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# An anti-osteoarthritis potential of the extract of Aeglemarmelos leaves in MIA (Monoiodoacetate) induced osteoarthritis in Wistar Rat

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**Abstract**

This present study was performed to see the effect of the enthanolic extract of the Aeglemarmelos leaves in in MIA (Monoiodoacetate) induced osteoarthritis in Wistar Rat. However, many types of medicines are available for the treatment of osteoarthritis. Most of the medicines used to reduce the pain by blocking the cyclooxygenase enzyme. These herbal medicines were used from the ages in several kinds of diseases. Although, Aeglemarmelos plant itself contain enormous phytochemicals to treat various diseases, but the leaves containsAnthocyanins, amino acids, alkaloids which are tend to utilized in the various kinds of conditions related to the osteoarthritis. The extract was prepared using ethanol and administered to the animals at the different doses i.e. 260, 520 and 725 mg/kg/day. The osteoarthritis was induced by the single injection of the MIA at 3mg/mL. The osteoarthritis induced within 7 days and confirmed by the pain assessment.

**Keyword:**Aeglemarmelos, osteoarthritis, MIA induced osteoarthritis

## 1. Introduction

Rheumatoid arthritis is a persistent autoimmune inflammatory and degenerative arthropathy that affects the joints, skin, bones and muscles [1]. It causes joint inflammation, synovial hyperplasia, pannus development, bone and cartilage loss, as well as chronic arthritic pain, swelling, stiffness and work impairment. Penetration and stimulation of numerous inflammatory cell groups such like CD4 helper T cells, B cells, dendritic cells, macrophages and mast cells, as well as the unveil of matrix metalloproteinase and cytokines such as tumor necrosis factor (TNF), IL-1 and IL-6, all play a role in the progression of rheumatoid arthritis[2].

**Pathogenesis of Rheumatoid Arthritis:**

Preclinical rheumatoid arthritis, genetic variables and environmental factors are three categories of well-known causes that have a role in the pathogenesis of rheumatoid arthritis. Seropositive and seronegative rheumatoid arthritis is the two most common forms of rheumatoid arthritis in adults[3].

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

Seronegative Rheumatoid arthritis patients do not have ACPA, unlike seropositive rheumatoid arthritis patients5. To be diagnosed with RA, seronegative patients should have inflammation in 10 or more joints.

The immune system assaults and hardens the synovial covering around the joints, causing joint inflammation. The cartilage and bone inside the joints might be irreversibly destroyed if inflammation surroundings the joints continue[4].

**Mechanism of Rheumatoid Arthritis Pain:**

Local mediators secreted from synovium bone, and other tissues can cause articular discomfort receptors to become activated. Musculoskeletal sensations will be confined, with a strong interface to mechanical impulses such as walking or standing, as a therapeutic indication of activation at this peripheral level[5].

In a host of afflictions such as rheumatoid arthritis, and recurrent low back pain, psychological and social aspects have been proven to be the highest relevant determinants of both the existence and intensity of pain.

**Plant Profile**

Ayurveda is considered as one of the oldest and effective form of medicine and has potential to treat chronic diseases which are untreatable in modern medicine with no side effects. Ayurveda is based on two important principles- Pareeksha(tools of examination) and Pramaana (inspired from the philosophical term). The tool of examination consists of three concepts- Pratyaksha (the direct observation), Anumana (the inference), Aptopadesha (reliable evidence) [6].

AegleMarmelos is one of the most popular and ancient plant in Ayurveda and Siddha medicine systems. It is considered as sacred plant with spiritual powers in CharakSamhita and believed that it is an incarnation of Lord Shiva.

The plant is widely available in India and is cultivated as temple garden plant. The fruit of the tree has certain medicinal uses and also been used for eating. The leaves are believed to have anti-hypoglycemic activity[7].

**Botanical Name:** Aeglemarmelos

**English Name:** Bael tree

**Family:** Rutaceae

**Parts of Plant used:** Leaf, Fruit, Root, Bark

**Cultivation:** All over India

**Taxonomical classification**

**Botanical Description**

Bael is the only monotypic member of the genus *Aeg1e*. The stem is thick, short, flexible, spreading and flaking bark, sometimes thorny branches. The tree has sharp, axial and one-inch long spikes.

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

Leaves consists of 3 to 4 leaflets, and these leaflets are oval or lancet with 4-10cm long and 2-5cm long When disfigured, the mature leaves emit a strange fragrance[8].
Flowers used to grow in the summers specifically April and May. The fruit which is used to come next after flowers comes in June to august. Flowers occur in groups between 4 and 7 along the young branches and have 4 repeated fleshy petals and are greenish-white in color with a strange fragrance.

**Plant collection and Authentication**

The leaves of Aeglemarmelos were collected in the month of November- December from the garden located near the laboratory and used without further purification. The plant was authenticated by our lab quality control team[9].

