Research Study

Effect Of Lampito Mauritii In Complete Freund’s Adjuvant Induced Arthritis In Wistar Rats

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ARTICLE INFO
ABSTRACT

The aim of present study focuses on anti arthritic activity along with the powder of Lampito mauritii against complete Freund’s adjuvant induced animal model

Method: Wister albino rats were selected for this study. The aqueous extract of Lampito mauritii (AQLM) was administered by the oral dose of 200mg/kg and 400mg/kg, for a period of 21 days duration. The negative control was used by induced complete Freund’s adjuvant. The Biochemical parameters, haematological parameters and histopathology of the joint were studied. The paw level also measured.

Result: It concludes that the both concentration of Lampito mauritii was elucidated and provide significant effect of anti-arthritic activity.
INTRODUCTION:
Rheumatoid arthritis (RA) is a chronic autoimmune disease, advancing, lingering and a crippling disorder characterized by swelling, pain and synovial joints stiffness (1). It affects about 1% of the population of world in a female and male ratio of 2.5:1 (2) and lead to loss of function affecting more than 10 million people in China and about 1% of the general population in the world (3). It is caused by pro-inflammaratory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines (4).

Although different categories like nonsteroidal anti-inflammatory drugs (NSAIDs), immunosuppressants and steroidal anti-inflammatory drugs are in use, the limitation is their potential side effects. The development of new safe, potent, less toxic antiarthritis drug is the growing concern all over (5) (6).

Earthworm (Lampito mauritii) plays a major role in the proper functioning of the soil ecosystem. Earthworm increases the soil fertility and is often referred as farmer’s friend. Earthworms have been used in medicine for various remedies since 1340 AD and recognized in oriental medicine as anti-inflammatory, analgesic and antipyretic agent. Anti arthritic activity also used but scientifically not proven. It shows anticancer effect by preventing excess glucose uptake. Microorganisms are known to play a major role in soil characteristics and invertebrates are believed to act as regulators of antimicrobial activity (7). The aim of present study focuses on anti arthritic activity along with the powder of Lampito mauritii against complete freund's adjuvant induced animal model.

MATERIALS AND METHODS

Collection and Identification

The species for the study is earthworm Lampito mauritii were collected in the month of December from the areas near by Theni, Tamil nadu, and was authenticated by Dr.V.Mariappan, Department of Zoology, Ayya Nadar Janki Ammal College, Sivakasi, Tamil Nadu, India.

Extraction

The cultured earthworms were used in the studies. The worms were placed into a distilled water for 5 hours to allow the soil was excreted. After wash the earthworms were placed in to filter paper to roll the worms for absorb the moisture content from the earth worm skin. After removal of moisture content, the earthworm was kept in petri dish to incubated for 24 hrs at a temperature of 55°C. After 24 hours, the earthworm liquid was removed and kept in petri dish and placed in a hotplate for 2 hours to adjust a temperature for 60°C and earthworm powder was removed by scrubbing a Petri dish. (8).

The earthworm powder was extracts by Soxhlet apparatus for continuous extraction process using Petroleum ether, chloroform, ethyl acetate, methanol and aqueous as the solvents. For each solvent, extraction was continued for 48 hours. The colour and percentage yield of each extracts were calculated (9).

Animal

Healthy young wistar albino rats weighing 100-150 gm were selected for study. The rats were kept in polypropylene cages properly numbered and given a standard diet and water ad libitum throughout the experimental period. The animals were maintained in 12 hr. light and dark cycle at 22°C (± 3°C) in a well-ventilated, they were acclimatized to laboratory conditions for 10 days prior to the commencements of the experiment. Paddy husk was used as bedding material and changed twice a week (10). The experimental protocol has been approved by institutional animal ethics committee, Ref no. SBCP / 2019-20/CPCSEA / IAEC /I(3) /F16/122

PHARMACOLOGICAL SCREENING

Complete Freund's adjuvant (CFA) induced arthritis

Treatment protocol

The animals were divided into six groups of animals each as follows:

- Group I– Normal saline (5ml/kg, p.o) (non-arthritic)
- Group II– CFA (1mg/ml) on sub planta region
- Group III– CFA (1mg/ml) + Indomethacin (10mg/kg, i.p)
- Group IV– CFA +AQLM (200mg/kg, p.o)
- Group V– CFA + AQLM (400mg/kg, p.o)
Arthritic control group was given a single injection of 0.1ml Complete Freund’s adjuvant in to subplantar region of right hind paw on day one under light ether anesthesia [11]. The oral administration of the samples and Indomethacin (10mg/kg, i.p) dosing of the groups starts from day1-12, once daily until day 21st. Anti Arthritic activity of AQLM was measured the paw level on day 0, 3, 6, 9, 12, 15 and day 21. The last day, all the animals were sacrificed under ether anaesthesia the blood was collected by retro-orbital route for haematological and Biochemical parameters. The joints were also removed for histology (12)

