### 3.2.2 Effect of *Altingia excelsa* aqueous extract on hind paw volume

In each statistical test, a significance level of  $p < 0.05$  was considered to indicate statistically significant findings, ensuring a robust and reliable interpretation of the experimental outcomes. Throughout the experiment, a consistent linear increase in paw volume was evident in CFA-induced arthritic rats when compared to the control group. However, noteworthy observations emerged when analyzing the effects of interventions. Specifically, both the group treated with methotrexate and the one administered with *Altingia excelsa* aqueous extract exhibited significantly reduced paw volumes compared to the CFA-induced arthritic rats. Furthermore, it's worth highlighting that there were minimal differences in paw volumes between the methotrexate-treated rats and those treated with *Altingia excelsa* aqueous extract, as detailed in Table 9 and Figure 9.

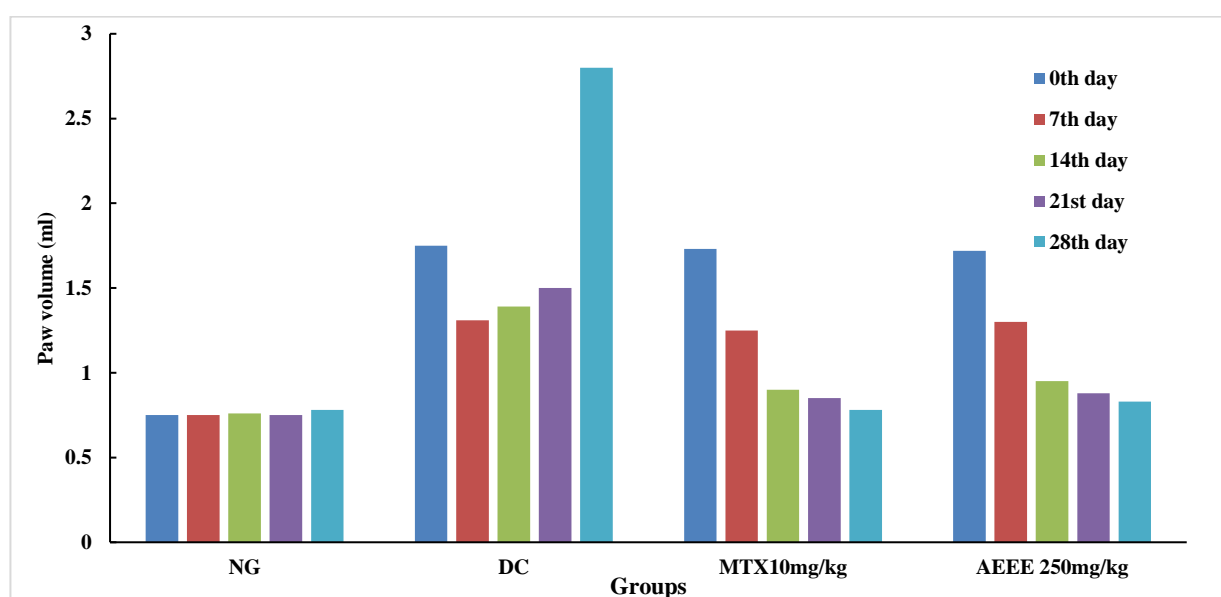
The left hind paw volume was measured weekly using plethysmometer to all groups. Increase in the paw volume of all FCA injected rats was evident on day 1. The increase in paw volume progressed till day 28 in normal group. Treatment of methotrexate 10mg/kg showed significant decrease in the paw volume of left hind paw at the day 21 and 28th with the % inhibition 42.33 and 72.14% respectively. *Altingia excelsa* aqueous extract 250mg/kg significantly ( $p < 0.001$ ) lower the paw volume from day 14 onwards as compared to control group with maximum inhibition (70.36 and 71.43%) respectively on day 28.

These findings underscore the potential anti-inflammatory impact of both methotrexate and *Altingia excelsa* aqueous extract in mitigating paw volume changes associated with arthritic conditions.

