

**ANTI-ARTHRITIS ACTIVITY OF *KALANCHOE TOMENTOSA*
IN FORMALDEHYDE INDUCED ARTHRITIS MODEL****Prachi V. Gupta*, J. V. Vyas, Vivek Paithankar, Anjali Wankhade****Department of Pharmacology, Vidyabharati College of Pharmacy, SRPF Camp Road, Near Collector Office, Maltekdi, Amravati-444602, Maharashtra, India***Corresponding Author's E mail: prachivgupta9881@gmail.com

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder that causes joint pain, swelling, stiffness, and progressive joint damage. The present study evaluated the anti-arthritis activity of ethanolic extract of *Kalanchoe tomentosa* in formaldehyde-induced arthritic rats. Animals were treated with the extract at doses of 200 mg/kg and 400 mg/kg, and the effects were compared with diclofenac sodium. The extract significantly reduced paw edema, swelling ratio, ESR, CRP, and TNF- α levels while improving body weight. Histopathological examination showed reduced inflammatory cell infiltration and protection against joint damage in treated groups, with the 400 mg/kg dose exhibiting greater efficacy. The anti-arthritis activity may be attributed to the presence of flavonoids, phenols, and other bioactive phytoconstituents. These findings suggest that *Kalanchoe tomentosa* possesses significant anti-inflammatory and anti-arthritis potential and may serve as a promising natural therapeutic agent for the management of rheumatoid arthritis.

Keywords: Rheumatoid arthritis, *Kalanchoe tomentosa*, Formaldehyde-induced arthritis, TNF- α , ESR, CRP.

INTRODUCTION

Rheumatoid arthritis (RA) is a long-term disease that causes inflammation in the joints, leading to pain, swelling, stiffness, and gradual damage to the joints. It affects about 1% of people worldwide, and more than 3 million new cases occur each year. RA can make it difficult for people to move and perform everyday activities, reducing their quality of life. The disease is also linked to a higher risk of health complications and a shorter life expectancy compared to healthy individuals. Therefore, developing effective treatments to prevent and control RA, as well as its associated complications, is very important¹.

Rheumatoid arthritis (RA) is a long-term autoimmune disease in which the body's immune system mistakenly attacks its own joints. It usually starts in the small joints of the hands and feet on both sides

of the body and can later affect larger joints. RA may also affect other organs such as the skin, eyes, heart, kidneys, and lungs.

Over time, the disease can damage bone and cartilage and weaken the tendons and ligaments around the joints. This can lead to joint deformities, bone erosion, and severe pain. Common symptoms of RA include joint pain, swelling, warmth, tenderness, morning stiffness lasting more than 30 minutes, tiredness, fever, and weight loss. Some patients may also develop small lumps under the skin called rheumatoid nodules.

RA most commonly occurs between 35 and 60 years of age and is characterized by periods when symptoms improve (remission) and periods when they worsen (flare-ups). A similar condition can also occur in children younger than 16 years, known as juvenile rheumatoid arthritis (JRA). Unlike adult RA, JRA usually does not show the rheumatoid factor in blood tests. RA affects about 1–2% of people in Western countries and approximately 1% of the world's population ². The present study was designed to evaluate the anti-arthritic potential of *Kalanchoe tomentosa* extract using a formaldehyde-induced arthritis model in experimental rats. The study aimed to investigate the effect of the extract on the progression of arthritis by assessing changes in paw volume, physical signs of inflammation, biochemical markers of inflammation and histopathological alterations in joint tissues.

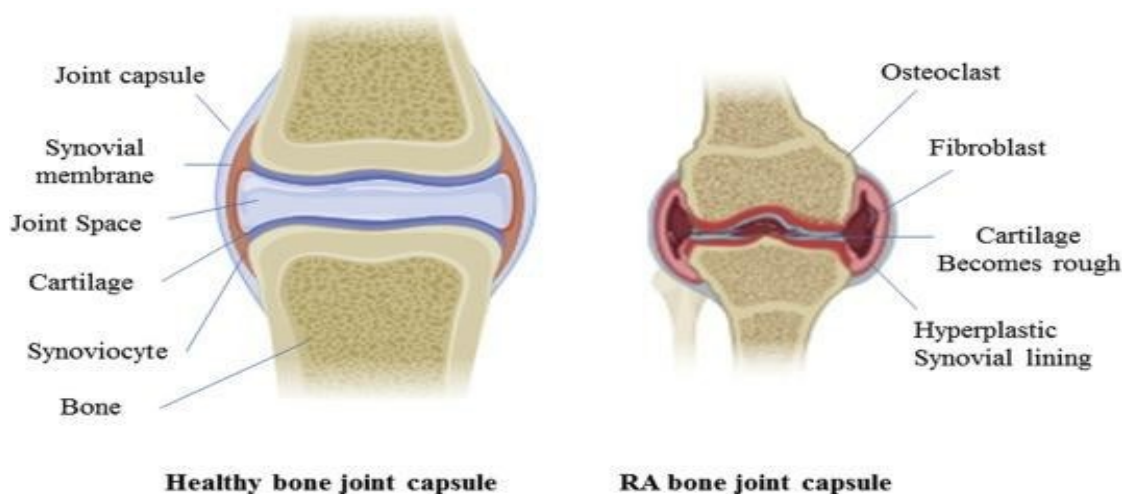


Figure no. 01. Bone joint differentiation of normal and Rheumatoid arthritis the pathophysiological mechanism of rheumatoid arthritis (RA) and the immune response involves a sequence of events ³