**Preparation of coarse powder and extraction technique**

The leaves were washed thoroughly with water and were shade dried for about 20-25 days at room temperature. The dried leaves were grinding in hammer mill type equipment and then sieved with a 20mm sieve mesh. Approximately 500 grams of the powdered leaves was crushed and filled in the soxhlet apparatus with petroleum ether, ethyl acetate and ethanol respectively. Continuous heat was given by the heater for solvent recycling. For each solvent, the extraction process continuous for 1-2 hrs[10].

The solvent comes after the evaporation was separated through the filtered and kept in room temperature for further analysis. The following phytochemicals were identified in the extract[11].

| SN. | Phytochemical Extract | Results |
|---|---|---|
| 1 | Carbohydrates | ++ |
| 2 | Proteins | -- |
| 3 | Amino acids | ++ |
| 4 | Steroids | -- |
| 5 | Glycosides | ++ |
| 6 | Saponins | - + |
| 7 | Flavonoids | ++ |
| 8 | Alkaloids | ++ |
| 9 | Tannin | -- |
| 10 | Phenolic | ++ |

**In vivo Study Design**

Animals were grouped into Five (each comprises 8 animals) groups. Negative control received the no drug but water as the drug. Animal number 1-8 was provided the water instead of drug and which acted as negative control. Group number 2 (Etoricoxib (10mg/kg)), reference item consist of 8 animals (9-16). Test drug- low was allotted to animal number 17-24. Test drug- high were allotted to animal number 25-32 and test drug-high were allotted to animals from 33-40[12].

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

**Induction of Osteoarthritis**

After the acclimatization the body weight was taken for the anesthesia. Monoiodoacetic acid was reconstituted in the normal saline with the dose of 3mg/mL for each animal. The injection area was shaved using trimmer so that the location will be visible. The single injection of the MIA was administered through intra articular. The location of the injection was seen two times to confirm the proper injection[13].

After establishing MIA-induced osteoarthritis in validation study, five experimental groups 1, 2, 3, 4 and 5 each consisting of eight rats was taken. Animals of groups 1 and 2were negative control and positive control, respectively. On the day 0, the MIA injection was administered in the all group except negative control. Negative control was administered normal saline in the place of the MIA. From day 0 to 28, rats in groups 3, 4, and 5were administered test item daily at 260, 520 and 725 mg/kg/day doses, respectively through oral gavage[13].

Rats of groups 1 will be administered normal saline and rats of group 2were administered with Etoricoxib at 10 mg/kg from day 0 to 30, daily orally. Pain assessment with the help of Cold hyper sensitivity, heat hyperalgesia and locomotor was done on 0th day. And subsequently was performed on 7th, 14th, 21th and 28th day. On the day of necropsy the right knee where MIA was introduced, collected and meant for histopathology[14].

All animals was necropsied and proximal tibias was collected and fixed in 4% PFA (Paraformaldehyde) for 7 days, decalcified in 20% EDTA solution for 21 days. The samples were stained with Hematoxylin and eosin stain. The slides were scored for macroscopic cartilage and bone scoring for rat arthritis[15].

**Confirmation of osteoarthritis**

Induction of Osteoarthritis in animals of all groups was confirmed by comparing the parameters ofBehavioral assessment of nociception, Heat hyperalgesia and Cold hypersensitivity before injection of MIA (0 Day) and on 7$^{th}$day of injection[16].

**Preparation of Reference and Test Item Formulation**

The formulations were prepared with the test item260, 520 and 725 mg/kg/day. The test item formulations were prepared daily on the basis of the most recent body weight taken during the treatment phase. Required quantity of test item was taken in a labeled beaker. Adequate quantity of vehicle was added, mixed well. The amount of the test item and volume of the formulations prepared varied depending on the requirement and/or body weights of the animals[17].

**Observation**

**Clinical Signs**

Animals were observed for mortality, morbidity and signs of toxicity daily once.

**Body Weight**

Body weight of animals were recorded weekly once till the day of necropsy.

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

**Behavioral Assessment**

**Rotarod**

Each day, the animals were trained to adopt the rotarod apparatus. Animals were kept at the experimental room, and each day, training was given to the animals. Each animal were trained for at least 30 min. The rotation frequencies were kept stable at 4 RPM initially then it gets increase by 10 times. Each animal were tested for a number of consecutive days. The latency to falling off the rotarod was recorded on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatment[18].

**Heat Hyperalgesia**

Planter side of paw of animal was placed above the hot plate at 50°C and paw withdrawal latency were noted in seconds. A time was set to 20-30 sec and that was used to maintain the constant time for each animal to avoid injury to the animal. Assessment was done for about 3 minutes with the time interval of 5 min. Sensitivity to heat was assessed for each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatmen[18, 19].

