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PRECLINICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *SIDA SPINOSA* FOR ITS ANTI-ARTHRITIC ACTIVITY IN MICE

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ABSTRACT: The present study aimed to evaluate the effect of hydroalcoholic extract of *Sida spinosa* L. (HYSS) in antigen induced rheumatoid arthritis in mice by intra articular administration of Bovine serum. Rheumatoid arthritis was induced by the mixture of antigen (Bovine serum albumin) and adjuvant (Imject alum) in 1:1 ratio on 1st, 7th and 21st day as an induction period. The mixture of antigen and adjuvant caused the joint destruction, chronic inflammation of synovium, pannus formation, erosion of bones and cartilage in mice. The HYSS administered at three different concentrations 100, 200 and 400 mg/kg,p.o. as test and Indomethacin (2 mg/kg, p.o) as reference standard for next 20 days as a treatment period in arthritis induced mice. The study was conducted for total 41 consecutive days as initial 21 days of induction and next 20 days as treatment period. Progression of arthritis in different group of animals was evaluated by biochemical parameters, histological changes, antioxidant activity and radiographic analysis in overall joint structure. HYSS at 200 and 400 mg/kg significantly recovered the Serum TNF-α level, NF-kβ, MPO activity, Radiographic Analysis (X-ray) as compared to antigen induced rheumatoid arthritis control group. Results of present study indicates the anti-arthritic potential of HYSS to heal antigen induced rheumatoid arthritis in mice. It can be concluded from the present study that HYSS at doses 200 and 400 mg/kg has the potential as anti-arthritic activity.

INTRODUCTION: Arthritis is referred as joint inflammation and pain from a variety of sources. More than 100 rheumatic diseases and ailments that affect joints, the tissues around the joints, and other connective tissue showing pain, swelling and stiffness as common indications and symptoms. Internal organs may also be affected by some rheumatic disorders. 15% of Indians have arthritis (about 180 million people) [1, 2].

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Although there is presently no cure for RA, Disease-Modifying Anti-Rheumatic Medications (DMARDs) can help to lessen systemic and local inflammation. Reduced inflammation improves discomfort, delays joint structural deterioration, and maintains function. Other therapies include simple analgesics and non-steroidal anti-inflammatory medications, which just alleviate pain and do not stop RA from eroding the patient's joints [3].

In individuals with active RA, low-dose oral glucocorticoids and local glucocorticoid injections are very effective at reducing symptoms, and continued treatment may have disease-modifying effects [4]. These medications also have side effects

such as hepatotoxicity, blood dyscrasias, and interstitial lung disease (serious) and nausea (minor) (DMARDS) [5], gastrointestinal ulcer, hemorrhage, perforation (NSAIDS), cataracts, hypertension, osteoporosis, hyperlipidemia (Corticosteroids) [6].

While the allopathic management of RA is getting better, remissions are still uncommon, and the management is still inadequate. As a result, research for efficient supplementary and alternative treatments for this illness is ongoing [7].

Looking forward to this concern, the present study was planned. The *Sida spinosa* plant is reported to have immuno-modulatory effect in Inflammatory Bowel Disease (IBD), hence to evaluate further its potential in another autoimmune disease like rheumatoid arthritis using hydroalcoholic extract. The hydroalcoholic extract was taken because it showed presence of more Phyto-constituents [8].

The most frequent type is osteoarthritis (OA), also known as degenerative joint disease, which is followed by rheumatoid arthritis (RA), gouty arthritis (GA), pseudo gout, also known as calcium pyrophosphate deposition disease (CPPD), psoriatic arthritis (PA), infectious arthritis (IA), spondylo-arthritis (SA), and other autoimmune diseases [9].

Rheumatoid arthritis (RA) is characterized by ongoing joint discomfort and inflammation, results in joint degeneration, disability, and a poor quality of life at significant societal and medical expense. Between 0.5 and 1% of adults in affluent nations have RA, and because of its long-term course, its prevalence increases with age [10]. In America, 1.3 million people suffer with rheumatoid arthritis. In this condition, the immune system of the body attacks the joint tissue, leading to inflammation. Bone and cartilage may be destroyed as a result [1].

The extracellular matrix (ECM), which is rich in collagen and proteoglycans, degenerates in OA because of dysregulation brought on by the presence of different bio factors. This degeneration leads to articular surface erosion, fibrillation, matrix calcification, and vascular invasion. OA is the leading cause of disability worldwide with a current prevalence of 15% and a projected increase to 35% by 2030. The rise in the number of obese and elderly people is most likely the cause of the increased prevalence of OA, even though the exact reason is yet unknown [11].

