

# EVALUATION AND EFFECTS OF BULBS OF *LILIUM CANDIDUM* ON RHEUMATOID ARTHRITIS FOR ITS ANTI-INFLAMMATORY POTENTIAL BY USING ALBINO RATS

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## Abstract-

The present research study were aids to evaluate the phytochemical screening and pharmacological activity of the bulbs extract powders of *Lilium candidum* by using Albino rats. The maceration process were used for extraction of bulbs of *Lilium candidum*. The bulbs powdered extract were given to the albino rats in the different dosage for evaluating the Anti- inflammatory activity in Rheumatoid Arthritis by employing Formaldehyde induced paw edema arthritis model. The powdered extract of *Lilium candidum* produced the significant anti- inflammatory effect in dose dependent manner. The various chemical constituents such as, alkaloids, phenolic acids, tannins and flavonoids might be responsible for its anti- inflammatory activity.

**Keywords-** *Lilium candidum*, maceration, anti- inflammatory activity, Rheumatoid Arthritis, flavonoids, etc.

## Introduction-

Rheumatoid arthritis (RA) is a long-lasting, regular, seditious autoimmune disease that firstly affects minor joints, developing to greater junctions, and ultimately the skin, eyes, heart, kidneys, and lungs. Frequently, the bone and cartilage of junctions are demolished, and ligaments and ligaments deteriorate [1]. All this injury to the junctions causes irregularities and bone destruction, frequently very tender for a patient. Mutual indications of RA include morning difficulty of the affected junctions for > 30 min, weakness, fever, weight loss, joints that are tender, inflamed and warm, and rheumatoid nodes under the skin. The start of this illness is usually from the age of 35 to 60 years [2,3,4,5].

In, China, the Liliaceae family plants of genus *Lilium* were extensively spread in the world and, there cultivation were done as in the form of ornamental plants, as well as they are also well known for their edible and organic pharmaceutical products. The genus *Lilium* has the many belongings such as tranquilizing the mind, nutrition of moisturised lungs and heart burn were also pure by the *Lilium*. Spirit trance, insomnia, cough, dreaminess, hemoptysis and nervousness were several uncontrolled environments, in which the *Lilium* is used [6].

There are numerous classes of genus *Lilium* plants, in which bulbs taking the high quantity of therapeutic belongings, such as *Lilium lancifolium* and *Lilium pumilum*. Conferring to many studies ingredients it is decided that the genus *Lilium* have the numerous biochemical ingredients such as sterols, saponins, phenyl propanoids, flavonoids, alkaloids, glycerides, polysaccharides and glycerols which exemplify the pharmacological action of hypoglycaemic effect, anti-tumor effect, hypolipidemic effect, anti-depression, anti-fatigue, anti-bacterial, hypoxia acceptance effects, blood lipid decrease effect, anti- inflammatory effect [7].

The plants of genus *Lilium*, is a herbaceous bulb comprising plant which belongs to the family Liliaceae. This genus *Lilium* has its origin from China and in the shares of Europe, North America and Eastern Asia, the plants of genus *Lilium* were cultivated as attractive plant. In the species of genus *Lilium* except *Lilium lancifolium thumb*, all classes are diploid [8].

The classes *Lilium candidum L* is also named white lily, or white Madonna lily, which is well recognized for its outmoded medications which were broadly used for the cures of infections, burns, healing wounds and for ulcers in the extract of *Lilium candidum L*. On the base of the hypothesis of the extract of the *Lilium candidum* possess the bio-protective movement due to connections of anti-mutagenic motion of natural compounds with antioxidant effects and contents of phytochemical ingredients from the flavonoids collections [9].

## Materials and Method-

### • Plants and Chemicals Required-

All the components used in this education were of standard pharmaceutical grade. *Lilium candidum L* are gained from the Jai Bharat Nursery, city branch-Hafizpur, Azamgarh, Uttar Pradesh. Formaldehyde were attained from R K. Pharmacy, Azamgarh. Methanol, Ethanol, Diclofenac, sodium picrate, dil. Sulphuric acid, chloroform, ammonia, glacial acetic acid, pyridine, sodium nitroprusside, ferric chloride, ninhydrin, were gained from the R. K. Pharmacy College, and of analytical reagent ranking.