MATERIAL AND METHOD:

Selection of plant:

The leaves and stems of *Kalanchoe tomentosa* were collected from a local farm in the Amravati District. The plant was identified and authenticated by Dr. Bansod from the Department of Botany, Vidyabharati Mahavidyalaya, Amravati. The collected leaves and stems were washed properly and

dried under shade. After drying, the plant material was stored in airtight containers for further experimental studies.

Experimental Animals

Healthy Wistar rats of either sex, weighing 150–200 g, were obtained from the Animal House of the Department of Pharmacology, Vidyabharati College of Pharmacy, Amravati (CPCSEA Registration No. 1504/PO/RE/S/11/CPCSEA). They were housed in cages under standard environmental conditions with a 12-hour light/12-hour dark cycle, temperature maintained at $25 \pm 1^\circ\text{C}$, and relative humidity of 50–55%. The experimental study was conducted according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) after obtaining approval from the Institutional Animal Ethics Committee (IAEC). The animals were provided with standard pellet diet and water ad libitum (free access) ⁴.

Drugs & Chemicals

Inducing agent: Formaldehyde was issued from store of Vidyabharati college of pharmacy, Amravati.
Standard drug: Diclofenac sodium 10mg tablet brought from pharmacy store Amravati
Treatment drug: Ethanolic extract of *kalanchoe tomentosa*

Preparation of Doses

Diclofenac sodium tablets were crushed and dissolved in distilled water to prepare a solution containing 10 mg/100 mL. Two doses of *Kalanchoe tomentosa* extract (200 mg/kg and 400 mg/kg) were prepared using distilled water. All solutions were freshly prepared before administration. The plant extract and vehicle (0.9% NaCl) were administered orally to the animals. Arthritis was induced by subplantar injection of 0.2% formaldehyde, while the standard drug, diclofenac sodium, was given orally ⁵.

Phytochemical Analysis

The ethanolic extract of *Kalanchoe tomentosa* was subjected to various phytochemical screening tests to identify the presence of different chemical constituents, such as flavonoids, alkaloids, tannins, steroids, and other phytochemicals.

A. Test for Glycoside

Legals Test: The extract was hydrolysed with HCl on a water bath. 1ml of pyridine was added to hydrolysate and few drops of sodium nitroprusside solution were added and then it made alkaline with NaOH solution, appearance of pink to red colour shows the presence of glycoside ⁶.

Killer-killani Test: 2ml of aqueous extract + glacial acetic acid, one drop 5% Ferric chloride and Concentration sulphuric acid. Reddish brown colour appears at junction of two liquid layer and upper layers appears bluish green ⁷.

B. Test for Flavonoid

1ml of extract + few drops of dil. NaOH was added. An intense yellow colour was produced which become colourless on addition of few drop of dil. HCl, indicate presence of flavonoids ⁸.

C. Test for sterols and triterpenoids:

Salkowski test: Different extract samples were treated with few drops of concentrated sulphuric acid. Appearance of red and yellow colour at the lower layer indicated the presence of steroids and triterpenoids respectively ⁹.

D. Test for Saponin

Froth Test: About 0.5gm of extract was dissolved in 10 ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allotted to stand in a vertical position and observed over 30 minutes period of time. If a "honey comb" froth above the surface of liquid persists after 30 minutes for sample is suspected to contain saponins ¹⁰.

E. Test for phenols

Ferric chloride test: 1 mg of the plant extract was dissolved in 1 mL of methanol and mixed with distilled water in a test tube. Then, 2 mL of ferric chloride solution was added. The appearance of a green or blue colour indicated the presence of phenols ^{11,12}.

F. Test for Alkaloids

Mayer's Test: 1ml of extract in 1ml of Mayer's reagent (potassium mercuric iodide solution), Whitish yellow or cream colour precipitate observed, which shows the presence of alkaloid ¹³.

Hager's Test: 1ml of extract in 3ml of Hager's reagent. Yellow colour precipitate observed, which shows the presence of alkaloid ¹⁴.

Wagner's Test: 1ml extract in 2ml of Wagner's reagent (iodine in potassium iodide). Formation of reddish- brown precipitate, which shows the presence of alkaloid¹⁵.

Dragendroff's Test: 1 ml of extract in 1ml of Dragendroff's reagent (potassium bismuth iodide solution). Reddish orange precipitate was observed, which shows the presence of alkaloid ¹⁶.

Extraction Process

The leaves of *Kalanchoe tomentosa* were collected, identified, and authenticated by a qualified taxonomist. The leaves were washed with distilled water to remove dust and impurities and then shade-dried at room temperature (25–30°C) for 7–10 days. After drying, the leaves were powdered using a grinder and stored in an airtight container.