Different Risk Factors Associated with Rheumatoid Arthritis: As RA is heritable, there is a significant amount of disease that may be caused by risk factors from the environment. It is generally agreed upon that deeper knowledge of the mechanisms behind illness development and progression requires an understanding of the intricate interactions between genetics and environment, as well as the roles they play in pathogenesis. The different risk factors involved in rheumatoid arthritis are age, sex, family history and smoking [12].

MATERIALS AND METHODS:

Materials: Imject alum was purchased from Thermo Fischer Scientific Inc. and Bovine serum albumin was purchased from Sigma Aldrich, Bangalore, India. Indomethacin was purchased from a local pharmacy store. All other chemicals were of analytical grade purchased from local suppliers.

According to the literature survey, the plant *Sida spinosa* was selected for the current research work. The aerial part of the *Sida spinosa* Linn. were procured from regions of Tirupati, Andhra Pradesh, and authenticated by botanist, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India.

Methods:

Preparation of Plant Extracts: The plant material was shade-dried and ground into a powder. A solvent hydroalcoholic mixture (70:30) was used to macerate the powder material (500g) for 72 hours at room temperature. Filtration was used to remove the residue, and the solvent was then evaporated at 42–45 °C in a rotary evaporator while under decreased pressure. The concentrated extract was kept in petri dishes and dried in a vacuum oven at 40 °C. Before drying completely, the solid extract was scraped, and it was then dried to a consistent weight. 20% of the extract was yielded, and it was stored in an airtight container until usage [8].

Preliminary Phytochemical Analysis of Extracts: The presence of different classes of phytoconstituents was also recorded by preliminary

phytochemical analysis of HYSS by using standard procedures [13].

Animals: Swiss albino mice of either sex weighing between 20-25 gms were used for the study. The Swiss albino mice procured from Bharat Serum and Vaccines, Narayangaon, Pune and Wistar rats procured from National Institute of Biosciences (NIB), Pune housed at animal house of Dr. D. Y. Patil College of Pharmacy, Pune in polypropylene cages at 25±2 ℃ temperature with 60% relative humidity and kept under 12:12 light -dark cycles. They were fed with standard pellet diet (Nutrivet life science, Pune) and water *ad libitum*. The mice were allowed to acclimatize to laboratory conditions prior to experimentation. All the procedures were carried out in daylight period. The experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC) (DYPCOP/IAEC/2022/05) and proper care of animals was taken as per guidelines of the Committee for the Control and Supervision of Experimentation on Animals (CPCSEA).

Acute Oral Toxicity of Extracts: Using OECD Guideline No. 423, the acute toxicity investigation for hydroalcoholic extract (HYSS) was carried out in mice. 2000 mg/kg dose was given orally to first animal and observed for 14 days. After 14 days, no mortality was found. Therefore, next two animals were taken and no mortality was observed. The same procedure was repeated for three animals. The dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg dose were selected for further study [14].

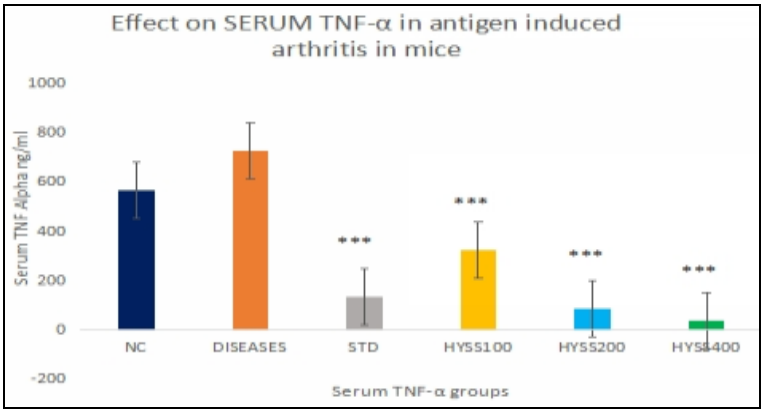
Induction of Rheumatoid Arthritis and Drug Treatment Schedule:

Antigen Induced Rheumatoid Arthritis (RIA): Rheumatoid Arthritis was induced as per the procedure described by Qasim [15]. The groups were divided into six each containing six animals. Group 1 (Normal control) received distilled water (10 ml/kg BW, p.o.) from day 1 to day 41. Group 2 (Induction control) received mBSA mixed with Imject alum (o.1 mg/0.1ml BW,s.c.) on 1st, 7th and 21st day. Group 3 (Reference standard) received Received mBSA+Imject Alum (o.1 mg/0.1ml BW, S.C. and I.A.) on 1st, 7th and 21st day and Indomethacin (2 mg/kg, p.o.) from day 21 to day 41 as a standard drug. Group 4 (Treatment group) received mBSA+Imject Alum (o.1 mg/0.1ml BW,s.c. and I.A.) on 1st,7th and 21st day and HYSS-100mg/kg (p.o.) From day 21 to day 41. Group 5 (Treatment group) received mBSA+Imject Alum (o.1 mg/0.1ml BW,s.c. and I.A.) on 1st,7th and 21st day and HYSS-200mg/kg (p.o.) From day 21 to day 41. Group 6 (Treatment group) received mBSA+Imject Alum (o.1 mg/0.1ml BW,s.c. and I.A.) on 1st,7th and 21st day and HYSS-400mg/kg (p.o.) From day 21 to day 41.