**Table 9. Effect of aqueous extract of *Altingia excelsa* on hind paw volume in arthritic rats**

Groups	Paw volume (ml)				
	0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
Normal Group	0.75±0.66	0.75±0.70	0.76±0.67	0.75±0.38	0.78±0.63
Control Group	1.75±0.83	1.31±0.42	1.39±0.62	1.50±0.61	2.80±0.72
MTX10mg/kg	1.73±0.74	1.25±0.71	0.90±0.29*	0.85±0.18**	0.78±0.01**
AEAE 250mg/kg	1.72±0.82	1.30±0.68	0.95±0.52	0.88±0.10**	0.83±0.01**

Data represents as SEM Indicates statistical significance compared to the normal group at  $p < 0.05$ . \*\* Indicates higher statistical significance compared to the normal group at  $p < 0.01$ . \*\*\* Indicates even higher statistical significance compared to the normal group at  $p < 0.001$ .



**Figure 9:** Effect of ethanol and aqueous extract of *Altingia excelsa* on hind paw volume in arthritic rats

### 3.2.3 Effect of *Altingia excelsa* ethanolic extract on Hematological Parameters:

Hematological parameters, including haemoglobin (HB) concentration, provide valuable insights into the extract's impact on blood composition and overall health. The determination of these parameters involves collecting blood samples from the retro-orbital plexus, a standard and minimally invasive technique. In this study, a noteworthy observation was a substantial decrease ( $p < 0.05$ ) in haemoglobin concentration at a dosage of 250mg/kg body weight. This reduction, while statistically significant, requires further exploration to understand its implications and whether it is a specific effect of the extract.

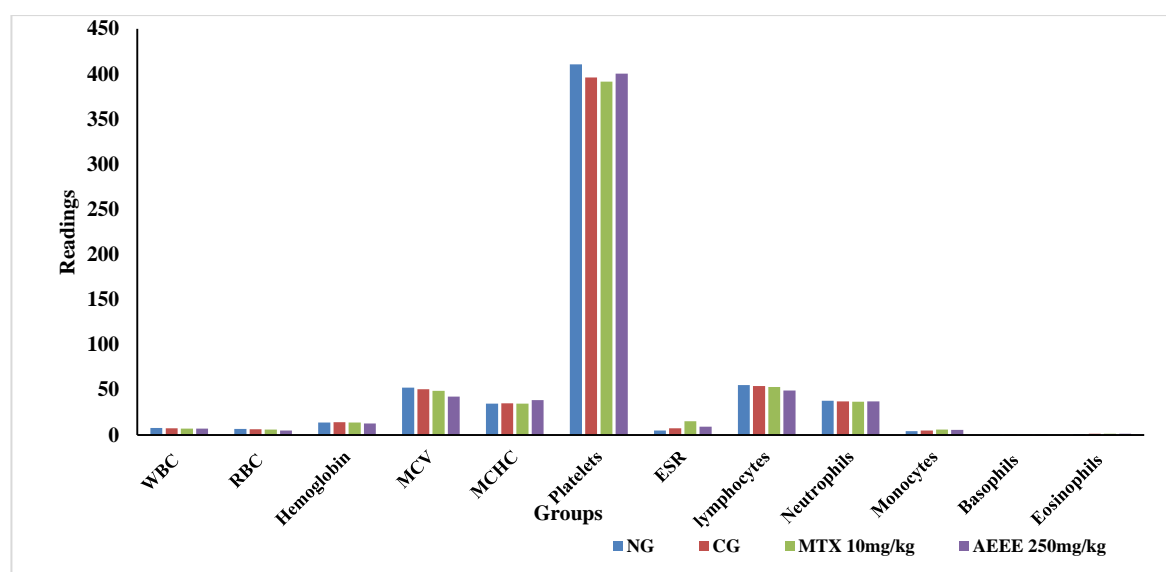
The decrease in haemoglobin concentration might prompt additional investigations into the extract's potential influence on erythropoiesis or red blood cell stability. The significance of this hematological change in the context of the extract's overall safety and therapeutic potential necessitates further exploration and consideration.

