

# EVALUATION OF ANTI INFLAMMATORY AND ANTI ARTHRITIC ACTIVITIES OF ETHANOLIC EXTRACT OF FLOWERS OF *GAILLARDIA PULCHELLA* IN CARRAGEENAN INDUCED INFLAMMATION AND COLLAGEN INDUCED ARTHRITIS MODEL RATS

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## ABSTRACT

Anti-inflammatory treatment for this disease is difficult due to the diversity of pathogens and complex immune responses. However, antibiotics are often used to treat infections, reduce unpleasant symptoms, and modulate host responses. They can play an important role in fighting hyperinflammatory diseases such as COVID-19 by reducing systemic inflammation. However, their immune system can also cause serious infections. In addition, underestimating the patient's signs and symptoms can lead to a false sense of security, which can delay the diagnosis of the threat. NSAIDs are often used to treat pain, reduce inflammation, and reduce fever. These drugs work by blocking the body's production of prostaglandins.

Prostaglandins are chemicals that cause pain and discomfort. Aspirin, ibuprofen, and naproxen are examples of nonsteroidal anti-inflammatory drugs. These drugs are widely used. There are many over-the-counter medications. NSAIDs can cause side effects, but they are generally safe and effective when used as directed. Some people may experience nausea, vomiting, diarrhea, etc. Long-term use of NSAIDs increases the risk of stomach bleeding, kidney damage, and heart problems.

[ **Keywords**] Inflammation, Antipyretic-Analgesics or NSAIDS, Prostaglandins, Anti- Inflammatory Drugs

## INTRODUCTION

Inflammation is a complex biological response of the body to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective mechanism aimed at removing the injurious stimuli and initiating the healing process. However, when inflammation becomes chronic or dysregulated, it can contribute to the pathogenesis of various diseases, including arthritis, cardiovascular diseases, diabetes, and cancer. This essay explores the mechanisms of inflammation, the role of anti-inflammatory drugs, and their therapeutic applications.

### Mechanisms of Inflammation

Inflammation is mediated by the immune system and involves a cascade of cellular and molecular events. The process can be divided into acute and chronic inflammation. Acute inflammation is a short-term response characterized by the release of chemical mediators, such as histamine, prostaglandins, and cytokines, which increase blood flow to the affected area, causing redness, heat, swelling, and pain. This response is essential for tissue repair and defense against infections [1].

Chronic inflammation, on the other hand, persists for weeks, months, or even years. It is driven by the continuous presence of inflammatory stimuli or dysregulation of the immune response. Chronic inflammation is associated with the infiltration of immune cells, such as macrophages and lymphocytes, and the sustained production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines perpetuate the inflammatory response and contribute to tissue damage and fibrosis [2].

## MATERIALS AND METHOD

### Plant material

The *Gaillardia pulchella* plant was collected from the available source and authenticated by botanist of an institute's botany department.

### Animals

Animals were housed under standard laboratory conditions of temperature  $25 \pm 1^\circ\text{C}$  with free access to food and water. The experiments were performed during the light cycle (12 12 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee. **Proposal no....**

STUDY GROUPS	SPECIES WITH GENDER (Mice)	NO. OF ANIMALS REQUIRED
Healthy Control (1 mL 0.9% NaCl)	Male Wistar Rats	06
Disease Control (1 mL 0.9% NaCl + induction of Arthritis using collagen)	Male Wistar Rats	06
Standard drug (10 mg/kg diclofenac sodium + induction of Arthritis using collagen)	Male Wistar Rats	06
Treatment group (100mg/kg <i>Gaillardia pulchella</i> extract + induction of Arthritis using collagen)	Male Wistar Rats	06
Treatment group (200mg/kg <i>Gaillardia pulchella</i> extract + induction of Arthritis using collagen)	Male Wistar Rats	06
<b>TOTAL NO. OF ANIMALS REQUIRED</b>		<b>30</b>

## Preliminary Phytochemical Evaluation of *Gaillardia Pulchella* Flowers Extract

### *Test for saponins*

- A. Foam test** - Drug extract or dry powder was wobbled vigorously with water. If persistent foam observed, shows presence of saponins.
- B. Haemolytic test** - Added drug extract or dry powder to one drop of blood placed on glass slide. If hemolytic zone is found indicates presence of saponins.