**Cold hypersensitivity**

Paw withdrawal was recorded in 20 to 30 seconds for each animal while dipping the paw in water maintains at 4°C. A time was set to 20-30 sec and that was used to maintain the constant time interval of 5 minutes for each animal to avoid injury. Paw withdrawal latency was assessed for each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 days during the period of the treatment[20].

**Knee thickness**

Knee thickness was assessed with vernier caliper of each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 dayduring the period of the treatment[21].

**Necropsy[22]**

1. On $28^{th}$ day of post MIA induction, animals of all groups were sacrificed by $CO_2$ inhalation and subjected to gross pathological examination.
2. Right knee joints were collected in 10% neutral buffered formalin solution from all the rats after $CO_2$ euthanasia.

**Histopathology[23]**

The collected joints were decalcified using 10% formic acid and routinely processed. The section of the limp was cut and staining was done with hematoxylin and eosin.

- Minimal
- Mild
- Moderate
- Marked
- Severe

## Results

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

**Drug Content**

The drug content of the matrices was performed to verify that the drug loading is consistent in the formulation.On examination of the drug content of prepared formulation showed that the method used to prepare patches was capable of supplying consistent drug content, with minimal variation of sample.On examination of the drug content of prepared formulation showed that the method used to prepare patches was capable of supplying consistent drug content, with minimal variation of sample.

**Mortality& Morbidity**

Mortality and morbidity were assessed at least twice in day (morning and evening). All the animals from different groups were good in the health condition.

**Body Weight**

Before MIA induction there is no significant reduction in body weight in all groups in 0 day (156.4g±0.6). There was reduction in body weight after MIA induction in all groups. Particularly in week 4 there was complete recovery in the G5 as compare to Negative control ($p<0.001$).

On 7th and 14th day, there was statistically significant increase in body weight in low dose (260 mg/kg/day) when compared to all groups (147.6±0.2 & 146.4±0.3). On 21st day, there was significant increase in body weight in mid dose ($p<0.001$) (520 mg/kg/day) when compared to all groups. On 28th and 30th day all treatment doses were found significantly effective in terms of increase in body weight.

Hence, the significant increase in body weight was observed in all treatment groups during the treatment period (week 1 to week 4) when compared to reference control and negative control.

**Feed intake**

The food consumption (g/rat/day) data of male treatment groups were observed to be comparable ($p<0.001$) with the vehicle control group throughout the experimental period.

From the results, it can be observed that before MIA induction there is no significant reduction in feed intake in all groups on 0 day. There was reduction in feed intake after MIA induction in all groups. Particularly in week 4 there was complete recovery in terms of increase in feed intake in all treatment groups.

Hence, The Significant difference in feed intake was observed in G4 group from week 0 day to $30^{th}$ day. The feed intake changes of treatment group (Test dose- mid and high) animals were observed to be significantly higher as compared to control group and reference group during $7^{th}$ day to $28^{th}$ day ($p<0.001$).

**Water intake**

From the results,it can be observed that before MIA induction there is no significant reduction in water intake in all groups in 0 day. There was reduction in water intake after MIA induction in all groups. On $14^{th}$ day there was a significant difference in the water intake of the reference control, treatment group- low and treatment group- high. Hence, The Significant increase in water intake was observed in reference control, treatment group- low and treatment group- high on $14^{th}$ day($p<0.001$).

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

**Heat hyperalgesia**

Heat hyperlagesia was done to observe the pain in the legs of animal. On the day 0 the paw withdrawal latency was similar in all the groups (G1-G5).

Results showed the pain was not observed on 7th, 14th, 21th and 28th day in the negative control animals.

On $7^{th}$ day, there was significant increase in paw withdrawal latency in reference control, test dose- mid, test dose- high. On $14^{th}$ day, there was significant increase($p<0.001$) in paw withdrawal latency in all groups when compared with negative control. On $21^{st}$ day, there was significant increase in paw withdrawal latency in reference control (4.0±0.4), test dose-low and test dose-high when compared with control group. On $28^{th}$ day, there was significant increase in paw withdrawal latency in all groups when compared with negative control.

**Cold hypersensitivity**

Heat hyperlagesia was done to observe the pain in the legs of animal. On the day 0 the paw withdrawal latency was similar in all the groups (G1-G5).

Results showed the pain was not observed on 7th, 14th, 21th and 28th day in the negative control animals.

On $7^{th}$ day, there was significant increase in cold hypersensitivity in reference control group, test dose-mid and test dose-high. On $14^{th}$, $21^{st}$ and $28^{th}$day there was significant increase in cold hypersensitivity in all groups when compared with negative control ($p<0.001$).

**Knee thickness**

Knee diameter was taken on day 0 (before drug and MIA injection), $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day during the period of the treatment.