In conclusion, the study provides compelling evidence of the *Altingia excelsa* ethanolic extract positive impact on hematological parameters in arthritic rats, indicating potential anti-inflammatory properties as shown in **Table 10 and Figure 9**. Simultaneously, the observed decrease in haemoglobin concentration prompts further investigation into the extract's influence on hematological parameters. These combined findings contribute to a comprehensive understanding of the extract's effects on both inflammatory and physiological aspects.

**Table 10: Effect of *Altingia excelsa* ethanolic extract on hematological parameters**

Parameters	Groups			
	NC	DC	MTX10	<i>Altingia excelsa</i> ethanolic extract 250mg/kg
WBC ( $10^3/\text{mm}^3$ )	7.82±0.15	7.35±0.75	6.94±0.60*	6.88±0.72*
RBC ( $10^6/\text{mm}^3$ )	6.78±0.75	6.30±1.24	5.85±1.14	4.98±1.85
Haemoglobin (%)	13.74±0.55	14.04±0.56	13.85±0.70*	12.50±0.70*
MCV( $\mu\text{m}^3/\text{red cell}$ )	52.40±0.65*	50.55±0.70*	48.65±0.45*	42.60±0.85*
MCHC(g/dL)	34.70±0.90	35.12±0.61	34.74±0.15*	38.40±0.55*
Platelets ( $10^3\text{cells}/\text{mm}^3$ )	410.65±0.62	395.85±0.10	391.45±0.08*	400.07±0.20*
ESR (mm/hr)	4.68±1.32	7.34±0.15	15.00±2.0	9.00±1.50
Lymphocytes (%)	55.30±0.95	54.00±0.15	53.00±0.65*	49.00±0.20*
Neutrophils (%)	37.75±0.68	37.25±0.90	36.80±0.20*	37.00±0.60
Monocytes (%)	4.00±0.50	5.00±0.10	6.00±0.20*	5.70±0.20*
Basophils (%)	0.02±1.15	0.04±1.52	0.06±1.08	0.05±0.50*
Eosinophils (%)	1.12±0.09	1.19±1.16	1.24±1.65	1.15±1.30

Each Value represents mean  $\pm$ SEM for 6 animals with statistical analysis by one-way ANOVA followed by Dunnet's test \* $p < 0.05$ , \*\*  $p < 0.01$  compared with standard drug

**Figure 9: Effect of ethanolic extract of *Altingia excelsa* on hematological profile**

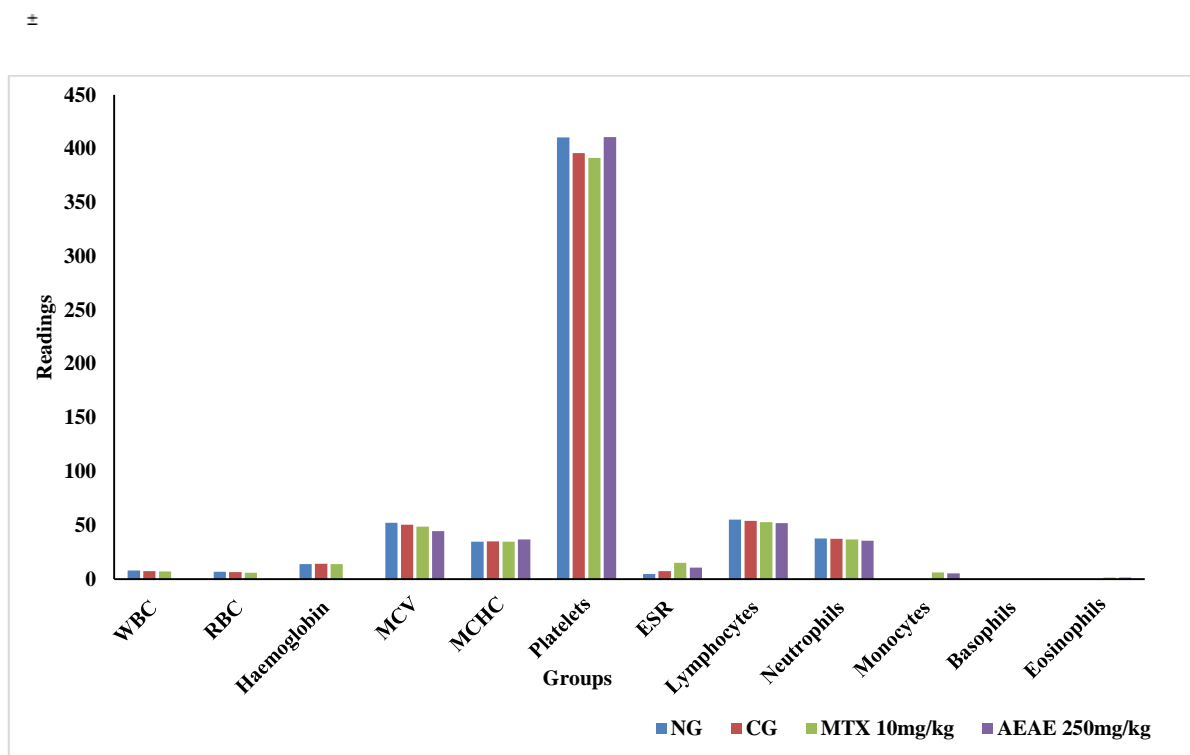
### 3.2.5 Effect of aqueous Extracts of *Altingia excelsa* on hematological parameters

