Evaluation of Anti-Inflammatory and Anti-Arthritic Activity of Balaguluchyadi Kwatham Tablets in Wistar Albino Rat Models

ABSTRACT

Objective: The current study was undertaken to evaluate the anti-inflammatory and anti-arthritic activity of Balaguluchyadi Kwatham tablets (BGKT) on Wistar albino rat models.

Materials and Methods: Anti-inflammatory activity was evaluated by Carrageenan and Histamine-induced paw edema models using a vernier caliper. Anti-arthritic activity was evaluated by using CFA induced arthritis. Paw diameter was recorded on days 0, 3rd, 7th, 14th and 21st and were measured using a vernier caliper. Body weight, serum, and hematological parameters were assessed on day 21 to determine the effectiveness of treatment.

Results: In the anti-inflammatory study, (P < 0.001, P < 0.0001) a significant reduction in paw thickness was observed between 3rd and 5th hours in both carrageenan and histamine-induced paw edema models after treatment with the formulation. In the anti-arthritic study, BGKT treated groups showed a significant reduction in paw diameter and changes in body weight of the animals on the 21st day compared with arthritic control. In the arthritic control group, hematological parameters such as WBC levels were increased; Hb and RBC levels were decreased. After treatment with the formulation, the altered levels were restored. Arthritic treated groups showed a marked inhibition of joint destruction (Histopathological and Radiological analysis).

Conclusion: Based on the above results, it was concluded that the BGKT possess significant anti-inflammatory and anti-arthritic activity in rat models.
INTRODUCTION

Ayurveda provides an integrated approach in preventing and treating illness through lifestyle interventions and natural therapies. [1] Inflammation is a complex biological response of vascular tissues to harmful stimuli such as microbial infections, irritants (Physical or corrosive chemical) and Tissue necrosis. It is a defense mechanism aimed to remove the injurious stimuli and initiate the tissue healing process. [2] It involves the participation of various cell types expressing and reacting to diverse mediators along a very precise sequence of events. [3] However, prolonged inflammation leads to numerous diseases including Rheumatoid arthritis (RA), Ankylosing spondylitis, Osteoarthritis, juvenile rheumatoid arthritis (JRA), fibromyalgia, sjögren's syndrome and systemic lupus erythematosus (SLE). Rheumatoid arthritis is an autoimmune systemic disease with chronic inflammation of the synovial joint and progressive destruction of cartilage and bone. RA is a chronic systemic inflammatory disorder that may affect many tissue and organs- skin, blood vessels, heart, lungs, and muscles but principally attacks the joints, producing a non-supportive proliferative and inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. [4] Rheumatoid arthritis is characterized by the infiltration of a variety of inflammatory cells into the joint. The synovial membrane becomes highly vascularized, synovial fibroblasts proliferate and inflammatory cells release numerous cytokines and growth factors into the joint. These agents subsequently cause synovial cells to release proteolytic enzymes resulting in the destruction of bone and cartilage. [5] Balaguluchyadi Kwatham tablets, a classical Ayurvedic formulation. It consists of a mixture of fine powders of dried pericarps of Bala (Sida Cordifolia Linn. Family - Mallow), Guduchi (Tinospora Cordifolia Wild. Family - Menispermaceae), Devdaru (Cedrus Deodara Roxb. Family - Pinaceae). It is recommended for inflammation, rheumatoid arthritis, hyperuricemia, analgesic, gout and synovitis. However, no scientific study is available on the anti-arthritic activity of this formulation. [6]

MATERIALS AND METHODS

Procurement of formulations:

Balaguluchyadi Kwatham tablets were procured from Arya Vaidya Sala, kottakkal, Kerala, India.
QUANTIFICATION OF TOTAL PHENOLICS AND FLAVONOIDS

Estimation of total phenolic content

The total phenolic content of the samples was determined by Folin-Ciocalteau assay method. To an aliquot 100 μl of both samples (1 mg/ml) and a standard solution of Gallic acid (10, 20, 40, 60, 80 and 100 μg/ml) were added, 50 μl of Folin-ciocalteau reagent followed by 860 μl of distilled water and the mixture was incubated for 5 mins at room temperature. 100 μl of 20% sodium carbonate and 890 μl of distilled water were added to make the final solution to 2 ml. It was incubated for 30 mins in dark to complete the reaction. After that absorbance of the mixture was measured at 725 nm against blank. Distilled water was used as the reagent blank. The total phenolic content was found out from the calibration curve of Gallic acid. In addition, it was expressed as milligrams of Gallic acid equivalents (GAE) per gram of samples.

