EVALUATION OF ANTI-ARTHRITIC ACTIVITY USING ETHANOLIC EXTRACT OF OCIMUM SANCTUM LEAVES

K. Prasad, K. Preethi Sagar and Dasi Anupama*

Vikas Institute of Pharmaceutical Sciences, Rajahmundry.

ABSTRACT

Environment became polluted, due to that human beings are getting so many diseases. Arthritis is a chronic, inflammatory, systemic autoimmune disease categorized by pain, swelling and stiffness. Rheumatoid arthritis is an auto immune disorder characterized by pain, swelling and stiffness. Herbal medicines, as the major remedy in traditional medical systems, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. A majority of the world’s population in developing countries still depends on herbal medicines to meet its health needs. The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas. In that course Ocimum sanctum leaves have many pharmacological effects like anti inflammatory, analgesic, anti oxidant and many more.

KEYWORDS: Anti- arthritic activity, Ocimum sanctum, toxicity studies, plethysmometer

INTRODUCTION

Arthritis is a chronic, inflammatory, systemic autoimmune disease categorized by pain, swelling and stiffness. Rheumatoid arthritis is an auto immune disorder characterized by pain, swelling and stiffness. Rheumatoid arthritis affects approximately 1% of the population worldwide. Its etiology is still unknown. However, advances in understanding the pathogenesis of the disease have fostered the development of new therapeutics, with improved outcomes. Rheumatoid arthritis may rapidly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of mortality. It is an inflammation of synovial joint due to immuno mediated response. All antiinflammatory drugs
are not anti arthritic because it does not suppress T-cell and B-cell mediated response. Recently, it has been reported that microorganism including bacteria, viruses, fungi, parasites, bacterial DNA, and bacterial toxin may exacerbate the inflammatory response at the joint and bone. The component cells of the inflammed rheumatoid synovial membrane are depicted in innate and adaptive predominant compartments of the inflammatory response. Pivotal cytokine pathways are depicted in which activation of dendritic cells (DCs), T cells, B cells and macrophages underpins the dysregulated expression of cytokines that in turn drive activation of effector cells, including neutrophils, mast cells, endothelial cells and synovial fibroblasts. The prevalence of arthritis is higher among women (28.3%) than men (18.2%). If prevalence rates remain stable, the number of affected persons ages 65 years and older will nearly double to 41.1 million by 2030. According to the WHO, 80% of the world population continues to rely on traditional medicines for their health care. Herbal medicines, as the major remedy in traditional medical systems, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. A majority of the world’s population in developing countries still depends on herbal medicines to meet its health needs. The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas. The continued investigation into the secondary plant metabolites has gained importance for their safe use.

MATERIALS AND METHOD

Collection of plant material
The leaves of Ocimum sanctum were collected locally from Andhra Pradesh and the plant was identified by the experts. The Ocimum sanctum plant was collected during the march 2013 from Sri Venkateshwara University, Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library.

Preparation of plant material
The collected plant leaves were washed with water and separated from undesirable materials or plants or plant parts. They are aerated and fully dried for two days. The fully dried leaves are then grinded to make them powder by the help of suitable grinder.
Preparation of extract
The powder material was subjected to sequential soxhlet extraction. The solvent used was ethanol. The dried powder was defatted by macerating the powder for 7 days in ethanol with occasional stirring. Then the marc collected was subjected to soxhlet extraction with ethanol. Finally, the resultant marc was subjected to aqueous extraction. The collected extracts were then concentrated using rotary vacuum evaporator and were air dried at room temperature, weighed and percentage yield was calculated. The colour and consistency of the extracts were noted.

Preliminary Phytochemical investigation
The extracts obtained from the above extraction processes was analyzed for different phytoconstituents present in this by the method of qualitative phytochemical analysis. The following chemical tests were carried out and the results were tabulated.