### • Apparatus Required-

Beaker, conical flask, Measuring cylinder, pipette, glass rod, funnel, test tubes, stand, test tube holder, Water bath, Round bottom flask, Condensor, Filter Paper, Sub-planter Injection, Plethresmometer etc were obtained in the Pharmacology research laboratory of R. K. Pharmacy College, Azamgarh.

### • Experimental Animal Required-

18 Albino rats of 100-150gm were accepted from the institutional animal ethical committee as per the way of CPCSEA for the in-vitro animal study such as anti-inflammatory movement, and acute toxicity learning.

### • Extraction of *Lilium candidum Bulb-*

- The Defatting of *Lilium candimum* were completed.
- *Lilium candimum* were shade dried at room temperature.
- The shade dried plant material was coarsely crushed and subjected to extraction with ethanol by maceration.
- The extraction was constant till the defatting of the solid had taken place.
- 100gm of dry plant solid were carefully extracted with Ethanol using maceration method for 48 hrs.
- Clarified and dried using vaccum evaporator at 40°C. Lastly the percentage produces were considered of the dried extracts [10].



**Figure 1- Cleaning of *Lilium candimum***



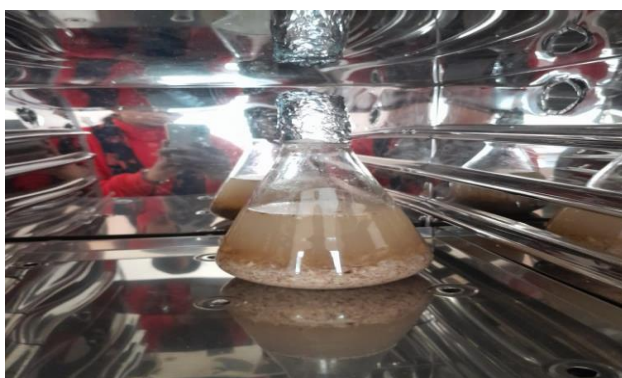
**Figure 2- Dried *Lilium candimum Bulbs***



**Figure 3- Grinded *Lilium Candidum* Dried Bulb**



**Figure 4-First day of *Lilium Candidum* Bulb Maceration**



**Figure 5-*Lilium candidum* Bulb Extracts Stored at Desired Temperature**



**Figure 6-Extracted *Lilium candidum* Bulb after 48 hours of Maceration by using Ethanol**



**Figure 7- Dried extract powder of *Lilium candidum* Bulb**

**Phytochemical Screening *Lilium candidum* Bulb Extract -**

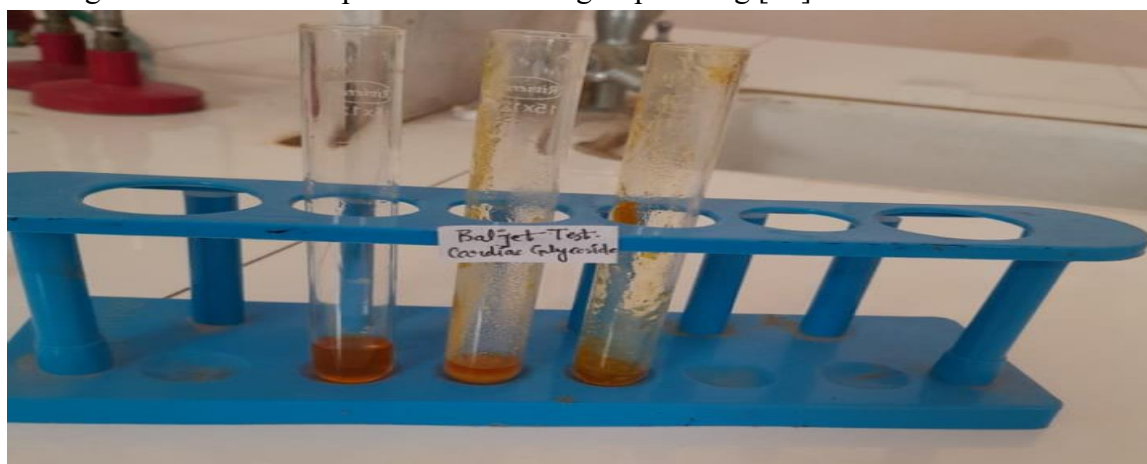
- **Baljet's test:** To the medication extract, sodium picrate elucidation was additional, yellow to orange colour was specified the existence of glycosides.
- **Legal's test:** Extract elucidation liquefied in pyridine then sodium nitroprusside elucidation was additional to it and finished alkaline. Pink red colour specified the existence of glycosides.
- **Test of saponins:** 1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in progressed cylinder for 15 minutes. One cm film of foam specified the existence of saponins.