The powdered material was extracted using ethanol as the solvent. For Soxhlet extraction, about 100–200 g of powder was extracted continuously for 6–8 hours. The extract was filtered and concentrated using a rotary evaporator to obtain a semi-solid mass, which was then stored for further studies ^{17,18}.

Grouping of animal:**Table 1: Grouping of animal**

Sr. no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I (Normal control)	6	Saline treatment	sub-plantar
2	II (Inducing group)	6	Single dose of formaldehyde (0.1ml/kg/day)	sub-plantar
3	III (Standard group)	6	formaldehyde (0.1ml/kg/day) + Diclofenac (10 ml/kg)	sub-plantar and Oral
4	VI (Treatment 1)	6	formaldehyde (0.1 ml/kg/day) + kalanchoe tomentosa extract (200 mg/kg bw)	sub-plantar and Oral
5	V (Treatment 2)	6	Formaldehyde (0.1 ml/kg/day) + kalanchoe tomentosa extract (400 mg/kg bw)	sub-plantar and Oral

Experimental Procedure

Wistar rats weighing 150–200 g were maintained under standard laboratory conditions with a 12-hour light and 12-hour dark cycle. Arthritis was induced by injecting 0.1 mL of 2% formaldehyde into the subplantar region of the left hind paw for 7 days in all groups except the normal control group.

The animals were divided into five groups (n = 6 per group): Normal Control, Arthritic Control, Standard Treatment, Test Group I, and Test Group II. The standard group received diclofenac sodium, while Test Group I and Test Group II received ethanolic extract of *Kalanchoe tomentosa* at doses of 200 mg/kg and 400 mg/kg, respectively, by oral administration for 4 weeks.

The anti-arthritic activity was evaluated by measuring paw diameter, paw swelling, body weight, and biochemical parameters such as ESR, CRP, and TNF- α levels. The formaldehyde-induced arthritis model was used to assess the anti-arthritic effect of the plant extract ¹⁹.

Erythrocyte sedimentation rate (ESR)

The Erythrocyte Sedimentation Rate (ESR) test measures how fast red blood cells settle at the bottom of a blood sample. A higher ESR value usually indicates the presence of inflammation in the body, which may be caused by infections, autoimmune disorders, or other inflammatory conditions ²⁰.

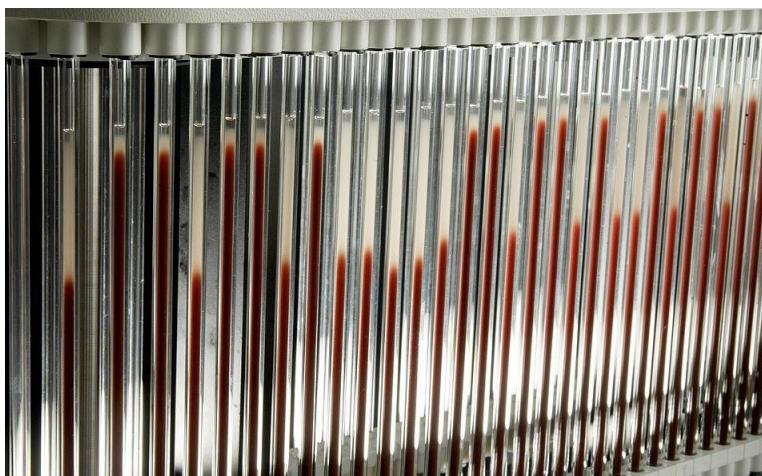


Figure 2: ESR Determination by Westergren method

CRP Test

The C-reactive protein (CRP) test is a blood test used to measure the level of CRP in the blood. CRP is a protein produced by the liver when there is inflammation in the body. Increased CRP levels indicate the presence of inflammation, which may be caused by infections, chronic diseases, or other inflammatory conditions ²¹.

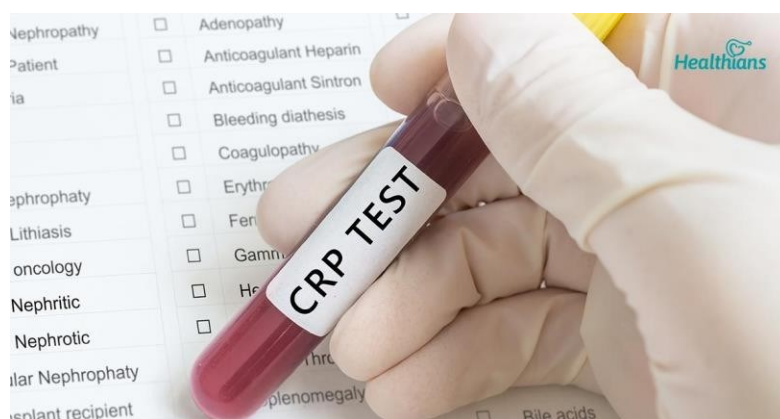


Figure 3: CRP test

Paw Diameter:

Paw diameter was measured using a Vernier caliper. The measurements taken on the day of arthritis induction were compared with those recorded after treatment with the two different doses of *Kalanchoe*

tomentosa extract and the standard drug. This comparison was used to evaluate the reduction in paw swelling and the anti-arthritic effect of the treatments ²².