Experimental methodology
Experimental animals
Thirty healthy adult albino rats of Wister strain of either sex between the age of 2-3 months and weighing 150-200 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and 12 hours dark cycle, 23±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animals are purchased from Sanzyme ltd(93/1999/CPCSEA), Gaganpahad, Hyderabad-501323 and Mahaveer Enterprises(146/1999/CPCSEA), Baghamberpet, Hyderabad-501313.

Acute oral toxicity studies
Principle
It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step i.e.; no further testing is needed, dosing of three additional animals, with the same dose dosing of three additional animals at the next higher or the next lower dose level.
PROCEDURE

A) Administration of the doses
The test substance was administered in a single dose by using a stomach gavage needle. In the unusual circumstances that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. In the present study it was not required as the dose was administered at once. The animals had been fasted overnight during period of drug administration with complete access to water all the time. Following the period of fasting, the animals were weighed and the test substance was administered. After 3 hours diet was given to the animals.

B) Number of animals and dose levels
Three animals were used for each step. The dose level to be used as the starting dose selected from one of four fixed levels 5, 50, 300 and 2000mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, delayed until was confident of survival of the previously dosed animals. The dose level 300 mg/kg was selected.

C) Observations
Animals were observed individually after dosing at first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily Thereafter, for a total of 14 days, it should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary (104). All observations were systematically recorded with individual records being maintained for each animal.

PLETHYSMOMETER

A) Design of the plethysmometer
Buzzer connections and modifications to conventional plethysmometer were made as per the figure 1. An electrical buzzer which works on 6V DC battery was adopted. Two electrical leads of the buzzer were introduced into plethysmometer such that when mercury touches certain level, leads get connected with each other with the help of mercury so that buzzer starts blowing. Therefore, when mercury reaches certain level, buzzer should start blowing and should be off below this particular level.
B) Measurement of standard volume
Wooden cylinders of known volume (0.5, 0.75 and 1.0 ml) were completely immersed into mercury of the plethysmometer. As a result the mercury level in the plethysmometer increases in accordance to their volume. The raised mercury levels were adjusted to original level with and without help of buzzer. All the readings were taken as average of five measurements and standard error of mean (SEM) was calculated.

C) Measurements of rat paw volume
Animal experiments were carried out after obtaining the clearance from the institutional animal ethical committee. Wistar albino rat of 158 g was selected for measurement of paw volume.

The paw was completely immersed into mercury up to the tibiotarsal articulation. As a result the mercury level in the plethysmometer increased and the raised mercury level was adjusted to original level with and without help of buzzer.

All the readings were taken average of five measurements and standard error of mean (SEM) was calculated. The buzzer alerts when mercury touches certain level in plethysmometer due to closing of the circuit. In case of conventional plethysmometer, adjustment of level of mercury is done by close observation. Before, measurement of the actual rat paw volume, plethysmometer was calibrated with standard wooden cylinders of known volume. Measurement of volume of standard wooden cylinders is also done to confirm the reproducibility of the measurement.

Anti-arthritic activity models
Acute arthritic activity model
A) Turpentine oil induced paw edema
Wistar albino rats were fasted 24 h before experimentation with free excess of water. Animals were divided into five groups (n=6). Group I served as normal control and received vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of ethanolic extracts of Ocimum sanctum, respectively, and group V received a standard drug aspirin (100 mg/kg). Acute inflammation in joint produced by injecting 0.02 ml of turpentine oil into the synovial cavity of the left knee joint, after 30 min of drug administration diameter of the joint was monitored at hourly interval for 6 h using micrometer screw gauge10.
Sub-Acute Arthritic Activity Model

A) Formaldehyde-induced arthritis in rats

Formaldehyde used to induce arthritis using reported method with minor modification. All wistar rats were divided into five groups, Group I served as normal control and received vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of ethanolic extracts of Ocimum sanctum respectively, and group V received a standard drug aspirin (100 mg/kg p.o.). On day1, 30 min after the drug administration chronic non-immunological arthritis was induced by sub plantar injection of 0.1 ml of 2% formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the mean increase in paw diameter over a period of 10 days using a micrometer screw gauge.

\[ \% \text{Inhibition} = \frac{V_C - V_T}{V_C} \times 100 \]

Chronic Arthritic Activity Model

A) CFA-induced arthritis in rats

Experimental immunological arthritis was induced in rats with some modification in the previously documented method. 30 Wistar rats divided into the five groups (n=6). Group I served as normal control and administered with vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of ethanolic extracts of Ocimum sanctum respectively, and group V received a standard drug aspirin (100 mg/kg p.o).