- **Keller Kiliani test:** Methanolic extract was liquefied in glacial acetic acid containing trace of ferric chloride one ml intense sulphuric acid was added carefully by the lateral of the test tube. A blue colour in the acetic acid film and red colour at the junction of the two liquid specified the existence of glycosides.
- **Dragendorff's Test:** Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.
- **Wagner's test:** Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.
- **Test for tannins-**To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

### Test for flavonoids

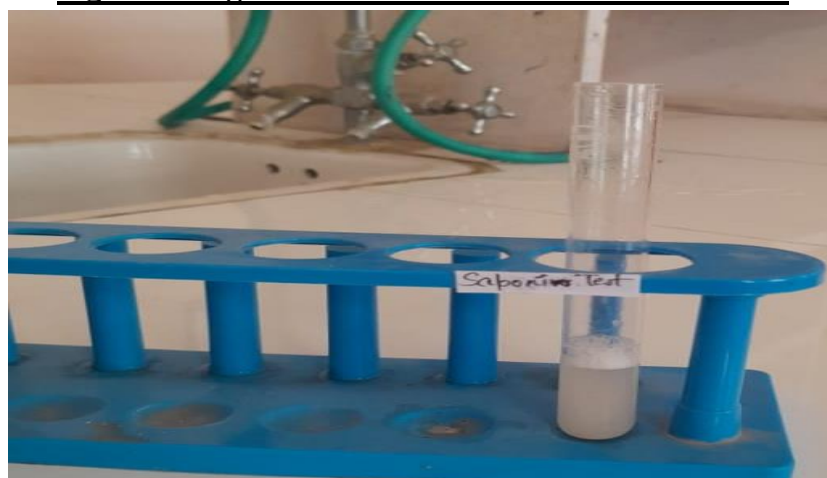
- **Shinoda test:** In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.
- **Trichloro acetic acid test:** Drug + saturated trichloro acetic acid → coloured precipitate → presence of triterpenes .
- **Modified brontrager's test :** 1 gm of drug sample + 5ml of dilute HCl + 5 ml of ferric chloride →Boil for 10 min. on waterbath → cool (on room temperature) →filter → extract of filtrate with carbon tetrachloride or benzene + equal amount of ammonia solution →appearance of pink to red colour → presence of anthraquinone moiety.
- **Libermann burchard test :** Alcoholic extract of drug → evaporated → dry →extracted with CHCl<sub>3</sub> + few drops of acetic anhydride + conc.salphuric acid ( from the side wall of test tube) → appearance of violet ring → blue colour → presence of sterol group in drug [11].