Estimation of total flavonoid content

The total flavonoid content of the samples was determined by using the Aluminium chloride colorimetric method. To an aliquot of 100 μl of both samples (1 mg/ml) and standard solutions of quercetin (10, 20, 40, 60, 80 and 100 μg/ml), ethanol was added separately to make up the solution up to 2 ml. The resulting mixture was treated with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415 nm against a blank, where a solution of 2 ml ethanol, 0.1 ml potassium acetate, and 2.8 ml of distilled water and 0.1 ml of aluminum chloride serve as the blank solution. The total flavonoid content was determined from the standard quercetin calibration curve and it was expressed as milligrams of Quercetin equivalents (QE) per gram of sample.[7]

PHARMACOLOGICAL STUDY

Experimental animal:

Wistar albino rats, 130-180g body weight, were procured from the animal house of KMCH College of Pharmacy, Coimbatore, Tamil Nadu. All the rats were housed and maintained under standard conditions of temperature (25°C ± 5°C), relative humidity (55 ± 10%), and 12/12 h light/dark cycles in the animal house. All the rats were provided with standard
commercial pellet diet and water *ad libitum* freely throughout the study. Protocol for the study was approved by the IAEC for animal care and was in accordance with the committee for the purpose of CPCSEA guidelines, Government of India.

**ACUTE TOXICITY STUDY**

Acute oral toxicity was performed as per the OECD-423 guidelines. The purpose of these studies is to know the safety & toxicity of the formulation doses. For this study, Wistar albino rats of weight 130-170g was selected & divided into four groups, each group consisting of 3 animals. The animals were fasted overnight with free access of water *ad libitum*. The Balaguluchyadi Kwatham tablets (BGKT) were suspended in water and administered to all the four groups at doses 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. All the animals were observed individually after administration for the first 30 minutes for gross behavioral and morphological profiles. [8]

**ANTI-INFLAMMATORY ACTIVITY**

Balaguluchyadi Kwatham tablets (BGKT) were evaluated for anti-inflammatory activity against carrageenan and histamine (inflames) induced rat paw edema methods.

**Carrageenan-induced paw edema in rats**

Wistar albino rats were divided into four groups of 6 animals each. The Balaguluchyadi Kwatham tablets (200 mg/kg and 400 mg/kg, p.o) and Standard (Diclofenac-20 mg/kg, p.o) were given orally half an hour before the administration of carrageenan suspension. The edema was induced by the subplantar injection of 0.1ml of 1% solution of carrageenan and the volume of the injected foot was measured periodically. The paw thickness was measured at 1, 2, 3, 4 and 5 hours after carrageenan injection by using vernier caliper. The percentage increase in paw thickness of the treated groups was compared with that of the control and the inhibitory effect of the drug was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. [9-11]

Percent inhibition of inflammation was calculated by using the formula,

\[
\text{% Inhibition} = \left( \frac{T_c - T_i}{T_c} \right) \times 100
\]

Where ‘Tc’ - Thickness of paw edema in control group.

*Citation: Jeevanantham Appusamy et al. Ijppr.Human, 2018; Vol. 12 (3): 323-339.*
‘Tt’ is the thickness of paw edema in the test compound treated group.

**Histamine–Induced Paw Edema:**

Wistar albino rats were divided into four groups of 6 animals each. The Balaguluchyadi kwatham tablets (200 mg/kg and 400 mg/kg) and Standard (Diclofenac-20 mg/kg) were given orally half an hour before the administration of histamine. The edema was induced by the subplantar injection of 0.1 ml of 1% w/w histamine in normal saline, the volume of the injected foot was measured periodically. The paw thickness was measured at 1, 2, 3, 4 and 5 hours after histamine injection by using vernier caliper. The paw thickness was measured before and after (1h) histamine injection and then every hour up to 5 hours for each group.\[11\]Percent inhibition of inflammation was calculated by using the formula,

\[
\% \text{ Inhibition} = \left( \frac{T_c - T_t}{T_c} \right) \times 100
\]

Where ‘Tc’ Thickness of paw edema in control group
‘Tt’ is the thickness of paw edema in the test compound treated group.