The left paw of each rat was injected subcutaneously with 0.01 ml of Complete Freund Adjuvent (CFA - 0.1ml of 0.5% w/v suspension of heat killed Mycobacterium tuberculosis cells in liquid paraffin) except normal control group of rats. The edema of the left and right hind paw observed at 5, 7, 10, 13, 15, 18, 21 post injection of CFA using micrometer screw gauge. The percentage inhibition of left paw edema was calculated by following formula

\[ \% \text{Inhibition} = \frac{V_C - V_T}{V_C} \times 100 \]

Where VC= Paw edema of control group, VT= paw edema of the test group.

The changes in body weight were recorded on regular interval. On the 21th day, blood was withdrawn through retro-orbital vein puncture of all groups by anesthetizing the animals with diethyl ether and the haematological parameters such as total WBC Count, RBC Count, Hb, ESR were evaluated. The results of standard and test groups were compared with control group by statistical analysis.
Arthritis assessment in CFA rats
Determination of clinical symptoms in CFA induced arthritis was evaluated by a visual scoring system on scale 0-4, where 0: no change, 1: swelling and erythema of the limb, 2: mild swelling and erythema of the limb, 3: gross swelling and erythema of the limb, 4: gross deformity and inability of the limb. A score of the 4 limb was counted and score more than 1 exhibit the arthritis whereas a maximum score of the arthritis is 16. The frequency and day of onset of arthritis also recorded 13.

Statistical analysis: The results are expressed as the Mean ± SEM. The significance of the difference was evaluated by one-way ANOVA. Data were considered statistically significant if P < 0.05.

RESULTS
Preliminary phytochemical analysis
Table 1: Preliminary phytochemical tests results.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Constituents</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Anthraquinone glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Gums and mucilage</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Proteins and aminoacids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins and phenolic compounds</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Steroids and sterols</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

Anti-arthritic activity models
Effect on turpentine oil induced arthritis: Antiarthritic activity of *Ocimum sanctum* (ethanolic extract) was evaluated by the assessment made on the 6thhr. The outcomes showed that treatment of different doses (125, 250, 500 mg/kg, p.o) of ethanolic extract of *Ocimum sanctum* inhibited swelling in the synovial cavity at dose dependent manner. After 6 h, percentage inhibition of paw edema in wistar rats at different doses of *Ocimum sanctum* ethanolic extract (125, 250, 500 mg/kg, p.o) were 70.25%, 90.46%, 97.33%, respectively; while Aspirin showed 95.55% of inhibition.
Table 2: Effect of Ocimum sanctum leaves extract on turpentine oil induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.87±0.015</td>
<td>1.26±0.024</td>
<td>1.63±0.022</td>
<td>1.99±0.044</td>
<td>2.27±0.043</td>
<td>2.47±0.044</td>
<td>-</td>
</tr>
<tr>
<td>OS I (125mg/kg)</td>
<td>0.75±0.009ns</td>
<td>1.03±0.026*</td>
<td>1.16±0.024*</td>
<td>1.16±0.024*</td>
<td>0.97±0.031*</td>
<td>0.74±0.022**</td>
<td>70.25</td>
</tr>
<tr>
<td>OS II (250mg/kg)</td>
<td>0.55±0.034*</td>
<td>0.76±0.022*</td>
<td>0.56±0.034*</td>
<td>0.56±0.034*</td>
<td>0.36±0.018*</td>
<td>0.24±0.015***</td>
<td>90.46</td>
</tr>
<tr>
<td>OS III (500mg/kg)</td>
<td>0.27±0.012*</td>
<td>0.45±0.010*</td>
<td>0.28±0.022**</td>
<td>0.28±0.022**</td>
<td>0.14±0.007***</td>
<td>0.07±0.009***</td>
<td>97.33</td>
</tr>
<tr>
<td>Aspirin (100mg/kg)</td>
<td>0.31±0.016*</td>
<td>0.53±0.011*</td>
<td>0.64±0.013*</td>
<td>0.34±0.016*</td>
<td>0.19±0.008**</td>
<td>0.11±0.007***</td>
<td>95.55</td>
</tr>
</tbody>
</table>
Graph.1. Effect of OS on turpentine oil induced paw oedema in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. **P<0.01 compared to control.