Figure 4: Paw diameter measurement by vernier caliper

Body weight:

Induction of osteoarthritis (RA) in the experimental animals caused a decrease in body weight in the induced group compared to the normal control group ²³.



Figure 5: Body weight

Swelling Ratio

The swelling ratio is used to measure the extent of swelling in the affected paw. It is calculated by comparing the paw size before and after swelling using a standard formula. A higher swelling ratio indicates greater inflammation, while a lower swelling ratio indicates a reduction in swelling. ^[24]

$$\text{Swelling Ratio} = \frac{DS - Dd}{Dd}$$

Where, Ds = Diameter After FA Induced

Dd = Diameter After Treatment/ Observations

TNF- α

The TNF- α (Tumor Necrosis Factor-alpha) test is performed using the ELISA method to measure the level of TNF- α in blood samples. TNF- α is an inflammatory protein that plays an important role in diseases such as arthritis. During the test, a color reaction occurs due to the binding of antigen and antibody. The intensity of the color is measured using a spectrophotometer, and a darker color indicates a higher level of TNF- α in the sample ^{25,26}.

Histopathology study:

Rats were sacrificed and the knee joint tissues were collected and preserved in 10% buffered formalin. The tissues were processed, embedded in paraffin wax, and sectioned into 5–6 μ m thickness. Thesections were stained with Hematoxylin and Eosin (H&E) stain and examined under a light microscope at 10X magnification for histopathological changes such as inflammatory cell infiltration, cartilage erosion, synovial hyperplasia, and bone destruction ^{27,28,29}.

Statistical Analysis

The data obtained from the screenings were subjected to statistical analysis following one- way ANOVA and Two-way ANOVA followed by Dunnett's Multiple Comparison Test and Tukey's multiple comparison test to assess the statistical significance of the results using GraphPad Prism version 11. software. P Values less than 0.05 were considered as statistically significant.

RESULT AND DISCUSSION:

Result:

Phytochemical analysis:

Phytochemical testing carried out to find out the secondary metabolite because secondary metabolic possess biological activity. Phytochemical studies of *Kalanchoe tomentosa* performed for the presence of Carbohydrate, Glycosides, sterols, Alkaloids, Saponins, Terpenoids, Flavonoids, Protein and Amino acids.

Table 2: Phytochemical Analysis of *Kalanchoe tomentosa*

Sr. No	Chemical Constituents	Results
1	Carbohydrate	-
2	Glycosides	+
3	Alkaloids	-
4	Sterols	+
5	Saponins	+
6	Phenols	+
7	Terpenoids	+
8	Flavonoids	+

Where (+) indicates Present & (-) indicates Absent

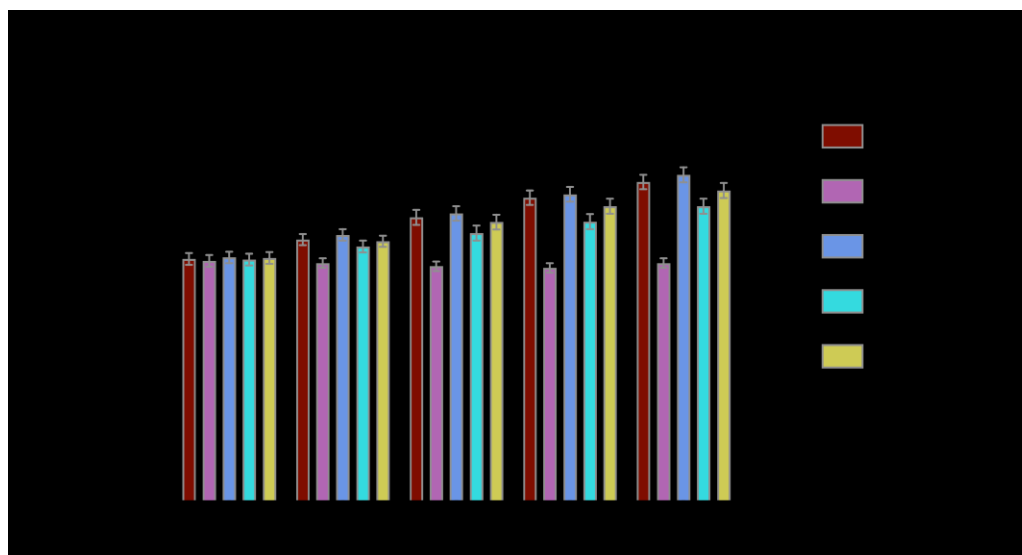
Pharmacological and Haematological Examination:

Body weight:

Table 3: Effect of *Kalanchoe Tomentosa* on Body weight

Day	Control	FA 0.1ml	DFS 10mg/kg	<i>Kalanchoe Tomentosa</i> 200mg/kg	<i>Kalanchoe Tomentosa</i> 400mg/kg
1	154.83 ± 1.52	153.50 ± 1.43	155.83 ± 1.35	154.50 ± 1.43	155.50 ± 1.35
7	167.17 ± 1.47	152.00 ± 1.22	170.17 ± 1.47	162.83 ± 1.47	166.17 ± 1.47
14	181.33 ± 1.88	150.00 ± 1.18	183.83 ± 1.88	171.33 ± 1.88	178.33 ± 1.88
21	193.83 ± 1.84	149.00 ± 1.18	196.00 ± 1.84	178.67 ± 1.84	188.50 ± 1.84
28	203.83 ± 1.84	152.00 ± 1.22	208.50 ± 1.92	178.67 ± 1.84	198.50 ± 1.84

All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to each group compared with each day on 0,7,14,21,28, (****p<0.0001) followed by Two-way ANOVA using Tuckey's test.