**Figure 8- Baljet Test of *Lilium candidum* Bulb Extract**



**Figure 9- Legal test of *Lilium candimum* Bulb Extract**



**Figure 10- Saponins test of *Lilium candimum* Bulb Extract**



**Figure 11- Keller Kiliani test of *Lilium candimum* Bulb Extract [Praveen morankar]**



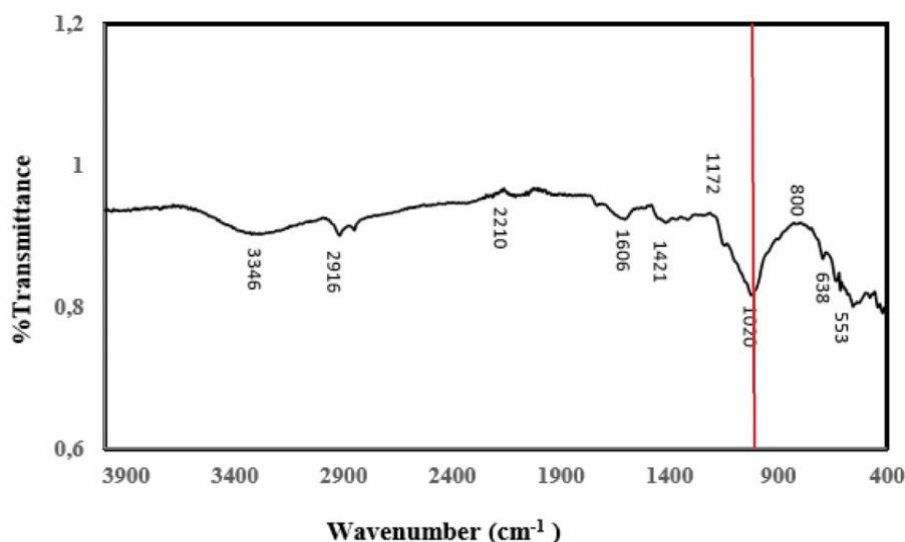
**Figure 12- Phytochemical Test of *Lilium candidum* Bulb Extract**

**Table 1-Phytochemical Test of Extracted powder Bulb**

S.No	Test	Present/Absent
1	Alkaloids	+
2	Cardiac Glycoside	+
3	Tannin	+
4	Steroid Glycoside	+
5	Saponin	+
6	Flavonoid	+
7	Anthraquinone Glycoside	+
8	Terpinoids	+

- **Fourier transform-infrared (FT-IR) spectroscopy analysis-**

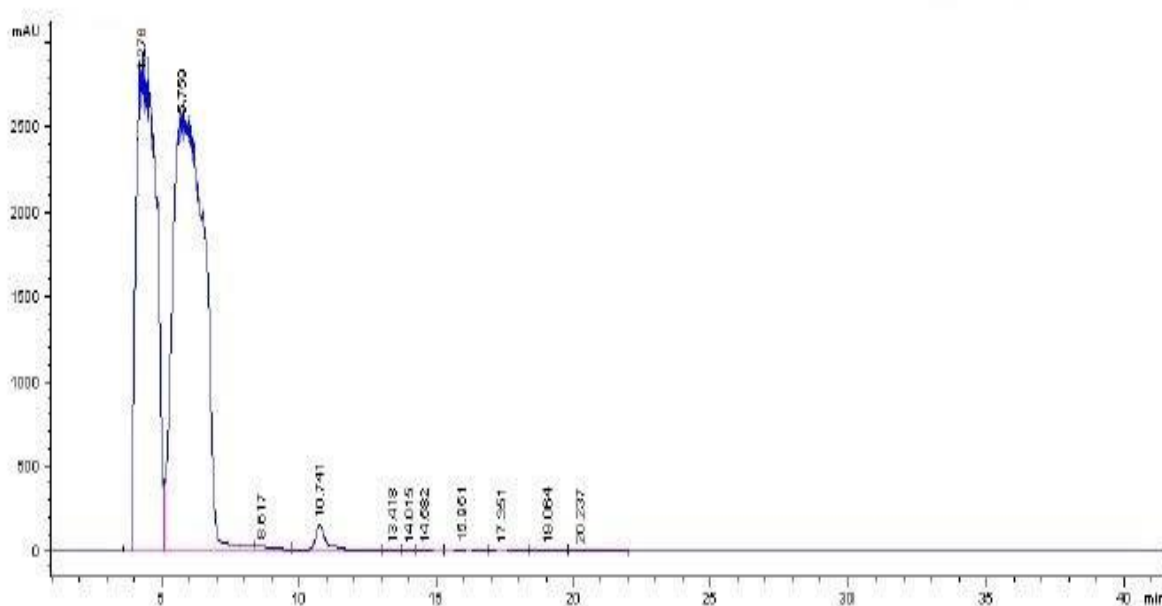
The FT-IR examination of ethanolic extract has been carried out as per the technique of Jagmohan [12]. The powdered plant models were mixed with potassium bromide (KBr pellet) and subjected to a pressure of nearby  $5 \times 10^6$  Pa to yield a pure translucent disc (diameter 13 mm) and width (1 mm). IR spectra in occurrence region  $400-4000 \text{ cm}^{-1}$  were documented at area heat on a PerkinElmer Fourier Transform Spectrometer prepared with an air-cooled deuterated triglycine sulphate (DTGs) sensor. For each spectrum, 100 scans were co-added at a spectral resolve of 4 cm. The incidences for all sharp groups were precise to 0.01 cm. All the spectral prices were expressed in percentage (%) transmittance [13].