**Statistical analysis**

Values are expressed as mean SEM. The mean differences in body weight and plasma biochemical analysis were analyzed using one-way ANOVA followed by Dunnett ‘t’ test. The difference between each group were considered statistically significant at analysis was performed using Graph Pad prism statistical software (version 5.03)

**RESULTS AND DISCUSSION**

The powder of *Lampito mauritii* was extracted with solvents like Petroleum ether, chloroform, ethyl acetate, methanol and aqueous respectively by extraction using soxhlet apparatus. The percentage yield of various extracts was mention table 1 and the presence of chemical constituents on the various extracts of *Lampito mauritii* was presented in Table-2. Based on the report of Chemical analysis we selected aqueous extracts for following studies.

**Table: 1 Percentage yield of various extracts of Lampito mauritii**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Solvent used</th>
<th>Amount of extract obtained (gm)</th>
<th>Percentage yield (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum Ether</td>
<td>5.1</td>
<td>11.98</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>6.3</td>
<td>14.79</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate</td>
<td>5.4</td>
<td>12.68</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>6.4</td>
<td>15.03</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous</td>
<td>6.6</td>
<td>15.50</td>
</tr>
</tbody>
</table>

**Table: 2 Chemical analysis of various extracts of Lampito mauritii**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical constituents</th>
<th>Pet-ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
<th>aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Triterpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Gums &amp; mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Fixed oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) sign denote the presence (-) sign denote the absence

**Complete Freund's adjuvant induced arthritis**

**Paw volume**

The mean difference in paw volume was found to be zero in normal control group. Paw volume difference was very much higher for the negative control group i.e., 0.30 on 21"day. The group of standard drug was found to be 0.02 in paw volume. The treated groups Aqueous extracts of *Lampito*
mauritii (AQLM) -200 mg/kg, p.o and 400 mg/kg, p.o were found to be 0.08 and 0.02 respectively. When compared with the negative control group. The paw volume of standard and extract at both concentrations were found to be decreased significantly. The results were shown in Table-3.

In normal control group, the serum level of SGPT and SGOT was found to be with 22.31 U/L and 58.13 U/L respectively. The SGPT and SGOT level of negative control group were increased. The Standard treated group was decreased the elevated level of SGPT and SGOT when compared to negative control group. The AQLM-200mg/kg p.o and 400mg/kg p.o treated groups were decreased the SGPT and SGOT level to 42.05 U/L and 24.03 U/L respectively when compared to negative control group. The SGPT and SGOT level of standard and AQLM 400mg/kg p.o groups were decreased and it is near to normal. The results were shown in Table-5.

In normal control group of alkaline phosphate (ALP) and Creatinine was found to be 57.52 U/L and 0.39 mg/dl respectively. The negative level of alkaline phosphate and Creatinine was increased when compared to control group. The Standard treated group (Indomethacin, 10mg/kg, i.p) was decreased the elevated level of alkaline phosphate and Creatinine when compared to negative control group. The ALP and Creatinine level of AQLM 200 mg/kg p.o and 400 mg/kg p.o groups were decreased and it was near to normal. The results were shown in Table-5.

In normal control group of haemoglobin value was found to be 12.21 g%. The negative control group was decreased potentially to 7.11 g%. The Standard group was increased the level of haemoglobin to 11.81 g% when compared to negative control group. The AQLM 200mg/kg p.o and 400mg/kg p.o treated groups were increased the haemoglobin level to 9.28 and 11.22 g% respectively when compared to negative control group. The results of haemoglobin level were found in Table-6.

The level of ESR was found to be 36.20% in normal control group and the negative control group was increased potentially to 61.07%. The indomethacin (Standard) was decreased the elevated level of ESR to 39.11% when compared to negative control group. The AQLM 200mg/kg p.o and 400mg/kg p.o treated groups were increased the haemoglobin level to 9.28 and 11.22 g% respectively, when compared to negative control group. The results of haemoglobin level were found in Table-6.

Haematological parameters

In normal control group, the RBC level was found to be $8.01 \times 10^6$/mm$^3$. The negative control group was decreased to $4.15 \times 10^6$/mm$^3$. The standard treated group was increased the level of RBC to $7.62 \times 10^6$/mm$^3$ when compared to negative control group. The AQLM 200mg/kg p.o and 400mg/kg p.o treated groups were increased the RBC level to 6.21 and 7.41 $\times 10^6$/mm$^3$ respectively and significantly changed, when compared to negative control group. The RBC level of standard and AQLM 400mg/kg p.o groups were increased and it was nearer to normal. The results were shown in Table-6.