The parameters evaluated were Serum TNF-α level, NF-kβ, MPO activity, Histopathological Evaluation, Radiographic Analysis.

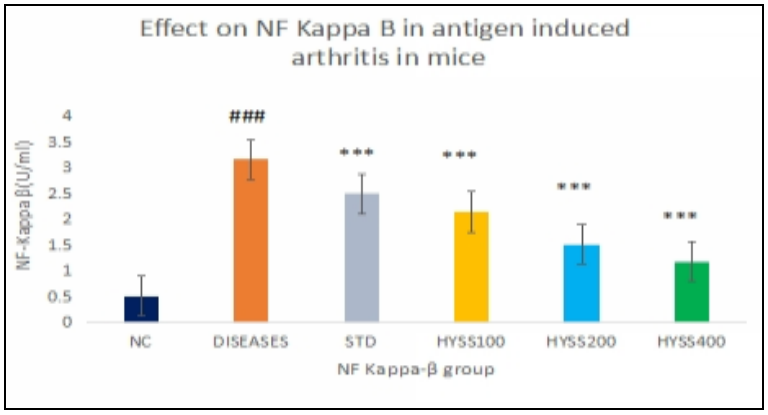
RESULT: Value are expressed as mean ± SEM; n=6; Data analyzed by one-way ANOVA test followed by Tukey multiple comparison test. Level of significance ### P< 0.01, *** P< 0.01.

Effect on SERUM TNF-α in antigen Induced Rheumatoid Arthritis in Mice: Serum TNF-α was found significantly high (p<0.001) in induction group. All treatment groups (100, 200 and 400 mg/kg) significantly lowered (p<0.001) TNF-α as compared to induction group.



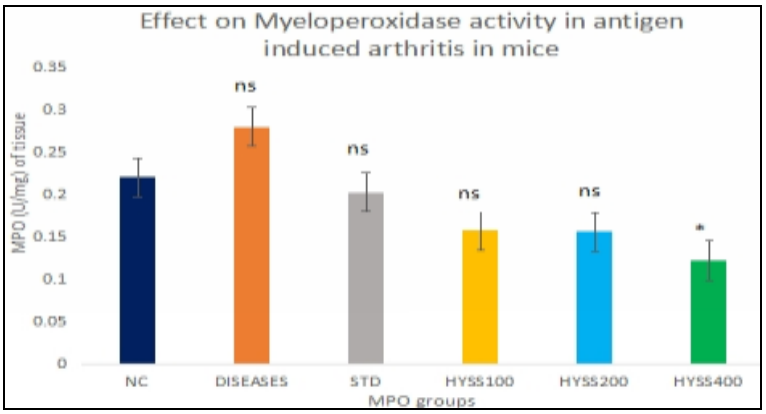
GRAPH 1: EFFECT ON SERUM TNF-A IN ANTIGEN INDUCED RHEUMATOID ARTHRITIS IN MICE

Effect on NF Kappa B in antigen induced Rheumatoid Arthritis in mice: NF Kappa B was found significantly high (p<0.001) in induction group. All treatment groups (100, 200 and 400 mg/kg) significantly lowered (p<0.001) NF-kβ as compared to induction group.



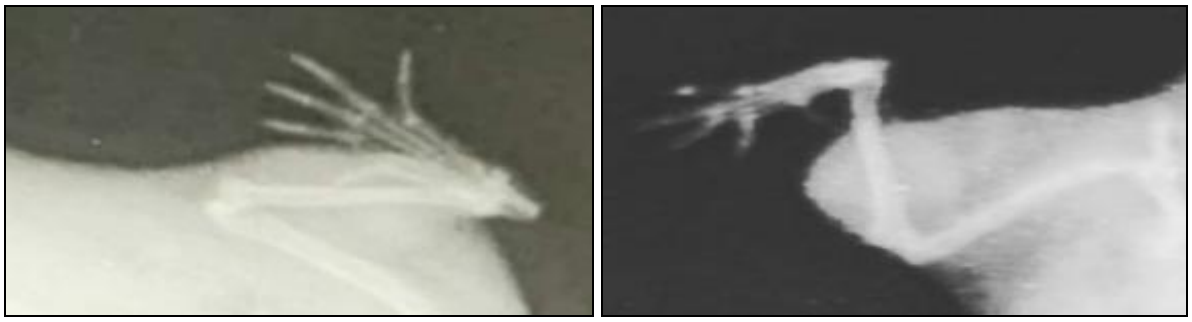
GRAPH 2: EFFECT ON NF-KB IN ANTIGEN-INDUCED RHEUMATOID ARTHRITIS IN MICE

Effect on Myeloperoxidase Activity in Antigen Induced Rheumatoid Arthritis in Mice: Myeloperoxidase activity (MPO) was found significantly high (p<0.001) in induction group. In treatment group, 400 mg/kg significantly lowered (p<0.05) as compared to 100 and 200 mg/kg.