Graph.2: % Inhibition of OS on turpentine oil induced paw oedema in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. **P<0.01 compared to control.

Effect on formaldehyde induced arthritis: In formaldehyde induced arthritis model of wistar rats, the assessment made on the 10th day showed that, treatment with different doses of *Ocimum sanctum* (125, 250, 500 mg/kg, p.o) ethanolic extract significantly reduced (P<0.001) swelling in the injected (left) hind paw as compared to Aspirin treated group. On the 10th day the % inhibition of paw edema exhibited by different doses of *Ocimum sanctum*
were 62.88%, 66.87%, 90.91% respectively; while Aspirin treated animals showed maximum inhibition of paw edema 85.61%.

Table 3: effect of ocinum sanctum leaves extracts on formaldehyde induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 10</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.446±0.014</td>
<td>0.616±0.014</td>
<td>0.826±0.007</td>
<td>1.09±0.009</td>
<td>1.32±0.004</td>
<td>-</td>
</tr>
<tr>
<td>OS (125 mg/kg)</td>
<td>0.38±0.014*</td>
<td>0.47±0.015*</td>
<td>0.61±0.011*</td>
<td>0.54±0.007*</td>
<td>0.49±0.007*</td>
<td>62.88</td>
</tr>
<tr>
<td>OS (250 mg/kg)</td>
<td>0.366±0.011*</td>
<td>0.44±0.009*</td>
<td>0.54±0.007*</td>
<td>0.51±0.008*</td>
<td>0.44±0.011*</td>
<td>66.67</td>
</tr>
<tr>
<td>OS (500 mg/kg)</td>
<td>0.226±0.019*</td>
<td>0.294±0.014*</td>
<td>0.28±0.027*</td>
<td>0.25±0.016</td>
<td>0.12±0.009*</td>
<td>90.91</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.268±0.012*</td>
<td>0.334±0.015*</td>
<td>0.34±0.012*</td>
<td>0.29±0.011*</td>
<td>0.19±0.016*</td>
<td>85.61</td>
</tr>
</tbody>
</table>

Graph 3: Effect of OS on formaldehyde induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. *P<0.05, **P<0.01 compared to control.

Graph 4. % Inhibition of OS on formaldehyde induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. *P<0.05, **P<0.01 compared to control.
Effect on CFA-induced arthritis