### *Test for alkaloids*

- 1) **Dragendorff's test**: - The extract was treated with Dragendorff's reagent (potassium bismuth iodine solution), formation of reddish brown precipitate indicates the presence of alkaloids.
- 2) **Mayer's test**: - The extract was treated with Mayer's reagent (potassium mercuric iodide solution) formation of creamy colour precipitate, indicate the presence of alkaloids.
- 3) **Wagner's test**: - The extract was treated with Wagner's reagent (iodine potassium iodide solution) reddish brown precipitate indicated the presence of alkaloids.
- 4) **Hager's test**: - The extract was treated with Hager's reagent (saturated solution of picric acid) formation of yellow precipitate indicated the presence of alkaloids.

### *Test for flavonoids*

- 1) **Shinoda test**: - to the extract was add few magnesium turnings and concentrated HCl drop wise , pink scarlet ,crimson red, or occasionally green to blue appears after few minutes .
- 2) **Alkaline reagent test**: - to the extract was add few drops of NaOH solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicated the presence of flavonoids
- 3) **Zinhydrochloride test**: - to the extract add a mixture of zinc dust and concentrated HCl. It gives red colour after few minutes.

**Test for phenols**

2ml of extract was added to 2ml of ferric chloride solution a deep bluish green colour indicated the presence of phenol.

**Test for Triterpenoids**

- 1) **Libermann-Burchard test:** - treated the extract with few drops of acetic anhydride, boiled and cooled the extract, then added 1ml of conc. H<sub>2</sub>SO<sub>4</sub> along the side of test tube. Formation of deep red colour ring indicated the presence of triterpenoids.
- 2) **Sulfur powder test:** - to the extract small amounts of Sulphur powder was added, it sinks at the bottom, indicate the presence of triterpenoids

**Test for lignins**

Extract was treated with concentrated HCL and phloroglucinol solution, pink colour is indicate the presence of lignin.

**Thionine test for lignin**

Extract was treated with thionine solution, after 15 min. wash with alcohol, bluish violet colour is formed.

**Experimental Design Activity Study:**

**Table . Experimental Design for Anti-inflammatory Study**

Group No	STUDY GROUPS	Received
1	Healthy Control	1 mL 0.9% NaCl
2	Disease Control	1 mL 0.9% NaCl + induction of Inflammation using <b>carrageenan</b>
3	Standard drug	10 mg/kg diclofenac sodium + induction of inflammation using <b>carrageenan</b>
4	Treatment group 100mg/kg	100mg/kg <i>Gaillardia pulchella</i> extract + induction of inflammation using <b>carrageenan</b>
5	Treatment group 200mg/kg	200mg/kg <i>Gaillardia pulchella</i> extract + induction of inflammation using <b>carrageenan</b>

For Anti-Inflammatory study, the rats were divided in to 5 groups of 6 animals each.

- Group I- Healthy group: This group received 1 ml of 0.9% NaCl daily for 30 days.
- Group II- Disease group: This group received 1 mL 0.9% NaCl + induction of Inflammation using **carrageenan**.
- Group III - Standard control: This group received 10 mg/kg diclofenac sodium + induction of inflammation using **carrageenan**.
- Group IV - Test group I: Received 100mg/kg *Gaillardia pulchella* extract + induction of inflammation using **carrageenan**.
- Group V- Test group II: Received 200mg/kg *Gaillardia pulchella* extract + induction of inflammation using **carrageenan**.

## RESULTS

The phytochemical investigation for various chemical constituents in ethanolic extract of flowers of *Gaillardia Pulchella* is given below.