Figure 6: Effect of *Kalanchoe Tomentosa* on Body weight

Paw Diameter:

Table No. 04: Effect of *Kalanchoe Tomentosa* on Paw Diameter

Day	Control	FA 0.1 ml	DFS 10mg/kg	Kalancho Tomentosa 200mg/kg	Kalanchoe Tomentosa 400mg/kg
1	3.092 ± 0.010	3.125 ± 0.0076	3.085 ± 0.0076	3.100 ± 0.0058	3.090 ± 0.0058
7	3.122 ± 0.006	5.908 ± 0.028	4.683 ± 0.022	5.308 ± 0.028	4.883 ± 0.022
14	3.150 ± 0.009	6.608 ± 0.028	4.483 ± 0.022	5.608 ± 0.028	4.783 ± 0.022
21	3.175 ± 0.007	6.908 ± 0.028	4.183 ± 0.022	5.408 ± 0.028	4.483 ± 0.022
28	3.092 ± 0.010	6.917 ± 0.028	3.595 ± 0.015	4.717 ± 0.022	3.895 ± 0.015

All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to each group compared with each day on 0,7,14,21, 28, (****p<0.0001) followed by Two-way ANOVA using Tuckey's test.

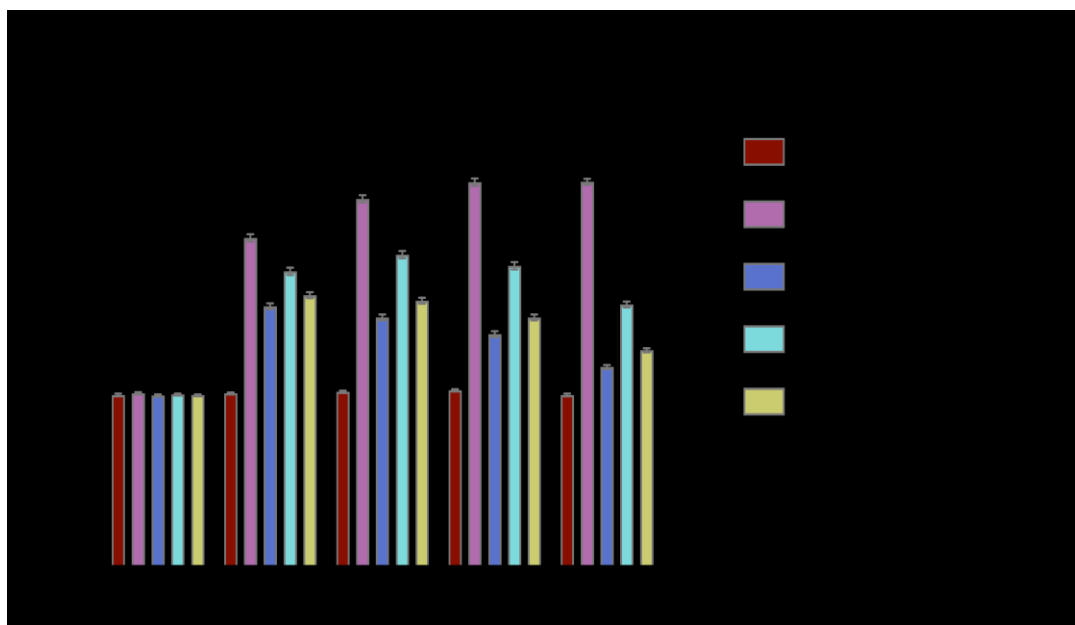


Figure 7: Effect of *Kalanchoe Tomentosa* on the Paw diameter

Swelling ratio:

Table No. 05: Effect of *Kalanchoe Tomentosa* on Swelling ratio

Day	Control	FA (0.1ml)	DFS 10mg/kg	Kalanchoe Tomentosa 200mg/kg	Kalanchoe Tomentosa 400mg/kg
1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	4.16 ± 0.04	52.50 ± 0.42	22.50 ± 0.42	37.50 ± 0.42	32.50 ± 0.42
14	3.16 ± 0.04	57.50 ± 0.42	16.00 ± 0.25	32.50 ± 0.42	27.00 ± 0.25
21	3.16 ± 0.04	54.50 ± 0.42	11.33 ± 0.33	30.00 ± 0.25	22.50 ± 0.42
28	3.16 ± 0.04	53.50 ± 0.42	9.16 ± 0.30	28.16 ± 0.30	19.16 ± 0.30

All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to each group compared with each day on 0,7,14,21, 28, (****p<0.0001) followed by Two-way ANOVA using Tuckey's test.