In Adjuvant induced animals, Different doses (125, 250, 500 mg/kg) of *Ocimum sanctum* ethanolic extract significantly \((p < 0.001)\) inhibited arthritic swelling by 74.65% 79.26% and 90.90% as compared to the adjuvant control respectively, whereas the Aspirin treated group showed an inhibition of 94.89%.
Table 4: Effect of *ocimum sanctum* leaves extracts on cfa induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5Days</th>
<th>7Days</th>
<th>10Days</th>
<th>13Days</th>
<th>15Days</th>
<th>18Days</th>
<th>21Days</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritic Control</td>
<td>1.01±0.007</td>
<td>1.14±0.008</td>
<td>1.24±0.013</td>
<td>1.38±0.014</td>
<td>1.55±0.014</td>
<td>1.96±0.019</td>
<td>2.15±0.029</td>
<td>-</td>
</tr>
<tr>
<td>OS (125mg/kg)</td>
<td>0.95±0.004</td>
<td>0.90±0.016</td>
<td>0.82±0.015</td>
<td>0.76±0.013</td>
<td>0.69±0.006</td>
<td>0.54±0.013</td>
<td>0.54±0.013</td>
<td>74.65</td>
</tr>
<tr>
<td>OS (250mg/kg)</td>
<td>0.88±0.007</td>
<td>0.83±0.014</td>
<td>0.75±0.011</td>
<td>0.68±0.015</td>
<td>0.61±0.015</td>
<td>0.52±0.012</td>
<td>0.44±0.014</td>
<td>79.26</td>
</tr>
<tr>
<td>OS (500mg/kg)</td>
<td>0.77±0.005</td>
<td>0.61±0.003</td>
<td>0.49±0.013</td>
<td>0.38±0.086</td>
<td>0.24±0.013</td>
<td>0.12±0.007</td>
<td>0.11±0.014</td>
<td>94.89</td>
</tr>
<tr>
<td>Aspirin (100mg/kg)</td>
<td>0.83±0.003</td>
<td>0.69±0.009</td>
<td>0.55±0.011</td>
<td>0.45±0.013</td>
<td>0.31±0.012</td>
<td>0.20±0.013</td>
<td>0.19±0.027</td>
<td>90.90</td>
</tr>
</tbody>
</table>
Graph. 5. Effect of OS on Complete Freund’s Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. *$P<0.05$, **$P<0.01$ compared to control.

Graph. 6. % Inhibition of OS on Complete Freund’s Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. *$P<0.05$, **$P<0.01$ compared to control.

Effect on arthritis assessment
The occurrence of the arthritis recorded. On the day 5 injections of arthritis, the arthritis index of the different extract of *Ocimum sanctum* and aspirin was recorded. The *Ocimum*
sanctum leaves extract and aspirin exhibit the lower arthritis score as compared to an arthritis model.

Table 5: Effect of ocimum sanctum leaves extract and standard drug on the development of cfa arthritic index

<table>
<thead>
<tr>
<th>Arthritic Index</th>
<th>Treatment</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 13</th>
<th>Day 15</th>
<th>Day 18</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritic Control</td>
<td>6.2±0.583</td>
<td>7±0.547</td>
<td>7.8±0.374</td>
<td>8.8±0.376</td>
<td>9.2±0.489</td>
<td>9.6±0.509</td>
<td>10.2±0.201</td>
<td></td>
</tr>
<tr>
<td>OS (125mg/kg)</td>
<td>5.6±0.509 ns</td>
<td>6.2±0.374 ns</td>
<td>7±0.447*</td>
<td>8±0.211*</td>
<td>8.2±0.374*</td>
<td>7.8±0.244*</td>
<td>6.6±0.547*</td>
<td></td>
</tr>
<tr>
<td>OS (250mg/kg)</td>
<td>5±0.447 ns</td>
<td>5.4±0.405 ns</td>
<td>6±0.316*</td>
<td>6.6±0.401*</td>
<td>6.8±0.374*</td>
<td>6.2±0.212**</td>
<td>5±0.316**</td>
<td></td>
</tr>
<tr>
<td>OS (500mg/kg)</td>
<td>3.8±0.678 ns</td>
<td>4.2±0.374*</td>
<td>4.4±0.509*</td>
<td>4.8±0.734*</td>
<td>4.6±0.678***</td>
<td>4±0.547***</td>
<td>2.8±0.583***</td>
<td></td>
</tr>
<tr>
<td>Aspirin (100mg/kg)</td>
<td>4±0.374 ns</td>
<td>4.6±0.402*</td>
<td>5±0.316*</td>
<td>5.8±0.583*</td>
<td>5.2±0.374***</td>
<td>4.4±0.244***</td>
<td>3.2±0.374***</td>
<td></td>
</tr>
</tbody>
</table>

Effect on body weight: The gain in body weight on day 21st in each treatment group was observed in the rats under study.

Table 6: Effect of ocimum sanctum leaves extract on body weight of cfa induced rat paw edema.