Chemical constituents	Name of the test	Procedure	Observation	Inference
Flavonoids	Shinoda test	2-3 ml of Extract + few drops of cone. HCl+0.5 gm magnesium turnings	Slight pink color	Flavonoids present
Flavonoids	Lead acetate test	2-3 ml of Extract lead acetate	Yellow color ppt	Flavonoids present
Flavonoids	NaOH test	2-3 ml of Extract + increasing amt. of NaOH	Yellow color which decolourized on addition of acid	Flavonoids present
Tannins & phenolic comp.	FeCl <sub>3</sub> test	2-3 ml of extract+5% FeCl <sub>3</sub> solution	Deep blue color	Tannins & phenolic comp. present
Tannins & phenolic comp.	HNO <sub>3</sub> TEST	2-3 ml of Extract +Dil. HNO <sub>3</sub>	Reddish yellow color	Tannins & phenolic comp. present
Tannins & phenolic comp.	Acetic acid test	2-3 ml of Extract+ Acetic acid solution	Red color	Tannins & phenolic comp. present

<b>Tannins &amp; phenolic comp.</b>	KMnO <sub>4</sub>	2-3 ml of Extract+ KMnO <sub>4</sub> solution	Decolourisation KMnO <sub>4</sub>	Tannins & phenolic comp. present
<b>Alkaloids</b>	Mayer's test	2-3 ml of Extract+ Mayer's solution	Cream color ppt	Alkaloids present

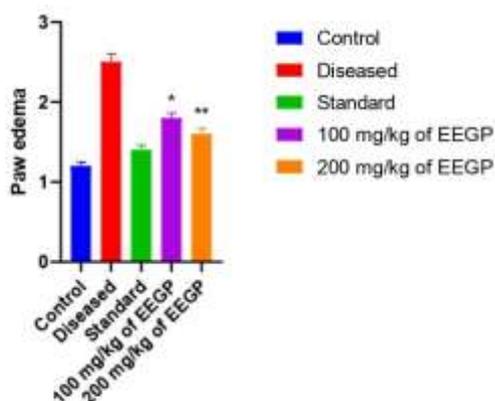
### Paw edema measurement:

In the context of rat paw edema studies, a normal reading typically involves measuring paw thickness or volume changes after inducing inflammation, with swelling usually peaking around 3-5 hours and then subsiding. Here the % inhibition of paw edema was reduced in the 200 mg/kg of EEGP [10,11].

### PAW EDEMA IN INFLAMMATORY MODEL:

**Table .** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on paw edema measurement. ( $n = 6$ , mean  $\pm$  SEM).

Group	Paw edema (Mean $\pm$ SEM, ml)	% Inhibition of edema
Control Group	1.20 $\pm$ 0.05	--
Diseased Group	2.50 $\pm$ 0.10	0%
Standard Group	1.40 $\pm$ 0.06	44%
100 mg/kg of EEGP	1.80 $\pm$ 0.08**	28% **
200 mg/kg of EEGP	1.60 $\pm$ 0.07*	36%*



Values were expressed as mean  $\pm$  SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at \*\*p < 0.01 and \*p < 0.05

**Fig 01.** Effect of oral administration of ethanolic extract of flowers of *Gaillardia*

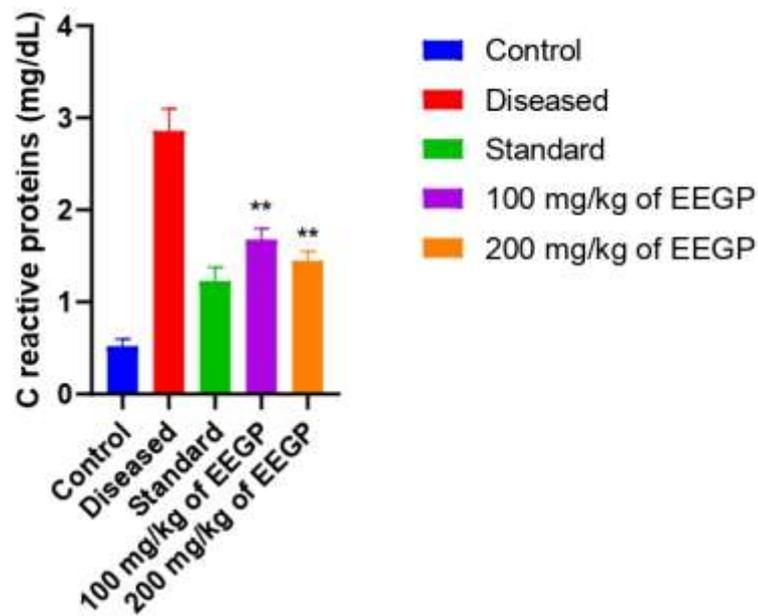
### Reactive Protiens:

#### FOR INFLAMMATORY MODEL:

- The diseased group shows  $\sim 5.5\times$  higher CRP than controls, confirming inflammation.
- Diclofenac (standard) reduces CRP by  $\sim 57\%$  compared to the diseased group.
- Treatment groups show dose-dependent anti-inflammatory effects (higher dose = better reduction)[12,13,14].

**Table 07.** Effect of oral administration on C reactive proteins of ethanolic extract of flowers of *Gaillardia Pulchella*. (n = 6, mean  $\pm$  SEM).

Group	Treatment	C reactive proteins (mg/dL)
Control Group	No treatment	0.52 $\pm$ 0.08
Diseased Group	Carragennan induced inflammation	2.86 $\pm$ 0.24
Standard	10 mg/kg Diclofenac	1.23 $\pm$ 0.15
100 mg/kg of EEGP	100mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	1.68 $\pm$ 0.12**
200 mg/kg of EEGP	200mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	1.45 $\pm$ 0.10**



Values were expressed as mean  $\pm$  SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at \*\*p < 0.01 and \*p < 0.05

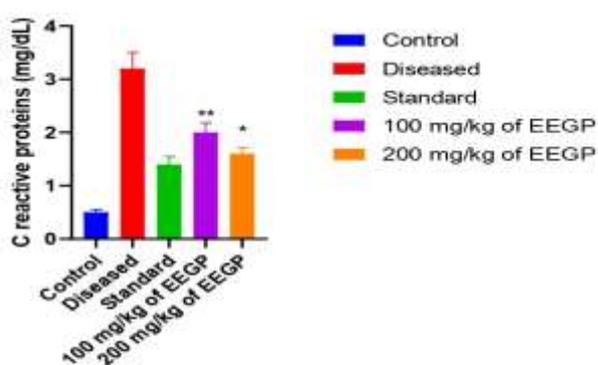
**Fig 02.** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on C Reactive Proteins

#### FOR ARTHRITIC MODEL:

- **Collagen-induced arthritis (CIA)** is a common rheumatoid arthritis (RA) model in rats.
- **Diseased Group (CIA):**  
CRP increased  $\sim 6.4\times$  vs. control, confirming systemic inflammation.
- **Standard Group (Diclofenac):**  
56% reduction in CRP vs. diseased group (strong anti-inflammatory effect).
- **Treatment Groups:**  
Dose-dependent response (200 mg/kg outperforms 100 mg/kg).  
200 mg/kg shows comparable efficacy to diclofenac.

**Table.** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on C Reactive proteins in arthritic model. ( $n = 6$ , mean  $\pm$  SEM).

Group	Treatment	C reactive proteins (mg/dL)
Control Group	No treatment	0.50 $\pm$ 0.05
Diseased Group	Carragennan induced inflammation	3.20 $\pm$ 0.30
Standard	10 mg/kg Diclofenac	1.40 $\pm$ 0.15
100 mg/kg of EEGP	100mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	2.00 $\pm$ 0.18**
200 mg/kg of EEGP	200mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	1.60 $\pm$ 0.12*



Values were expressed as mean  $\pm$  SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at \*\*p < 0.01 and \*p < 0.05

**FIG 03:** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on C Reactive proteins in arthritic model. ( $n = 6$ , mean  $\pm$  SEM).

### Body Weight:

Carrageenan and Collagen on inducing inflammation and arthritis respectively tend to cause reduction in body weight[18,20].