To assess hematological parameters, blood samples will be collected from the retro-orbital plexus. It's noteworthy to mention that there were notable and statistically significant decreases ( $p < 0.05$ ) observed in hemoglobin (HB) concentration at a dosage of 250 mg/kg body weight. This finding underscores the impact of the administered substance on haemoglobin levels and merits careful consideration in the overall evaluation of the study. **Table 11 and Figure 10** shows effects of aqueous extract of leaves of *Altingia excelsa* on hematological parameters.

**Table 11: Effect of aqueous Extracts of *Altingia excelsa* on hematological parameters**

Parameters	Groups			
	NG	CG	MTX10mg/kg	AEAE 250mg/kg
WBC ( $10^3/\text{mm}^3$ )	7.82±0.15	7.35±0.75	6.94±0.60	7.15±0.56*
RBC ( $10^6/\text{mm}^3$ )	6.78±0.75	6.30±1.24	5.85±1.14	4.90±2.25
Haemoglobin (%)	13.74±0.55	14.04±0.56	13.85±0.70*	12.98±0.50*
MCV ( $\mu\text{m}^3/\text{red cell}$ )	52.40±0.65*	50.55±0.70*	48.65±0.45*	44.50±0.75
MCHC (g/dL)	34.70±0.90	35.12±0.61	34.74±0.15	36.70±0.35*
Platelets ( $10^3\text{cells}/\text{mm}^3$ )	410.65±0.62	395.85±0.10*	391.45±0.08*	410.78±0.50*
ESR (mm/hr)	4.68±1.32	7.34±0.15	15.00±2.0	10.50±1.45
Lymphocytes (%)	55.30±0.95	54.00±0.15	53.00±0.65*	52.00±0.20*
Neutrophils (%)	37.75±0.68	37.25±0.90	36.80±0.20*	35.50±0.40*
Monocytes (%)	4.00±0.50*	5.00±0.10	6.00±0.20*	5.40±0.70*
Basophils (%)	0.02±1.15	0.04±1.52	0.06±1.08	0.04±0.50*
Eosinophils (%)	1.12±0.09	1.19±1.16	1.24±1.65	1.28±1.10

Data represented as mean SEM; n=6, \* $p < 0.05$  compared to NC group and \*\* $p < 0.05$  compared to standard drug (one-way ANOVA followed by Tukey's multiple comparison test).