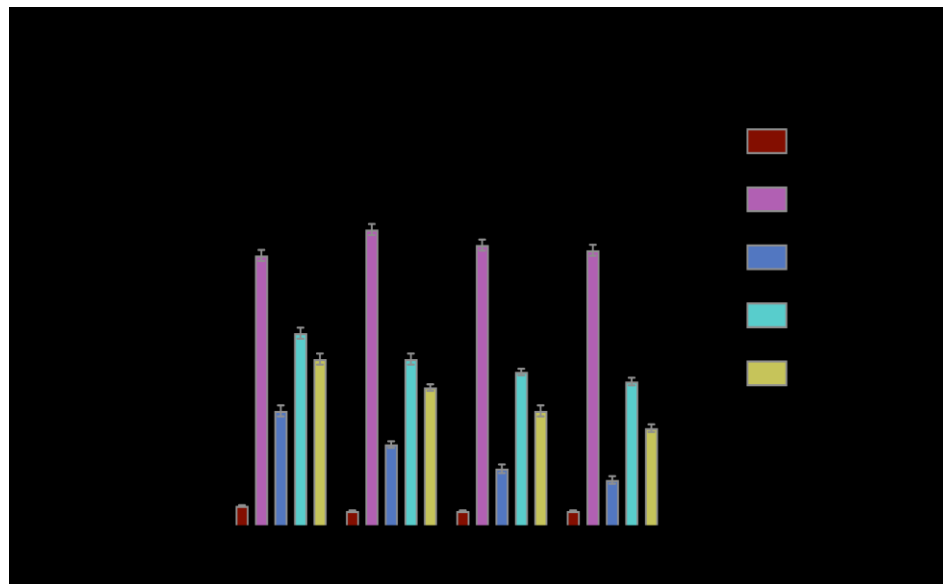
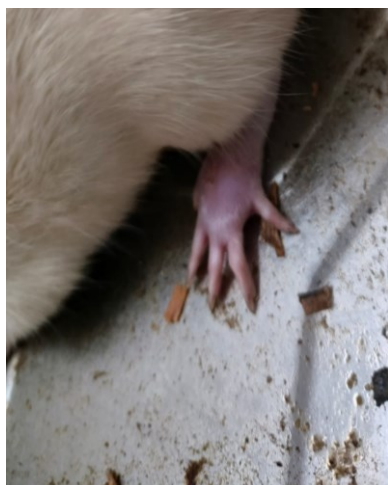


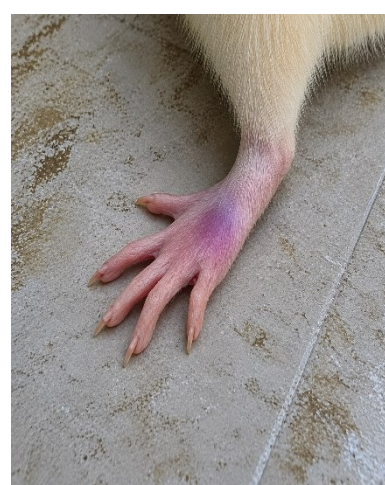
Figure 8: Effect of *Kalanchoe Tomentosa* on swelling ratio



Day 1



Day 7



Day 14



Day 21



Day 28

Erythrocyte sedimentation rate (ESR):

Table No. 06: Effect of *Kalanchoe Tomentosa* on ESR

Group	Treatment	ESR in (mm/hr)
I	Control	6.83 ± 0.10
II	Formaldehyde (0.1 ml)	18.82 ± 0.17
III	Diclofenac sodium (10mg/kg) + Formaldehyde (0.1)	9.85 ± 0.10
IV	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 200mg/kg	13.92 ± 0.11
V	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 400mg/kg	11.33 ± 0.09

All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to Formaldehyde induced group, (****p<0.0001) F (4, 25) = 1535 followed by One way ANOVA using Dunnett's test

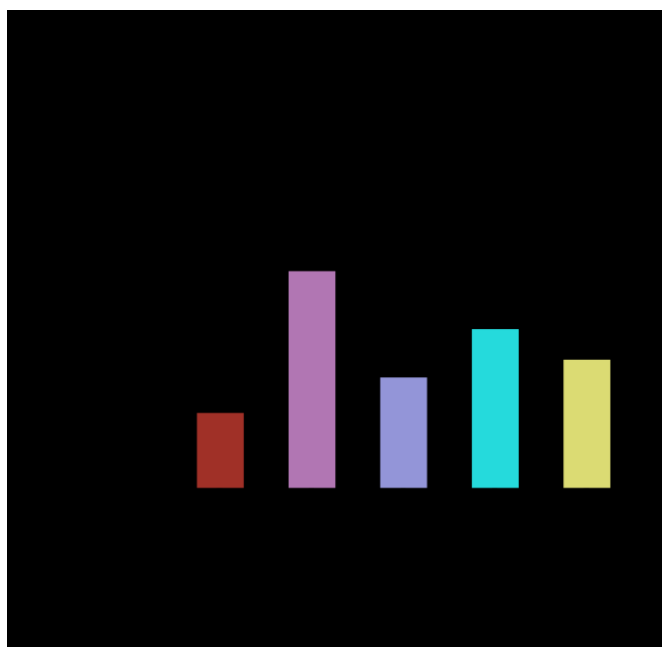


Figure 9: Effect of *Kalanchoe Tomentosa* on the ESR

CRP Test (C-reactive protein test):