**ANTI-ARTHRITIC ACTIVITY**

**Complete Freund’s adjuvant (CFA) induced arthritis**

Thirty animals were divided into five groups of six animals each as follows:

- **Group I:** Normal animals: Normal saline.
- **Group II:** Control animals: Complete Freund’s adjuvant (0.1ml)
- **Group III:** Arthritic animals received Leflunomide at a dose (10 mg/kg, p.o.).
- **Group IV:** Arthritic animals received BGKT at a dose (200 mg/kg, p.o.)
- **Group V:** Arthritic animals received BGKT at a dose (400 mg/kg, p.o.)

On day zero, all rats were administered with 0.1 ml of Complete Freund’s Adjuvant (CFA) into the subplantar region of the left hind paw. The adjuvant contained heat-killed *Mycobacterium tuberculosis* in sterile paraffin oil (10 mg/ml). Treatment with the test and standard compounds were started on the first day and continued for 21 days. The severity of
Arthritis was recorded by the scoring system. The body weight of animals was measured by animal laboratory balance and paw thickness was measured by compressing the joint by rotating the screw of Digital Vernier Caliper. Paw Diameter measurements were carried out on day 0, 3rd, 7th, 14th, and 21st.[12, 13]

**Arthritic Score**

The severity of arthritis was assessed by visual observation. The rats were observed periodically for the severity of joint inflammation on day 0, 3rd, 7th, 14th and 21st. The severity of arthritis was graded on a five-point scale with, 4 indicating edema and erythema from the ankle to the entire leg, 3 indicating moderate edema and erythema from the ankle to the tarsal bone, 2 indicating slight edema and erythema from the ankle to the tarsal bone, 1 indicating slight edema and limited erythema, 0 indicating no edema or swelling. The arthritis score for each rat was the sum of the severity in all the left hind paw (maximum four points for individual rats).[14]

**Blood analysis in CFA induced arthritis**

At the end of day 21, the animals were anesthetized with anesthetic ether and blood was obtained from the retro-orbital vein of animals to analyze hematological parameters such as RBC, WBC and Hb. Blood was centrifuged at 3500 rpm for 20 min and serum was separated for estimation of serological parameters like C - reactive protein (CRP) and Rheumatoid Factor (RF) using RELAX-CRP and RF kits (TULIPS Diagnostics).[15, 16]

**Radiological analysis**

The rats were anesthetized on the 21st day and were subjected to radiographical analysis using X-ray instrument (FUJIFILM, FCR PRIMA II). The instrument was operated at 75 kV peak, 50 mA and 2 s exposure time. Radiological changes were evaluated on the basis of 1) joint space narrowing, 2) joint space destruction and 3) degree of bone erosion.[17]

**Histopathological analysis**

The animals were sacrificed on day 21st by cervical dislocation. Ankle joints were separated from the hind paw, weighed and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5 μ thickness. The sections were stained with hematoxylin and eosin and evaluated under a light
microscope for the presence of hyperplasia of synovium, inflammatory cell infiltration, and bone necrosis.[18]

STATISTICAL ANALYSIS

The data were analyzed by one way ANOVA followed by Dunnet’s comparison test and Tukey’s multiple comparison tests using Graph pad 5.0 software. The values are expressed as Mean ± SEM. P<0.05 was considered significant.

RESULTS:

QUANTIFICATION OF TOTAL PHENOLS AND FLAVONOIDS CONTENTS

The total phenolic content of the Balaguluchyadi Kwatham tablets was found to be 59.44 mg/g calculated as gallic acid equivalent. The total flavonoid content of the Balaguluchyadi Kwatham tablets was found to 22.34 mg/g calculated as quercetin equivalent.

ACUTE TOXICITY TEST

Preliminary studies showed that there was no perceptible change in the autonomic and behavioral patterns of animals on administration of BGKT in the prescribed dose. There was no mortality in any group even after 14 days. No signs of toxicity were observed in any group.