**Fig 13- FTIR Spectra of *Lilium candidum* Bulbs Extract**

• **High Performance Liquid Chromatography (HPLC)-**

RP-HPLC with C18 columns is the most popular technique for the analysis of polyphenols of different foods, despite the fact that the separation of procyanidins is not satisfactory [14]. A UV-vis multiwavelength detector was used because all phenolic compounds show intense absorption in the UV region of the spectrum. The present method is simple, easy to use, and effective enough for the identification and quantification of major phenolic compounds in aromatic plants. A similar technique has been reported by other authors for the analysis of major flavonoid aglycons [15,16]. A typical HPLC profile of the phenolic constituents of an analyzed plant is presented in Figure 14.



**Fig 14- High Performance Liquid Chromatography (HPLC) of *Lilium candidum* Bulbs Extract**

**Anti-inflammatory activity in Rheumatoid Arthritis by using Formaldehyde Induced Arthritis Model-**

Afterwards the injection of formaldehyde the development of edema in the paw of the rat, the histamine release at the place of injection. At the place of administration, the formaldehyde-induced arthritis by denaturing protein produces immunological response against product tarnished [17].

➤ **Experimental Animal procedure-**

- Albino rats weighing between 100 and 150 g were separated into four sets of four rats in each.



- The first group served as usual control, which was given with distilled water only.
- The second group served as toxicant control, given with 0.1 ml of formaldehyde (2% v/v) into the hind paw.
- The third group served as test group treated with *Lilium candidum* bulb extract at a dose of low (100 mg/kg) and high (200 mg/kg). Groups B, C, and D were intoxicated with 0.1 ml of formaldehyde (2% v/v). Daily the paw volume was measured for the subsequent 10 days
- The fourth group served as standard medication which was given with Diclofenac (10 mg/kg) [18].

➤ **Paw volume-**

The rat paw length was measured using plethysmometer on days 1, 2, 4, 6, 8, and 10 of experimental period. Radiological inspection was ended for the knee joints on day 10 after the last dose administration of test extract and standard drug [19].

➤ **Radiographic (X-ray) Evaluation-**

Radiographic alterations in RA situations are beneficial investigative actions which specify the harshness of the illness. Soft tissue inflammation is the prior radiographic symbol, whereas significant radiographic fluctuations such as bony erosions and narrowing of joint places can be detected only in the advanced stages (final stages) of arthritis. The radiographic explanation of the rat joints in formaldehyde-induced arthritis model is exposed in underneath figure. In formaldehyde-induced arthritic rat, soft tissue bulge along with the decrease of the joint spaces was detected which indicates the bony demolition in arthritic disorder. The standard drug Diclofenac (10 mg/kg) cured groups have banned this bony demolition and also there is no inflammation of the joint. The plant *S. grandiflora* cure for 10 days has exposed important inhibition alongside bony demolition by showing less soft tissue bulge and thinning of joint spaces when equaled with formaldehyde-induced group [20,21].