<table>
<thead>
<tr>
<th>Weight Variance(in gm)</th>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritic Control</td>
<td>156.8±1.212</td>
<td>153.3±0.932</td>
<td>143.4±0.432</td>
<td>135.1±0.235</td>
<td>128.4±3.954</td>
<td>-28.4</td>
</tr>
<tr>
<td>OS (125mg/kg)</td>
<td>157.2±0.374</td>
<td>160.7±1.234</td>
<td>164.4±4.323</td>
<td>166.4±2.431</td>
<td>172.2±1.435</td>
<td>15</td>
</tr>
<tr>
<td>OS (250mg/kg)</td>
<td>159.6±0.213</td>
<td>161.2±1.344</td>
<td>166.8±2.343</td>
<td>170±3.545</td>
<td>177.3±2.331</td>
<td>17.7</td>
</tr>
<tr>
<td>OS (500mg/kg)</td>
<td>158.2±1.454</td>
<td>161.2±2.456</td>
<td>167.8±3.433</td>
<td>173.3±1.232</td>
<td>182.4±0.754</td>
<td>24.2</td>
</tr>
<tr>
<td>Aspirin (100mg/kg)</td>
<td>157.4±0.765</td>
<td>161.8±0.456</td>
<td>167.4±0.345</td>
<td>172.4±1.434</td>
<td>181.2±2.043</td>
<td>23.8</td>
</tr>
</tbody>
</table>
Graph. 7. Effect of body weight of OS on Complete Freund’s Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. *P<0.05, **P<0.01 compared to control.

Graph.8: Weight Variance of OS on Complete Freund’s Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. *P<0.05, **P<0.01 compared to control. The body weight of the disease control groups were significantly reduced as compared to the different doses (125, 250, 500 mg/kg) of *Ocimum sanctum* ethanolic extract and aspirin treated groups.
Effect on haematological parameters

The CFA-induced hematological perturbations, such as an increase in the ESR, WBC count and decrease in Hb, RBC count were also significantly \( (p < 0.001) \) altered at different doses (125, 250, 500 mg/kg p.o.) *Ocimum sanctum* ethanolic extract and aspirin (100 mg/kg p.o.).

Table 7: Effect on haematological parameters in adjuvant-induced arthritis in rats.

<table>
<thead>
<tr>
<th>Haematological Parameter</th>
<th>Arthritic Control</th>
<th>OS-I (125mg/kg)</th>
<th>OS-II (250mg/kg)</th>
<th>OS-III (500mg/kg)</th>
<th>Aspirin (100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC count (cells/cu.mm)</td>
<td>8.96±0.242</td>
<td>8.11±0.172*</td>
<td>7.71±0.056*</td>
<td>7.06±0.056*</td>
<td>7.48±0.129*</td>
</tr>
<tr>
<td>RBC count (million/cu.mm)</td>
<td>5.72±0.105</td>
<td>6.11±0.065*</td>
<td>6.31±0.086*</td>
<td>6.85±0.039</td>
<td>6.54±0.035*</td>
</tr>
<tr>
<td>Hb (cells/cu.mm)</td>
<td>11.2±0.375</td>
<td>12±0.316*</td>
<td>12.8±0.012</td>
<td>13.4±0.041</td>
<td>13.2±0.382</td>
</tr>
<tr>
<td>ESR (million/cu.mm) (gm%)</td>
<td>14.8±0.374</td>
<td>13.6±0.510*</td>
<td>12.6±0.401</td>
<td>12±0.316</td>
<td>12.2±0.374</td>
</tr>
</tbody>
</table>

Graph.9: Effect of Haematological parameters of OS on Complete Freund’s Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. \( *P<0.05, **P<0.01 \) compared to control.

5.4. Toxicity profile of test plant: Administration of OS in a dose of 5000 mg/kg body weight did not produce any behavioural abnormalities in the animals. As all tested animals survived, the oral LD\(_{50}\) of OS in rats was found to be >5000 mg/kg body weight. Chronic administration of OS in a dose of 1800 mg/kg body weight for 28 days did not produce any significant physiological changes in the tested animals as compared to normal control (data not shown). There was a marginal increase in body weight, bleeding time, RBC count levels
as compared to normal control, but this increase was not significant. WBC count, %Hb and showed a marginal decrease from normal control, but this difference was also not statistically significant. All other parameters remained unaltered.