In WBC level of normal control group was found to be $8.22 \times 10^3$/mm$^3$ and the negative control group was increased potentially to $12.20 \times 10^3$/mm$^3$. The Standard treated group was decreased the elevated level of WBC to $8.13 \times 10^3$/mm$^3$ when compared to negative control group. The AQLM 200mg/kg p.o and 400mg/kg p.o groups were decreased and it was close to normal. The results were shown in Table-6.

In normal control group of haemoglobin value was found to be 12.21 g%. The negative control group was decreased potentially to 7.11 g%. The Standard group was increased the level of haemoglobin to 11.81 g% when compared to negative control group. The AQLM 200mg/kg p.o and 400mg/kg p.o treated groups were increased the haemoglobin level to 9.28 and 11.22 g% respectively, when compared to negative control group. The results of haemoglobin level were found in Table-6.

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### Table 3: Effect of LM on mean paw volume change in Complete Freund’s adjuvant (CFA) induced arthritis in albino rats

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Drug and dosage</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline (5ml/kg, p.o)</td>
<td>0.00±0.00***</td>
<td>0.00±0.00**</td>
<td>0.00±0.00***</td>
<td>0.00±0.00**</td>
<td>0.00±0.00***</td>
<td>0.00±0.00***</td>
<td>0.00±0.00**</td>
<td>0.00±0.00***</td>
<td>0.00±0.00***</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CFA (1mg/mL) on sub plantar region</td>
<td>0.14±0.04</td>
<td>0.18±0.06</td>
<td>0.22± 0.01</td>
<td>0.27± 0.03</td>
<td>0.29± 0.05</td>
<td>0.31± 0.01</td>
<td>0.31± 0.04</td>
<td>0.32± 0.01</td>
<td>0.31±0.03</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td>3</td>
<td>CFA (1mg/mL) + Indomethacin (10mg/kg, i.p)</td>
<td>0.16±0.01**</td>
<td>0.13±0.03**</td>
<td>0.14±0.003***</td>
<td>0.12±0.02***</td>
<td>0.10±0.03***</td>
<td>0.09±0.02***</td>
<td>0.07±0.03***</td>
<td>0.04±0.05***</td>
<td>0.03±0.02***</td>
<td>0.02±0.01***</td>
</tr>
<tr>
<td>4</td>
<td>CFA + AQLM (200mg/kg, p.o)</td>
<td>0.15±0.03*</td>
<td>0.16±0.02</td>
<td>0.14± 0.05***</td>
<td>0.12± 0.02***</td>
<td>0.11± 0.04***</td>
<td>0.10± 0.01***</td>
<td>0.10± 0.06***</td>
<td>0.09± 0.03***</td>
<td>0.08± 0.01***</td>
<td>0.08± 0.03***</td>
</tr>
<tr>
<td>5</td>
<td>CFA + AQLM (400mg/kg, p.o)</td>
<td>0.16±0.05**</td>
<td>0.14±0.02**</td>
<td>0.11± 0.01***</td>
<td>0.09± 0.04***</td>
<td>0.06± 0.05***</td>
<td>0.04± 0.03***</td>
<td>0.04± 0.07***</td>
<td>0.03± 0.06***</td>
<td>0.02± 0.02***</td>
<td>0.02± 0.01***</td>
</tr>
</tbody>
</table>

n=6, Data were expressed as Mean± SEM, one-way ANOVA followed by Dunnett’s test. All groups were compared with Negative control, *P˂0.05, **P˂0.01, ***P<0.001

### Table 4: Effect of LM on the biochemical parameters in Complete Freund’s adjuvant (CFA) induced arthritis in albino rats

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Drug and dosage</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Protein (g/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline (5ml/kg, p.o)</td>
<td>58.13±1.1***</td>
<td>22.31±0.6**</td>
<td>57.52±0.5***</td>
<td>6.5±0.11**</td>
<td>0.39±0.02**</td>
</tr>
<tr>
<td>2</td>
<td>CFA (1mg/mL) on sub plantar region</td>
<td>117.11±0.86</td>
<td>67.02±0.10</td>
<td>145±0.15</td>
<td>3.9±0.29</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>3</td>
<td>CFA (1mg/mL) + Indomethacin (10mg/kg, i.p)</td>
<td>59.31±0.77***</td>
<td>23.01±0.12***</td>
<td>72.10±0.61***</td>
<td>6.58±0.10**</td>
<td>0.42±0.01***</td>
</tr>
<tr>
<td>4</td>
<td>CFA + AQLM (200mg/kg, p.o)</td>
<td>84.11±0.21***</td>
<td>42.05±0.15**</td>
<td>84.21±1.15***</td>
<td>6.50±0.21**</td>
<td>0.52±0.07***</td>
</tr>
<tr>
<td>5</td>
<td>CFA + AQLM (400mg/kg, p.o)</td>
<td>63.10±0.2***</td>
<td>24.03±1.7***</td>
<td>60.12±0.41***</td>
<td>6.45±0.11***</td>
<td>0.44±0.02***</td>
</tr>
</tbody>
</table>