**Fig 15- Albino rat hind paw at initial stage**





**Fig 16- Albino rat hind paw after induction of Arthritis**



**Fig 17- X-ray of Albino rat hind paw at initial stage**



**Fig 18- X-ray of Albino rat hind paw after inducing Arthritis**



**Fig 19- X-ray of Albino rat hind paw after inducing low dose of *Lilium candidum* bulb extracts**



**Fig 20- X-ray of Albino rat hind paw after inducing high dose of *Lilium candidum* bulb extracts**



**Fig 21- X-ray of Albino rat hind paw after inducing standard drug Diclofenac**

**Table 2-The effect of *Lilium candidum* bulb extract in albino rats paw volume (in ml)**

Days	0	1	2	3	4	5	6	7	8	9	10
<b>Group</b>	<b>Paw Volume in ml</b>										
<b>Control</b>	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02	0.90± 0.02
<b>Induced</b>	0.90± 0.02	1.82± 0.03	1.82± 0.03	1.84± 0.08	1.84± 0.08	1.80± 0.08	1.80± 0.08	1.80± 0.08	1.78± 0.10	1.77± 0.11	1.76± 0.12
<b>Test(Low dose)</b>	0.90± 0.02	1.78± 0.04	1.78± 0.04	1.80± 0.02	1.81± 0.01	1.67± 0.01	1.68± 0.01	1.60± 0.02	1.60± 0.02	1.52± 0.01	1.50± 0.02
<b>Test(High dose)</b>	0.90± 0.02	1.72± 0.01	1.72± 0.01	1.72± 0.01	1.64± 0.01	1.63± 0.02	1.60± 0.02	1.50± 0.03	1.41± 0.02	1.34± 0.02	1.21± 0.04
<b>Standard</b>	0.90± 0.02	1.68± 0.05	1.68± 0.05	1.78± 0.07	1.68± 0.03	1.65± 0.02	1.61± 0.04	1.41± 0.06	1.16± 0.01	1.16± ±0.01	1.13± 0.02

Where, n=4 (mean ± standard deviation)

#### **Acute toxicity activity-**

- The severe poisonousness of aqueous extract of the plants of *Lilium candidum* was observed by giving a sole quantity of several considerations of the plant species aqueous extract to overnight fasted wister albino rats.
- Individual rats were reviewed for a total 14 days following medicating at least once through the first 24 hours, which superior consideration so long as through the first 4 hours, and daily there after [22].

#### **Statistical analysis-**

- All the standards were articulated as mean ± SD. The data were measured using one way examination of alteration (ANOVA) followed by student t' test. Statistical importance was established at  $p < 0.05$ .

#### **Result and Discussion-**

In our study, the bulbs of *Lilium candidum* were calculated for its anti- inflammatory action in Rheumatoid Arthritis by using Maceration procedure and Formaldehyde induced paw edema arthritis model. There was important and dose dependent anti- inflammatory action of bulb extract in the Formaldehyde induced paw edema arthritis model. Edema was significantly inhibited irrespectively of the amount level of the extract used and was similar to the standard Diclofenac cure. The bulbs extract of *Lilium candidum* at the amount levels of 100 mg/kg at low dose and 200 mg/ kg at high dose caused a dose dependent inhibition of localized bump triggered by Formaldehyde as shown in table 2. There are total four groups of animals were divided in which the first one is the control group and paw volume inflammation due to arthritis were found to be 0.90±0.02 ml, the second group were the induced group/ toxicant control group and paw volume inflammation due to arthritis were found to be reduced on 10<sup>th</sup> day as 1.76±0.12 ml, the third group were the test group and it were subdivided into two group such as low dose and high dose, and paw volume inflammation due to arthritis were found to be reduced on 10<sup>th</sup> day as 1.50±0.02 ml and 1.21±0.04 ml, and the fourth group were the Standard group and paw volume inflammation due to arthritis were found to be reduced on 10<sup>th</sup> day as 1.13±0.02 ml. The administrations of *Lilium candidum* bulb extracts in rats did not show mortality at fixed dose 2000 mg/kg body weight. Moreover, there were no visible signs of acute toxicity, i.e., food and water consumptions were unaffected; and salivation, aggression, rising furs, and writhing were not observed for 14 days. Herbal products are expended in outmoded medical arrangements as

practical food additions or as drugs in many countries. In recent years, indication has gathered to propose that balancing medicine for action of numerous illnesses is another more standard excellent. Numerous plant extracts of botanical therapeutic herbs have been shown to discharge disease indications similar to those gotten from allopathic drugs. So, harmless mixtures of usual goods with less side effects are desirable. In this study, demonstration have created novel explanations for the first time that the leaves extract of *Lilium candidum* possess anti-inflammatory effects in Formaldehyde induced hind paw arthritis model.

## Conclusion-

From this research paper, we were concluded that, the *Lilium candidum*, which is also known as Madonna Lily or White Lily, is a plant with white beautiful flowers and elongated green leaves and beneficial bulbs and roots which is full of various medicinal benefits. As a natural compound, the *Lilium candidum* is very useful on the basis of pharmacological aspects in the past and in the future too. The *Lilium candidum* consist of a number of medicinal activity and here by performing this research work we can conclude that, the bulbs of *Lilium candidum* have better efficacy as an anti-inflammatory agent in the Rheumatoid arthritis inflammation.

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