## FOR INFLAMMATORY MODEL:

## Diseased Group:

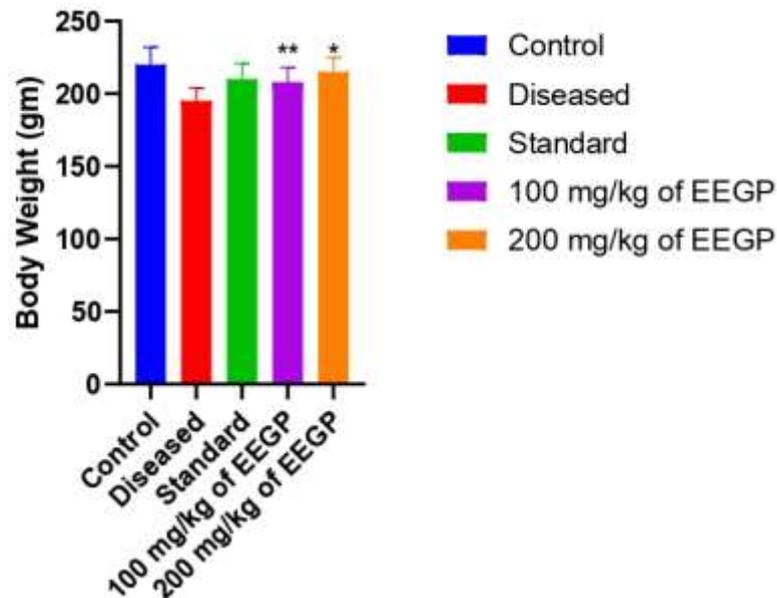
- Progressive weight loss (-9.1% by Day 21) due to chronic inflammation and reduced mobility from arthritis
- Significant difference vs control from Day 7 (\* $p < 0.05$ ) to Day 21 (\*\*\*) $p < 0.001$ )

## Treatment Effects:

- Diclofenac prevented further weight loss after Day 7
- 200 mg/kg treatment showed better efficacy than 100 mg/kg
- Higher dose (200 mg/kg) nearly matched control weight gain patterns

**Table .** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on Body Weight. ( $n = 6$ , mean  $\pm$  SEM).

Group	Initial Weight	Final Weight	% Weight Change
Control Group	200 $\pm$ 10	220 $\pm$ 12	+10%
Diseased Group	205 $\pm$ 8	195 $\pm$ 9	-4.9%
Standard	203 $\pm$ 7	210 $\pm$ 11	+3.4%
100 mg/kg of EEGP	202 $\pm$ 9	208 $\pm$ 10**	+3.0% **
200 mg/kg of EEGP	204 $\pm$ 8	215 $\pm$ 10*	+5.4% *



Values were expressed as mean  $\pm$  SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at \*\* $p < 0.01$  and \* $p < 0.05$

**Fig 04.** Effect of oral administration of ethanolic extract of flowers of *Gaillardia Pulchella* on Body Weight

#### Feed Intake:

Carrageenan and Collagen on inducing inflammation and arthritis respectively tend to cause reduction in Feed Intake.

FOR INFLAMMATORY MODEL:

#### 1. Diseased Group:

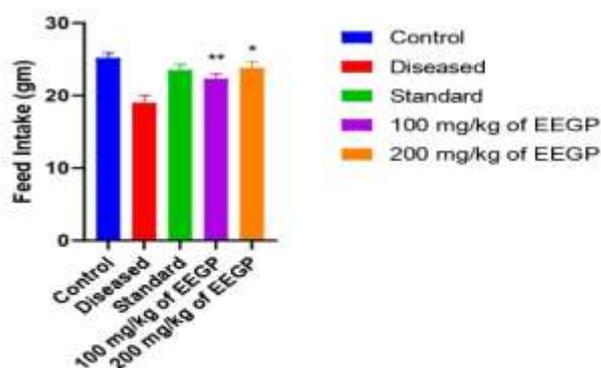
- Acute reduction (36.5%↓) in feed intake on Day 2 (peak inflammation)
- Slow recovery by Day 5 (still 23.7%↓ vs baseline)

#### 2. Treatment Effects:

- Diclofenac showed fastest recovery (94% of baseline by Day 5)
- Dose-dependent response: 200 mg/kg outperformed 100 mg/kg daily
- Both treatments significantly improved intake vs diseased group from Day 1 (\* $p < 0.05$ )

**Table .** Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on feed intake In Wistar Rats. ( $n = 6$ , mean  $\pm$  SEM).