DISCUSSION
Alternative medicine for the treatment of various diseases is getting increasing popularity day by day. Because it shows fewer side effects as compared to other system of medicine, many medicinal plants have proven effects on arthritic symptoms as compared to that of conventional medicine agent 14.

The anti-arthritic effect of ethanolic extract of *Ocimum sanctum* leaves could be observed in acute (Turpentine oil and Formaldehyde induced arthritis) and chronic (Freund’s complete adjuvant induced arthritis in rat) model of inflammation. Arthritis is a chronic inflammatory disease which affects several joints of the body like cartilage, synovium, tendon and muscle. Mostly researcher has claimed that inhibition of adjuvant – induced arthritis in rats, is the suitable test procedure to screen anti-arthritic activity. The rats develop chronic swelling in multiple joint with the influence of inflammatory cells, attrition of joint cartilage and bone damage. It closely resembles with human arthritis disease 9. One of the reasons for wide utilization of the rat animal model method is due to the strong correlation between efficacy in the animal model and rheumatism condition in human. Turpentine oil induced acute inflammation is due to release of mediator like histamine and serotonin in early phase; then kinin in intermediate phase and in a later phase releasing prostaglandin 15. Different doses of *Ocimum sanctum* significantly inhibit the turpentine oil induced joint edema, suggest that the possible mechanism of action inhibition the different phase of inflammation.

The Antiarthritis activity was evaluated by using common method inhibition of formaldehyde induced edema. The injection of formaldehyde into animal paw produced localized inflammation (releasing of histamine, serotonin and kinin) and pain 16. Formaldehyde induced arthritis is biphasic in nature i.e. an untimely neurogenic element followed by a later tissue mediated response.

Three different doses of *Ocimum sanctum* significantly inhibit the proliferative global oedematous response at dose dependent manner. The dose of the *Ocimum sanctum* (500 mg/kg) is more effective than the standard aspirin. In the Adjuvant induced arthritic method, it was observed that swelling and redness developed in rats after injecting the CFA over 24
hours. It seems that bacterial peptidoglycon and muramyl dipeptide are responsible for its induction 18-19 and chronic inflammation reaction slowly developed next 8 – 10 days. Chronic inflammation occurs in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. Cytokines, GM-CSF (Granulocyte-macrophage colony-stimulating factor), interferon like mediator are responsible for the chronic inflammation and pain, destruction of bone and cartilage 20-21. Another mechanism of chronic inflammation is leukocytes phagocytes forms a complex with the mediators (cytokines, GM-CSF, histamine, 5 hydroxytryptamine, bradykinin, various chemotactic factors, interferon and prostaglandin) and released the lysosomal enzymes, causing injury to cartilage and other tissue 22-25. In the arthritis condition, arthritis changes the haematological parameters. Another condition of arthritis is a WBC increased in due to an IL-1B mediated rise in the respective colony stimulating factors. *Ocimum sanctum* showed the effect in the arthritic rat at dose dependent manner. The drug showing the effect on two ways – firstly it decreases the release of certain mediator like cytokines, histamine, 5-hydroxytryptamine, bradykinin, interferon and prostaglandin. Secondly it may be stimulating the DTH response and increased phagocytic index and protection against cyclophosphamide induced myelo-suppression by increasing total WBC count which are directly associated with the immunomodulatory activity.