SCREENING FOR ANTI-INFLAMMATORY ACTIVITY

Effects of BGKT on carrageenan-induced edema in rats

The results of the effect of BGKT against carrageenan-induced paw edema in rat revealed time and dose-dependent inhibition. The results showed that the BGKT at 200 and 400mg/kg exhibited significant activity (P < 0.0001) at 5 h were compared to control. The decrease in paw thickness (mm) and maximum inhibition of 42.37% was observed with 400mg/kg of BGKT at 5 h, while the 200mg/kg showed 36.85%. Standard Diclofenac showed 44.99% inhibition at 5 h of drug treatment (Table 1).
Table 1: Effect of BGKT on carrageenan-induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>Paw thickness (mm) and % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Carrageenan 1%w/v (0.1ml)</td>
<td>Saline</td>
<td>5.440 ± 0.162</td>
</tr>
<tr>
<td></td>
<td>Diclofenac 20mg/kg</td>
<td>4.543 ± 0.210**</td>
</tr>
<tr>
<td></td>
<td>BGKT 200mg/kg</td>
<td>4.795 ± 0.0892*</td>
</tr>
<tr>
<td></td>
<td>BGKT 400mg/kg</td>
<td>4.610 ± 0.107**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=6); (One way ANOVA, Dunnet’s comparison test). Standard and Test groups vs. Control. (** P<0.001, * P< 0.05, ns-non significant).

Effects of Histamine-induced Edema in Rats

BGKT exhibited significant (P < 0.0001) decrease of paw thickness (mm) with maximum percentage inhibition of paw edema at 5 h for Diclofenac was showed 34.56% and BGKT (200mg/kg and 400 mg/kg) were showed 30.22% and 34.24% in histamine treated group respectively (Table 2).

Table 2: Effect of BGKT on histamine-induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>Paw thickness (mm) and % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Histamine 1%w/v (0.1ml)</td>
<td>Saline</td>
<td>5.330 ± 0.310</td>
</tr>
<tr>
<td></td>
<td>Diclofenac 20mg/kg</td>
<td>4.278 ± 0.264*</td>
</tr>
<tr>
<td></td>
<td>BGKT 200mg/kg</td>
<td>4.580 ± 0.158**</td>
</tr>
<tr>
<td></td>
<td>BGKT 400mg/kg</td>
<td>4.318 ± 0.209*</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SEM; (n=6); (One way ANOVA, Dunnet’s comparison test). Standard and Test groups vs. Control. (** P<0.001, * P< 0.05, ns-non significant).

SCRENNING FOR ANTI-ARTHRITIC ACTIVITY

Complete Freund's adjuvant-induced arthritis

In the arthritic control group, a significant increase in paw diameter of rats was observed when compared to the normal group. A considerable decrease in paw diameter was observed in the BGKT (200 and 400 mg/kg) and Leflunomide treated groups after day 7. Leflunomide and BGKT (400mg/kg) showed significant (P < 0.0001, P < 0.001) reduction in paw diameter from day 7 compared to arthritic control group (Table 3).

Table 3: Effect of BGKT on CFA induced arthritis paw diameter

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Increase in paw diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>6.323 ± 0.12</td>
</tr>
<tr>
<td>II</td>
<td>1% CFA (0.1mL) Sub plantar</td>
<td>6.538 ± 0.14</td>
</tr>
<tr>
<td>III</td>
<td>CFA + Leflunomide (10 mg/kg)</td>
<td>6.540 ± 0.13</td>
</tr>
<tr>
<td>IV</td>
<td>CFA + BKT (200 mg/kg)</td>
<td>6.522 ± 0.10</td>
</tr>
<tr>
<td>V</td>
<td>CFA + BKT (400 mg/kg)</td>
<td>6.533 ± 0.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=6); (one way ANOVA, Tukey’s multiple comparison test). Control vs. Normal. Standard and Test groups vs. Control. (** P<0.0001, * P< 0.05, ns-non significant).

Effects of BGKT on the arthritic score

Subplantar administration of CFA results in significant increase in arthritic score in all CFA treated rats as compared to normal rats. The arthritic score of the control group remained high.
till the end of the experiment, that is, up to the 21 days as compared to normal rats. Rats treated with BGKT (400 mg/kg) and Leflunomide showed significant ($P<0.0001$) decreased in arthritic score from day 7 to day 21 as compared to control rats (Table 4) (Figure 1).

**Table 4: Effect of BGKT on arthritic score**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Arthritic score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>I</td>
<td>1% CFA (0.1mL) Subplantar</td>
<td>1.5 ± 0.223</td>
</tr>
<tr>
<td>II</td>
<td>CFA + Leflunomide (10 mg/kg)</td>
<td>0.66 ± 0.210</td>
</tr>
<tr>
<td>III</td>
<td>CFA + BKT (200 mg/kg)</td>
<td>1.333 ± 0.210</td>
</tr>
<tr>
<td>IV</td>
<td>CFA + BKT (400 mg/kg)</td>
<td>0.833 ± 0.307</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=6); (one way ANOVA, Dunnet’s comparison test). Standard and Test groups vs. Control. (*** $P<0.0001$, ** $P< 0.001$, * $P< 0.05$, ns-non significant).