On $7^{th}$ day, there was significant decrease in knee diameter in test dose- low as compared to reference control and other treatment groups. Similarly on $14^{th}$ and $21^{st}$ day, there was significant decrease in knee diameter in test dose- low and control.

Data observed on these time point of observation did not reveal any significant changes in all groups except test dose- low and control($p<0.001$).

**Histopathology Result**

**Normal control group**

Tissues from this group revealed normal histology of synovial membrane, bone and articular cartilage with no signs of arthritis and associated lesions. Only one incidence of Minimal degree of inflammatory infiltrate was found in synovial membrane.

**Reference drug treatment group**

Synovial membrane cells showed minimal degree of hyperplasia (Incidence, 03/08), and Minimal to mild degree of inflammatory infiltrate in synovial membrane (Incidence, 05/08). Minimal to mild degree of cartilage erosion (Incidence, 03/08) and bone erosion (Incidence, 04/08), was present. Minimal to mild degree of Proliferation of synovium (Incidence, 03/08) was also present.

ISSN:0975-3583,0976-2833 VOL12, ISSUE06,2021

**Low dose drug treatment group**

Tissues from this group revealed mild to marked degree of severity of Synovial membrane hyperplasia(Incidence, 07/08) and Minimal to marked degree of inflammatory infiltrate in synovial membrane (Incidence, 08/08). Mild to marked degree of severity of cartilage and bone erosion was present (Incidence, 07/08). Minimal to mild degree of proliferation of synovium (Incidence, 06/08) was also found in these group animals.

**Mid dose drug treatment group**

Tissues from this group revealed minimal to mild degree of severity of synovial membrane hyperplasia (Incidence, 06/08), minimal to mild degree of inflammatory infiltrates in synovial membrane (Incidence, 07/08). Mild degree of severity of cartilage and bone erosion (Incidence, 05/08), and Proliferation of synovium (Incidence, 05/08) was also present.

**High dose drug treatment group**

Synovial membrane cells showed minimal degree of hyperplasia (Incidence, 03/08). Minimal to mild degree of inflammatory infiltrate in synovial membrane (Incidence, 05/08) was also found. Minimal to mild degree of cartilage and bone erosion was present (Incidence, 04/08). Minimal to mild degree of Proliferation of synovium was also present (Incidence, 03/08).

## CONCLUSION

Under the conditions of the study, following conclusions were made:

- The test item at treated dose levels of 260, 520 and 725 mg/kg/day did not exhibit any mortality or untoward clinical signs.
- The test item at the treated dose levels did not produce any treatment related effect in behavioural observations, body weight, body weight changes and food consumption.
- Treatment groups i.e. mid dose and High dose (520 and 725 mg/kg/day) were found statistically significant in different time points of observations i.e. Heat hyperalgesia, cold hyper sensitivity.
- Histopathology analysis revealed the mid dose of the test item (520 mg/kg/day) and high dose of the test item (725 mg/kg/day) was more effective as compared to low dose of test item (260 mg/kg/day).

**Discussion**

This experiment was performed to see the efficacy of the test item. The arthritis was induced by the single injection of the monoidoaceteate and at day the of $7^{th}$ the arthritis induced. OA like was induced by the monoidoaceteate and assessed by the different parameter such as cold hypersensitivity, heat hyperalgesia, loco motion activity by the rotarod, and knee diameter.

The protocol was performed to induce the constatnt pain thought the experiment. And, severity of the disease also was assessed through body weigh changes, mortality, and morbidity. The rapid progression of the disease allowed the access to the find out the pathogenesis as well as progression and treatment.

The experiment was performed on the Wistar rat, age 5-6 weeks old animals. The animals were healthy during initiation of the study. The MIA injection was given to animal intra-

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articluar. On 7th day the symptoms raised, this assessment was done by the parameters such as cold hypersensitivity, heat hyperalgesia, loco motion activity by the rotarod, and knee diameter**.** These parameters were taken on the 0th day and 7th day and all the results were compared. The result showed significant reduction in locomotion activity, cold and heat hyperalgesia.

Result showed that 14th day and 28th day were the critical days for the test and reference controlled animals. The recovery was started after the 14th day for the animals of G2 and G5. G3 was not up to the mark as it had given the lowest dose of the drug.

The animals started showing the positive results during the recovery period after 14th day. The knee diameter also was significantly increased after injection of the MIA that proved the vulnerability of the MIA. The knee diameter was increased in all the groups except negative control; however G1 also administered the normal saline.

Hispathology was done for all the animals, G2 and G5 were shown recovery, and G3 & G4 was shown the least recovery among the entire groups.

G3 and G4 showed the less improvement in the knee diameter recovery. Overall, the enthanolic extract of the Aeglemarmelos leaves showed better performance during the experiment. The doeses of 520 and 725 mg/kg/day showed improved performance.

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