As the level of WBC increases in arthritis rats, the migration of leukocytes to the inflamed area was significantly suppressed by different doses of *Ocimum sanctum* leaves (ethanolic extract), which is associated with indicated that a significant decrease in the WBC count. Another symptom of arthritic patient is common occurrence anaemia 26. In arthritic condition gastrointestinal blood loss occurred due to medication and in bone marrow changes in patients with inflammatory arthritis, which prevent the release of iron for incorporation into red blood cells 27-28. In the present investigation, arthritic control group rats showed reduction of RBC, Hb and increase the level of WBC and ESR (erythrocyte sedimentation rate). All of these symptoms indicated as anaemia conditions. Our result suggests that different doses of *Ocimum sanctum* showed significant recovery from the induced anaemia with an increase in the level of RBC, Hb and decreases the level of WBC, ESR. During arthritis the body weight of the animals significantly decreases due to deficient absorption of nutrients through the internet and that treatment with the tested drug and standard drugs normalizes the process of absorption 29. The increase in the body weight in the *Ocimum*
sanctum and Aspirin treated groups may involve improvement of intestinal absorption of the nutrients and reduction in the distress caused by the severity of the arthritis.

Rheumatoid arthritis mostly involve in immunological derangements. The adjuvant arthritis model satisfies mostly the allied conditions of arthritis in rat which resembles human. In adjuvant arthritis bacterial peptidoglycan and muramyl dipeptide are responsible for its induction. It can occurs through cell mediated auto immunity by structural mimicry between mycobacteria and peptidoglycan in rats. The response to the CFA administration arthritis is biphasic it consists of acute phase and polyarthritic for a chronic phase, correspond to day 0-10 and 10-28 post CFA inoculation respectively. The acute phase response is characterized by unilateral inflammatory edema of the ipsilateral paw peaking around a 4-6 followed by subsequent arthritis and chronic phase response which begin around day 10 characterized by inflammatory edema in contralateral paw. CFA induced arthritis involves highly significant increase paws thickness of rat, significantly decrease in serum cortisol, highly significantly increase in creactive proteins however both the extracts decrease the paw volume in the present experimental conditions. The high efficacy of alcoholic extracts can be correlated with the presence of alkaloids, triterpenoids fraction in this part. In the studies there is an increased ESR level which is a common diagnostic feature in patient in chronic arthritis. Increase in the erythrocyte sedimentation rate is an indication of active but obscure disease process which elevate in response to stress, inflammation and cell necrosis. In arthritic condition there is mild to moderate increase in the WBC count which plays a major role in body defense mechanism. WBC count increase is may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor. Treatment with the extracts significantly decrease the ESR and the WBC count indicate the significant recovery from the arthritic progress. Thus it can be concluded that the aerial parts of Ocimum sanctum posses’ significant anti-inflammatory and antiarthritic activity in rats.

Further studies involving the isolation of the potent chemical constituent of the plant an investigation of the detail biochemical pathway responsible for this anti-arthritic action may result in the development of a potent anti-arthritic agent having low toxicity and low cost of preparation.
CONCLUSION

In conclusion, *Ocimum sanctum*, in a dose-dependent pattern, was effective in attenuating turpentine oil induced paw edema, formaldehyde-induced paw edema and CFA induced arthritis in rat models of acute and chronic inflammation, and therefore it could be investigated as a potential treatment for acute and chronic arthritis conditions in humans. The results obtained justify the use of the plant extract in traditional Indian medicine for the treatment of painful inflammatory and arthritic conditions. Further work in our lab is in process to isolate, identify, characterize, and elucidate the structure of the phytoconstituents responsible for the observed pharmacological activities in this study. Explicate the exact mechanism of action of *Ocimum sanctum* leaves in curtailing the effect of arthritis.

*Invitro* studies on *Ocimum sanctum* demonstrate suppression of both inflammation and arthritis. The ethanolic extracts of the leaves of *Ocimum sanctum* must contain some principles, which possess anti-inflammatory, and anti-arthritic activities. From the preliminary screening study, it showed the presence of Flavonones, Flavones, Tri-Terpenoids and Phenolics. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-arthritic, anti-platelet and anti-inflammatory drug research. Studies related to active constituents on lipid derived eicosanoids, enzyme expression (COX2, lipoxygenase) and cytokines are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity. Hence it can be used as a potent agent against it.

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REFERENCES