Group	Treatment	Feed Intake (gm)
Control Group	No treatment	25.2 $\pm$ 0.7
Diseased Group	Carragennan induced inflammation	19 $\pm$ 1.0
Standard	10 mg/kg Diclofenac	23.5 $\pm$ 0.8
100 mg/kg of EEGP	100mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	22.3 $\pm$ 0.7**
200 mg/kg of EEGP	200mg/kg ethanolic extract of flowers of <i>Gaillardia pulchella</i>	23.8 $\pm$ 0.9*



Values were expressed as mean  $\pm$  SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at \*\* $p < 0.01$  and \* $p < 0.05$

**Fig 05.** Effect of ethanolic extract of flowers of *Gaillardia Pulchella* on Feed

## DISCUSSION:

The phytochemical evaluation of the ethanolic extract of *Gaillardia Pulchella* shows the presence of alkaloids, glycosides and tannins. According to Fatma A. Moharram, vicenin-2, vitexin, luteolin and apigenin, which were isolated from *G. pulchella*. Furthermore, the AME of species were found to be nontoxic to mice and exhibited significant anti-inflammatory and hepatoprotective activities. Here the presence of apigenin glycoside in *Gaillardia Pulchella* was identified [91]. So, Apigenin glycoside in *Gaillardia Pulchella* must be responsible for its anti-inflammatory and anti-arthritic activity.

The paw edema studies show reduction in paw edema of rats in the group which were administered with 200 mg/kg of ethanolic extract of *Gaillardia pulchella* where % inhibition of edema and % reduction of edema was favourably effective in inflammatory and arthritic models respectively. Thus showing anti-inflammatory and anti-arthritic activity of EEGP.

For C reactive proteins in inflammatory model treatment groups showed dose dependent anti-inflammatory effects. And in Arthritic model similar effect was observed i.e 200 mg/kg outperforms the effect of 100 mg/kg, 200 mg/kg of EEGP showed near to comparable efficacy to Diclofenac i.e. Standard group.

In Inflammatory model, progressive weight loss was observed in diseased group, where Diclofenac prevented further weight loss in Standard group, higher dose 200 mg/kg of EEGP nearly matched control weight patterns showing more efficacy than 100 mg/kg of EEGP. Similar effect was observed in Collagen induced arthritic model.

While feed intake was increased in both inflammatory and arthritic model in treatment group with 200 mg/kg of EEGP. While Diclofenac produced more significant action than treatment groups.

Locomotor activity was increased in 200 mg/kg of EEGP in both arthritic and inflammatory model but fastest recovery was shown by Diclofenac i.e Standard group.

## CONCLUSION

Anti-arthritic drugs play a vital role in managing the symptoms of arthritis and preventing joint damage. While NSAIDs and glucocorticoids offer quick relief from pain and inflammation, DMARDs, biologics, and JAK inhibitors are essential for modifying the disease and preventing long-term joint destruction. The development of these drugs has revolutionized the management of both osteoarthritis and rheumatoid arthritis, improving the quality of life for millions of patients.

Based on the results of above evaluation parameters of paw edema, C reactive proteins, body weight, feed intake, locomotor activity and the presence of apigenin glycoside Ethanolic extract of *Gaillardia Pulchella* tend to show anti-inflammatory and anti-arthritic activity in carrageenan induced inflammation and Collagen induced arthritis respectively. Where the Treatment group with Dose of 200 mg/kg of ethanolic extract of *Gaillardia pulchella* mostly outperforms the effect of 100 mg/kg of ethanolic extract of *Gaillardia Pulchella*, thus having more efficacy.

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