**Figure 10: Effect of aqueous Extract of *Altingia excelsa* on hematological parameters**

**3.2.6 Biochemical profile:** As the culmination of the study approached, the biochemical estimation was scheduled for the last day. The study was conducted on a group of Wistar rats, a commonly used strain in scientific research. The methodology involved the collection of blood samples from the retro-orbital venous plexus of rats, with a time interval of 12 hours between the last extract administration and sample collection. This specific venous plexus is chosen for its accessibility and the minimal stress it imposes on the animals. The collected blood samples underwent meticulous biochemical analysis, focusing on crucial parameters such as SGOT or AST, SGPT or ALT, TB, and ALP. These biochemical markers serve as indicators of liver function and overall metabolic health.

Upon analysis, the extract demonstrated a significant and dose-dependent reduction in the levels of the aforementioned biochemical parameters. This reduction was not only statistically significant but also comparable to both the control group and the group treated with the standard extract. The specific values and statistical details can be referenced in **Table 12**.

These findings suggest a favorable impact of the extract on liver function and metabolic processes. The dose-dependent reduction in SGOT, SGPT, TB, and ALP levels indicates a potential hepatoprotective effect, mitigating the biochemical alterations that might occur under inflammatory or pathological conditions. The comparability to the control group ensures that these effects are specific to the extract and not a result of spontaneous variations.

In conclusion, the biochemical assessment conducted on the last day of the study reaffirms the extract's ability to positively influence key markers of liver function and metabolism. This not only contributes valuable insights into the extract's safety profile but also hints at potential therapeutic applications, especially in conditions where hepatic health is a critical factor.

**Table 12: Effect of *Altingia excelsa* ethanolic extract on biochemical parameters**

Groups	Parameters			
	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	TB (mg/dL)
NC	158 ± 1.15	170 ± 0.75	195 ± 0.60	0.743±0.05
DC	280 ± 1.75	320 ± 0.24	354 ± 1.14	0.894±1.02
MTX10	220 ± 0.05*	249 ± 1.06 *	278 ± 0.70*	1.027±0.02*
<i>Altingia excelsa</i> Ethanolic extract 250mg/kg	192±0.01***	175±3.03*	160±1.00**	0.309±0.05*

Values are expressed as mean ± SD for six animals. One-way ANOVA followed by Dunnet test  
Statistical significance at \*p < 0.05, \*\*p<0.01, \*\*\*p<0.001 compared to the standard group

### 3.2.7 Effect of *Altingia excelsa* aqueous extract on Biochemical parameters

The biochemical estimation is scheduled for the final day of the study, conducted on a group of Wistar rats. After a 12-hour interval, blood samples will be taken from the rats' retro-orbital venous plexus. The extract exhibited a dose-dependent and significant reduction in the levels of biochemical parameters, including SGOT (AST), SGPT (ALT), TB, and ALP. These reductions were comparable to both the control group and the aqueous extract group. **Table 13** represents effect of *Altingia excelsa* on biochemical parameters.

**Table 13: Effect of aqueous extract of *Altingia excelsa* on biochemical parameters**

Groups	Parameters			
	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	T B(mg/dL)
NC	158 ± 1.15	170 ± 0.75	195 ± 0.60	0.743±0.05
DC	280 ± 1.75	320 ± 0.24	354 ± 1.14	0.894±1.02
MTX10	220 ± 0.05*	249 ± 1.06 *	278 ± 0.70*	1.027±0.02*
<i>Altingia excelsa</i> aqueous extract 250mg/kg	210±1.01**	185±1.70	170±01**	0.427±0.09*

Values are expressed as mean ± SD for six animals. One-way ANOVA followed by Dunnet test  
Statistical significance at \*p < 0.05, \*\*p<0.01, \*\*\*p<0.001 compared to the standard group

#### 4. DISCUSSION

The exploration of natural sources for therapeutic agents has gained significant attention in recent years. Among the various plant extracts studied, *Altingia excelsa* have shown promise in managing inflammation and arthritis. This discussion aims to delve deeper into the findings of a study that investigated the ethanol and aqueous extracts of these plants, focusing on their yields, chemical compositions, pharmacological activities, and potential applications in the treatment of inflammatory conditions.