![Figure 1: Effect of BGKT on the arthritic score](image)

**Figure 1: Effect of BGKT on the arthritic score**

**Effect of BGKT of body weight in CFA induced arthritis:**

The body weight in the normal group gradually and significantly increased during the experiment, while in the control group, a significant decrease in animals weight was observed.
from day 7 to day 21. With the treatment of the animals by BGKT, the body weight of animal slowly increased but this was significant only at the dose of 400 mg/kg. Changes in body weight were significant in Leflunomide treated group (Table 4).

Table 4: Effect of BGKT on rat body weight in CFA-induced arthritic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Body weight (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>162.3 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.88</td>
</tr>
<tr>
<td>II</td>
<td>1% CFA (0.1mL) Subplantar</td>
<td>149.2 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>III</td>
<td>CFA + Leflunomide (10 mg/kg)</td>
<td>153.8 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td>IV</td>
<td>CFA + BKT (200 mg/kg)</td>
<td>152.7 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>V</td>
<td>CFA + BKT (400 mg/kg)</td>
<td>150.5 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.88</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=6); (One way ANOVA, Tukey’s multiple comparison test). Standard and Test groups vs. Control. (*** P<0.0001, ** P< 0.001, * P< 0.05, ns-non significant).

EFFECT OF BGKT ON BLOOD PARAMETERS IN CFA INDUCED ARTHRITIS

Effect of BGKT on hematological parameters: At the end of the study, the Arthritic control group showed a significant increase in the total WBC level and significant decrease along the Hb and RBC level of control animals as compared to normal rats. However, the treatment with BGKT (200 and 400 mg/kg) showed a significant increase in the Hb and RBC when compared to control group. At the same time, the BGKT (200 and 400 mg/kg) significantly decrease WBC levels. (Table 5).
Table 5: Effect of BGKT on hematological parameters in CFA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Haemoglobin (mg/dl)</th>
<th>RBC (1x10⁶ cells/mm³)</th>
<th>WBC (1x10³ cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.76 ± 0.46</td>
<td>6.607 ± 0.12</td>
<td>10.77 ± 0.21</td>
</tr>
<tr>
<td>Arthritic control</td>
<td>10.58 ± 0.24***</td>
<td>5.572 ± 0.29**</td>
<td>12.69 ± 0.32**</td>
</tr>
<tr>
<td>CFA + Leflunomide (10 mg/kg)</td>
<td>13.01 ± 0.31**</td>
<td>6.365 ± 0.10*</td>
<td>11.08 ± 0.29*</td>
</tr>
<tr>
<td>CFA + BGKT (200 mg/kg)</td>
<td>12.17 ± 0.39*</td>
<td>5.673 ± 0.19ns</td>
<td>11.81 ± 0.48ns</td>
</tr>
<tr>
<td>CFA + BGKT (400 mg/kg)</td>
<td>12.78 0.43**</td>
<td>5.970 ± 0.13*</td>
<td>11.22± 0.31*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=6); (One way ANOVA, Tukey’s multiple comparison test). Standard and Test groups vs. Control. (*** P<0.0001, ** P< 0.001, * P< 0.05, ns-non significant).

Effect of BGKT on the Serological parameter:

The absence of RF and CRP (IgM class) in the serum with the latex reagent (Standard and sample) was confirmed by the absence of agglutination in standard and test groups. No agglutination was observed when serum sample of the normal group tested. Agglutination was spotted in arthritic control group indicating the presence of RF and CRP (greater than 0.6 mg/dl) in the serum (Figure 2).

Figure 2: Qualitative analysis of Rheumatoid factor and C-reactive protein
1. Negative control  
2. Positive control  
3. CFA treated group  
4. Leflunomide- 10mg/kg  
5. BGKT- 200 mg/kg  
6. BGKT- 400 mg/kg

**Radiographical investigation:**

Radiographs were analyzed for joint space narrowing, joint space destruction, and bone erosion. Increased bone erosion and joint space reduction, and joint deformation were observed in the arthritic control group. The standard and BGKT treated groups showed mild and visible signs of bone and joint deformation (Figure 3).