n=6, Data expressed as Mean± SEM, one-way ANOVA followed by Dunnett’s test. All groups were compared with Negative control, *p<0.05, **p<0.01, ***p<0.001

### Table 5: Effect of LM on Hematological parameters in formaldehyde induced arthritis in albino rats

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Drug and dosage</th>
<th>RBC x10⁹/mm³</th>
<th>WBC x10⁶/mm³</th>
<th>Haemoglobin (g%)</th>
<th>ESR (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline (5ml/kg, p.o)</td>
<td>8.01±0.27***</td>
<td>8.22±0.24***</td>
<td>12.21±0.06***</td>
<td>36.20±63***</td>
</tr>
<tr>
<td>2</td>
<td>CFA (1mg/mL) on sub plantar region</td>
<td>4.15±0.26</td>
<td>12.2±0.45</td>
<td>7.11±0.16</td>
<td>61.07±1.2</td>
</tr>
<tr>
<td>3</td>
<td>CFA (1mg/mL)+Indomethacin (10mg/kg, i.p)</td>
<td>7.62±0.29***</td>
<td>8.13±0.37*</td>
<td>11.81±0.2**</td>
<td>39.11±0.44**</td>
</tr>
<tr>
<td>4</td>
<td>CFA + AQLM (200mg/kg, p.o)</td>
<td>6.21±0.27**</td>
<td>9.62±0.24**</td>
<td>9.28±0.23***</td>
<td>48.23±0.40***</td>
</tr>
<tr>
<td>5</td>
<td>CFA + AQLM (400mg/kg, p.o)</td>
<td>7.41±0.36***</td>
<td>8.39±0.39*</td>
<td>11.22±0.34**</td>
<td>42.11±0.39***</td>
</tr>
</tbody>
</table>

n=6, Data expressed as Mean± SEM, one-way ANOVA followed by Dunnett’s test. All groups were compared with Negative control, *p<0.05, **p<0.01, ***p<0.001
Group I Normal Control: Sections shows normal structure with small joint space. No inflammation is seen.

Group II Negative Control: Sections shows very large joint space, severe hyperplasia, granulomas, cells infiltration

Group III Standard: Sections show Bone and cartilage surrounding the joints appeared normal

Group IV AQLM 200mg/kg: Sections show bony tissue and connective tissue. No inflammation is seen.

Group V AQLM 400mg/kg: Sections show bony trabeculae and fibro muscular connective tissue. There is no evidence of inflammation

CONCLUSION

From the above-mentioned data, it was concluded that the both concentration of aqueous extracts of *Lampito mauritii* provide significant effect. In the dose of aqueous extracts of *Lampito mauritii* 400mg/kg was observed the effectively used to treat the diseases when compared to aqueous extracts of *Lampito mauritii* 200mg/kg. The aqueous extracts of *Lampito mauritii* (AQLM) was produced to cure the arthritis in dose dependent manner. Therefore, focus should be taken in the analysis part to identify and isolate the active constituent for future studies.

ACKNOWLEDGEMENT

I hereby express my sincere gratitude to respected Mr. S. Sriram Ashok (Correspondent, SB College of Pharmacy, Sivakasi, Tamilnadu, India) for his motivation and suggestions with latest infra structural facilities and for providing good environment which was encouraged me to carry out this project work successfully.

ABBREVIATIONS

AQLM: Aqueous extract of *Lampito mauritii*

RA: Rheumatoid arthritis

NSAIDs: Nonsteroidal anti-inflammatory drugs

CFA: Complete Freund's adjuvant

SGOT: Serum glutamic-oxaloacetic transaminase

SGPT: Serum glutamic pyruvic transaminase

ALP: Alkaline phosphate

RBC: Red blood cells

WBC: White blood cells

ESR: Erythrocyte sedimentation rate
REFERENCE


5) G. Kingsley, J. Lanchbury, and G. Panayi, “Immunotherapy in rheumatic disease: An idea whose time has come—or gone?” Immunology Today, 1996; 17(1) : 9–12.


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