The study commenced by evaluating the extraction yields of *Altingia excelsa* using ethanol. The ethanol extraction from plant had a yield of 5.75% w/w while aqueous extract had a yield of 3.42% w/w. These yields provide an initial insight into the extractive efficiency of the plants. Further, the chemical composition of the extracts was assessed by determining the total flavonoid and phenolic contents. *Altingia excelsa* ethanolic extract had values of 3.67% of flavonoid and 18.90% for phenolic, respectively and for aqueous extract had values of 1.95% for flavonoid and 11.65% for phenolic. The reference compound, quercetin, was employed for this analysis, ensuring a standardized comparison.

Pharmacological investigations were conducted to evaluate the potential therapeutic applications of the extracts. The study employed *in vitro* DPPH radical scavenging, *in vivo* carrageen-induced paw edema, and AIA tests in rats.

Acute toxicity investigations up to 5000 mg/kg for two weeks demonstrated the safety of the extracts. The extracts showcased significant *in vivo* anti-inflammatory activity, surpassing the effects of indomethacin in the carrageenan-induced paw edema model. This result is particularly noteworthy, as inflammation is a central component of various pathological conditions, including arthritis.

AIA tests in rats further substantiated the anti-arthritic potential of the extracts. Positive outcomes in joint stiffness, mobility, and gait tests indicated that *Altingia excelsa* extracts could be effective in managing arthritis. The extracts demonstrated a capacity to significantly reduce inflammation compared to control groups.

- **Hematological and Biochemical Parameters:**

Evaluation of hematological parameters revealed a notable decrease in hemoglobin (HB) concentration at 250 mg/kg body weight. This observation suggests a potential impact on blood parameters, warranting further investigation into the extracts' hematological effects. Biochemical estimates on the last day of the study in Wistar rats demonstrated a dose-dependent reduction in SGOT, SGPT, TB, and ALP levels. These reductions were comparable to the control group, suggesting a potential role of the extracts in modulating biochemical

markers associated with inflammation.

- **Paw Volume and Anti-Arthritic Activity:**

Paw swelling, a sensitive indicator of anti-inflammatory efficacy, increased in CFA-induced arthritic rats but decreased in the methotrexate-treated and extract-treated groups after 14 days. This finding further supports the anti-arthritic activity of the alcoholic as well as aqueous extract of *Altingia excelsa* leaves.

- **Free Radical Scavenging Activity:**

Free radical scavenging activity was determined as percentages compared to the standard at varied extract concentrations. This assay provides insights into the antioxidant potential of the extracts, which is relevant given the role of oxidative stress in inflammatory conditions.

## 5. CONCLUSION

In conclusion, the study on *Altingia excelsa* extracts presents a comprehensive investigation into their potential therapeutic efficacy in inflammation and arthritis. The extracts exhibited notable anti-inflammatory and anti-arthritic activities with a favourable safety profile. Ethanolic extracts, in particular, demonstrated superiority in anti-arthritic effects. The detailed exploration of their chemical composition, pharmacological activities, and differential effects between ethanol and aqueous extracts provides a solid foundation for future research. Further studies should focus on identifying specific bioactive compounds responsible for these effects and elucidating their mechanisms of action, paving the way for the development of natural therapeutics for inflammatory conditions.

## LIST OF ABBREVIATIONS

1. AIA	Adjuvant-Induced Arthritis
2. DPPH	1,1-Diphenyl-2-picrylhydrazyl
3. CPCSEA	Committee for the purpose of control and supervision of experiments on animals
4. CFA	Complete Freund's Adjuvant
5. NSAIDs	Non-Steroid Anti-inflammatory Drugs
6. DMARDs	Disease - Modifying Anti-Rheumatic Drugs
7. ESR	Erythrocytes Sedimentation Rate
8. MCV	Mean Corpuscular Volume
9. SGOT	Serum Glutamic Oxalo acetic transaminase
10. SGPT	Serum Glutamic Pyruvic transaminase
11. ALP	Alkaline Phosphatase
12. SEM	Standard Error Mean
13. TB	Total Bilirubin

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