![Radiographs showing joint space narrowing, joint space destruction, and bone erosion.](image)

**Figure 3: Effect of Balaguluchyadi Kwatham tablets and Leflunomide on X-ray analysis in the CFA-treated rats.**

**Effect of BGKT on histology of ankle joint**

The joint tissue sections of CFA injected arthritic control revealed major pathological changes when compared with joints of normal rats. In CFA induced arthritic control, Dermis showed dense infiltration of lymphocytes, plasma cells, macrophages and mature bony trabeculae. Inflammation extending into the muscular layer. A mild evidence of necrosis was
seen. Treatment with BGKT (200 mg/kg and 400 mg/kg) showed a clear reduction in histological injury and changes as compared to arthritic control (Figure 4).

![Normal group vs Arthritic control vs Leflunomide](image)

**Figure 4: Effect of Balaguluchyadi Kwatham tablets and Leflunomide on Histopathological analysis in the CFA-treated rat.**

**DISCUSSION**

The present study was undertaken to evaluate the anti-inflammatory and anti-arthritic of Balaguluchyadi Kwatham tablets in Wistar albino rats.

The presence of phenols and flavonoids in BGKT might have contributed to its anti-inflammatory action. Phenols and flavonoids inhibit inflammatory mediators like reactive oxygen species (ROS) and nitric oxide (NO), cyclooxygenases (COXs) and inducible nitric oxide synthase (iNOS). Phenols and flavonoids reduce the production and expression of cytokines and the modulation of transcription factors such as the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and activating protein-1 (AP-1).[19]
The BGKT at doses of 200mg/kg and 400mg/kg decreased the paw thickness by acting at both phases of the carrageenan-induced inflammation. Thus, the formulation may inhibit the synthesis or release of mediators leading to the acute phase, like histamine and other pro-inflammatory mediators, which usually appear in the early phase of inflammation moreover, the effect in the second phase of inflammation may be through the inhibition of COX-2, which leads to the inhibition of prostaglandin synthesis. The BGKT-treated group showed a significant reduction in paw thickness suggesting the anti-inflammatory activity of the formulation. The Potency of BGKT was found nearly similar to that of diclofenac (20mg/kg). The BGKT and diclofenac, a reference drug, significantly decreased inflammation 3 hours after histamine injection.

In the present study, Complete Freund’s Adjuvant (CFA) induced arthritis in rats were selected to induce arthritis, the first and acute phase (0–10 days) is caused by various mediators such as histamine, serotonin, kinins, and prostaglandins, released by leukocytes that migrate to the affected region and provoked a vascular-exsudatifs phenomena responsible for edema. The second and chronic phase (10–21days) is due to cellular inflammatory mediators such as cytokines (interleukin-1β [IL-1β], IL-6, tumor necrosis factor [TNF-α]), granulocyte-macrophage-colony-stimulating factor), IL-1 and IL-6, and prostaglandins. These substances are responsible to the synovial hyperplasia; cartilage destruction, nociceptive stimulation and pro-inflammatory mediator's production.[20] In this study’s BGKT-treated animals showed significant inhibition of paw diameter inflammation as well as arthritic scores compared to control rats. Whereas, Leflunomide exhibited a significant decrease in paw diameter on the 21st day.

In arthritic control rats, CFA administration leads to rising in total WBC count due to the release of IL-IB inflammatory response, IL-IB increases the production of both granulocyte and macrophages colony stimulating factors, decrease in RBC count and Hb concentration was also observed. In arthritic control rats, a significant decrease in RBC and Hb levels and a marked increase in WBC levels were observed. The decrease in RBC and Hb level reflects the anemic condition. The important cause might be the decreased level of plasma iron due to the sequestering of iron in the reticuloendothelial system and synovial tissue that lead to failure of bone marrow to respond to anemia. The increase in WBC counts might be due to the stimulation of immune system against the invading pathogenic microorganism.[21] The BGKT at a dose 400mg/kg and standard drug Leflunomide 10mg/kg significantly decreased
the WBC count and restored the Hb level and RBC count as compared to arthritic control group.

CONCLUSION

From the results obtained in the present study, it may be concluded that Balaguluchyadi Kwatham tablets possess significant anti-inflammatory and anti-arthritic activity. Further experimentation is needed in order to understand the precise mechanism of action of this formulation.

ACKNOWLEDGMENT

The authors are thankful to Chairman and Managing Trustee of KMCH College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore, Tamil Nadu for providing the facilities and support for the successful completion of the work.

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