Table 7: Effect of *Kalanchoe Tomentosa* on CRP Count

Group	Treatment	CRP Level in (mg/dL)
I	Control	3.32 ± 0.05
II	Formaldehyde (0.1 ml)	12.92 ± 0.10
III	Diclofenac sodium (10mg/kg) + Formaldehyde (0.1)	5.03 ± 0.06
IV	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 200mg/kg	8.70 ± 0.06
V	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 400mg/kg	6.15 ± 0.04

All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to Formaldehyde induced group, (****p<0.0001) F (4, 25)=3348 Followed One way ANOVA using Dunnett's test.

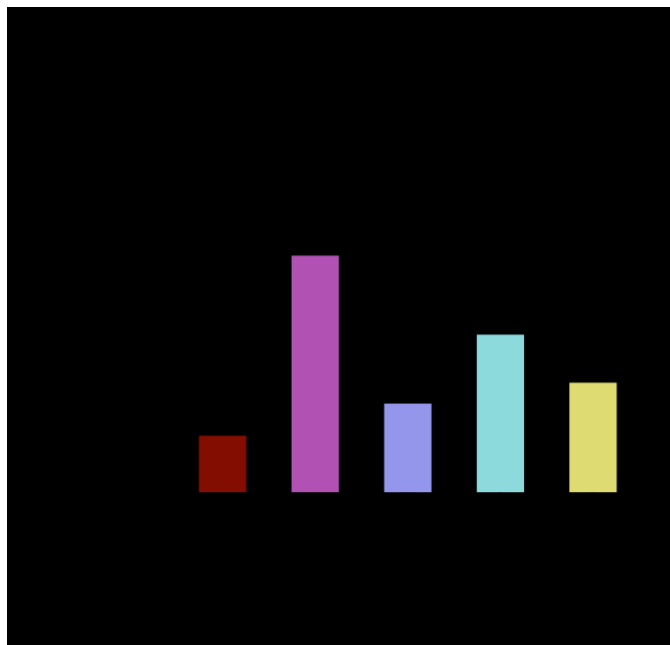
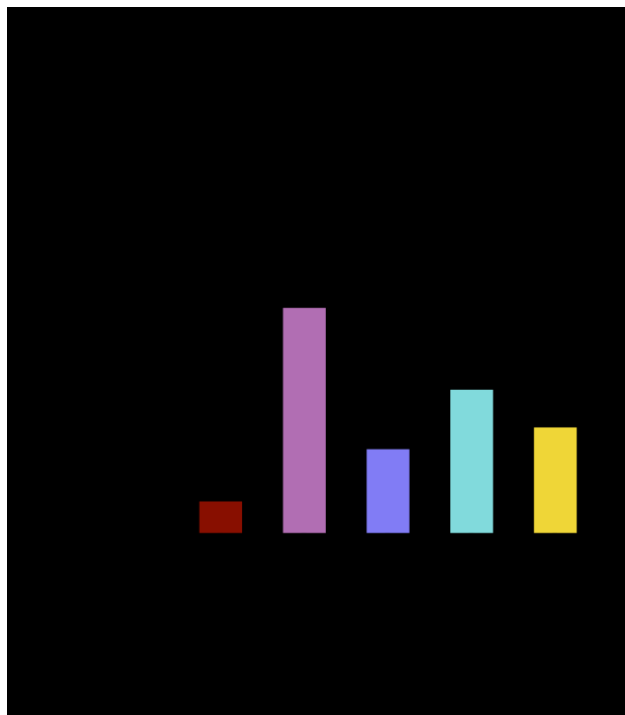


Figure 10: Effect of *Kalanchoe Tomentosa* on the CRP

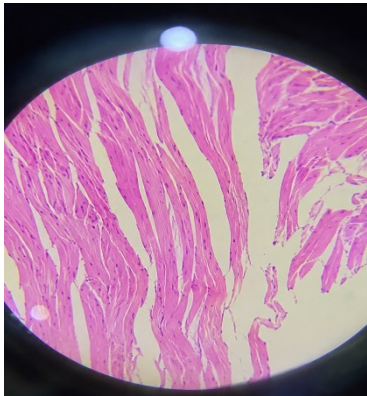
TNF-alpha Test (Tumor Necrosis Factor):**Table 8: Effect of *Kalanchoe Tomentosa* on TNF Count**

Group	Treatment	TNF-a (pg/mL)
I	Control	17.17 ± 0.60
II	Formaldehyde (0.1 ml)	103.83 ± 1.80
III	Diclofenac sodium (10mg/kg) + Formaldehyde (0.1)	40.50 ± 0.76
IV	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 200mg/kg	67.17 ± 0.87
V	Formaldehyde (0.1) + <i>Kalanchoe Tomentosa</i> 400mg/kg	50.33 ± 0.88

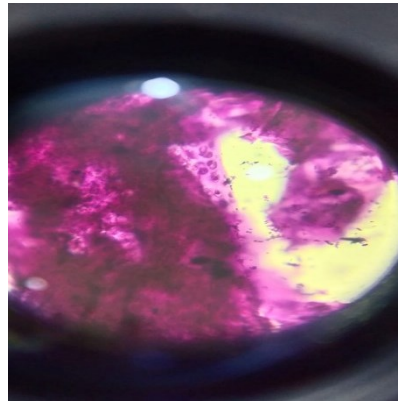
All Values were expressed as mean ± SEM, (n=6), (****p<0.0001) compared to Formaldehyde induced group, (****p<0.0001) F (4, 25) = 839.3 followed by One way ANOVA using Dunnett's test

**Figure 11: Effect of *Kalanchoe Tomentosa* on the TNF-alpha**

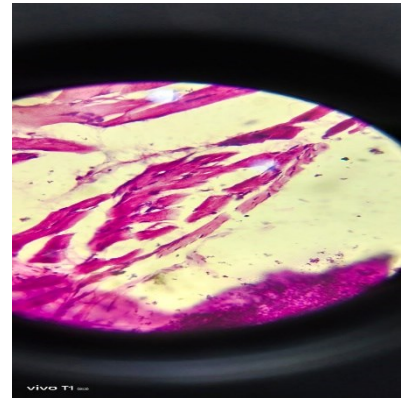
Histology:



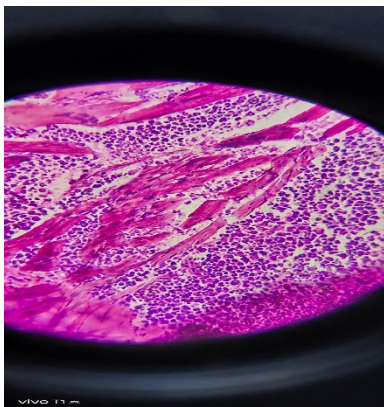
I (control)



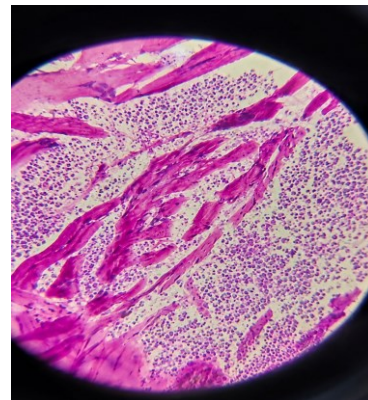
II (induce)



III (standard)



IV (treatment 200 mg/kg)



V (treatment 400 mg/kg)

- I. Control (Normal) group: Showing normal joint architecture with intact cartilage and absence of inflammatory cell infiltration.
- II. Induced (Formaldehyde) group: Showing severe inflammatory cell infiltration, cartilage erosion, and disrupted joint architecture.
- III. Standard (Diclofenac sodium 10 mg/kg) group: Showing reduced inflammation with partial restoration of cartilage and preserved bone architecture.
- IV. *K. tomentosa* 200 mg/kg group: Showing moderate reduction in inflammatory changes with mild protection of cartilage structure.
- V. *K. tomentosa* 400 mg/kg group: Showing significant restoration of normal joint structure with minimal inflammatory infiltration.

Discussion:

The present study evaluated the anti-arthritis activity of *Kalanchoe tomentosa* extract using the formaldehyde-induced arthritis model in rats. Formaldehyde administration produced significant paw edema, increased

inflammatory markers, reduction in body weight, and histopathological alterations, indicating successful induction of arthritis.

Treatment with *Kalanchoe tomentosa* extract at doses of 200 mg/kg and 400 mg/kg significantly reduced paw swelling and inflammatory responses compared with the arthritic control group. The higher dose showed greater anti-arthritic activity, suggesting a dose-dependent effect. The observed activity was comparable to diclofenac sodium, indicating potent anti-inflammatory potential of the plant extract.

The extract-treated groups also showed improvement in body weight and reduction in ESR, CRP, and TNF- α levels. Elevated TNF- α is closely associated with synovial inflammation and joint destruction in rheumatoid arthritis; therefore, reduction of this cytokine suggests suppression of inflammatory pathways involved in disease progression.

Histopathological findings further confirmed the protective effect of *Kalanchoe tomentosa*. Treated groups demonstrated reduced inflammatory cell infiltration and preservation of normal joint architecture compared with the arthritic control group.

The anti-arthritic activity of *Kalanchoe tomentosa* may be attributed to the presence of flavonoids and phenolic compounds, which possess significant anti-inflammatory and antioxidant properties. Overall, the study suggests that *Kalanchoe tomentosa* possesses promising anti-arthritic potential and may serve as a natural therapeutic agent for the management of rheumatoid arthritis.

CONCLUSION

The present study demonstrated that *Kalanchoe tomentosa* possesses significant anti-arthritic activity in formaldehyde-induced arthritic rats. The extract reduced paw edema, inflammatory markers, and histopathological alterations, with the 400 mg/kg dose showing greater efficacy. The observed activity may be attributed to flavonoids and phenolic compounds present in the plant. These findings suggest that *Kalanchoe tomentosa* has promising potential as a natural therapeutic agent for the management of rheumatoid arthritis.

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