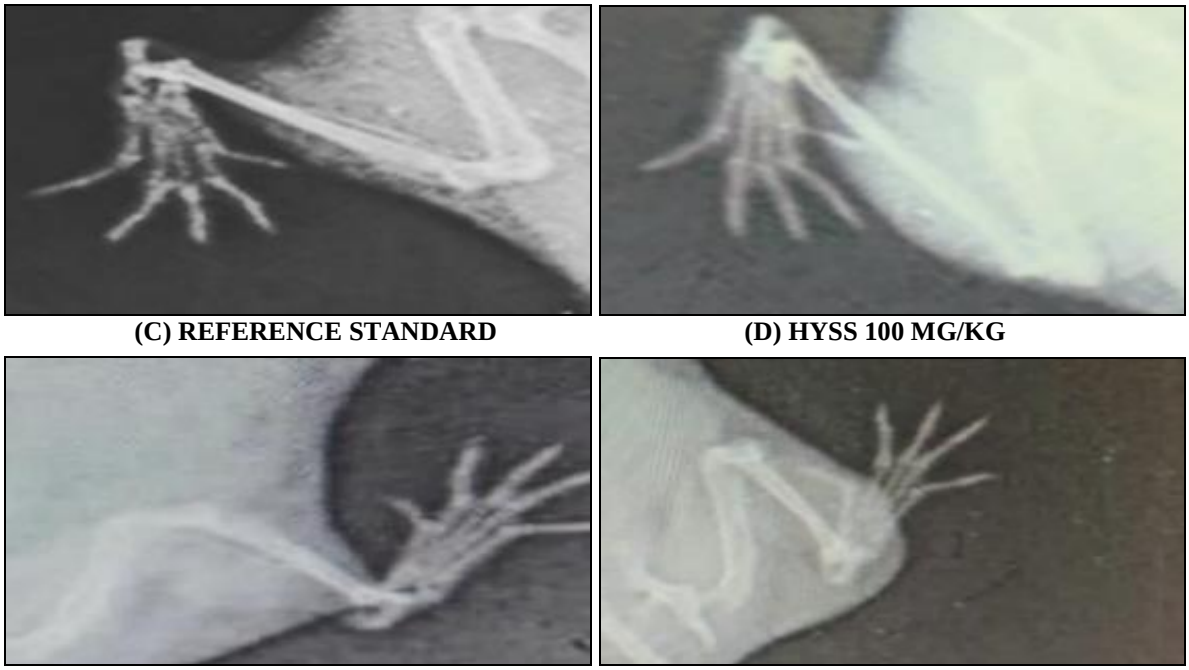


GRAPH 3: EFFECT ON MYELOPEROXIDASE ACTIVITY IN ANTIGEN INDUCED RHEUMATOID ARTHRITIS IN MICE

Radiographic Analysis: For arthritic joints to confirm degenerative changes, a radiographic scan is necessary [16]. It's general knowledge that arthritis is accompanied by bone and cartilage degradation, which results in the fusing of bones and a reduction in joint space [17, 18]. The radio graphic evaluation of the ankle joint in the current study showed loss of joint spaces as a result of its fusion in a sick state. In arthritic conditions, macrophage recruitment at the cartilage junctions was seen [19], and it has now been discovered that these cells release a variety of cytokines that further aid in the breakdown of bone and the recruitment of osteoclast precursors [20].



(A) NORMAL CONTROL **(B) INDUCTION CONTROL**



(C) REFERENCE STANDARD **(D) HYSS 100 MG/KG**

(E) HYSS 200 MG/KG **(F) HYSS 400 MG/KG**

FIG. 1: RESULT OF RADIOGRAPHIC ANALYSIS

Radiographic study of diseased animals was done in order to assess the disease's state and remission in the presence of HYSS. Additionally, it was discovered that the HYSS at dose 400 mg/kg successfully decreased bone fusion and bone loss, which resulted in the reduction of joint gaps in the arthritic animals as seen by the radio graphs as compared to HYSS doses at 100 and 200 mg/kg.

Histopathological Results: RA is prominently morphologically and pathologically characterized by histopathology [21].

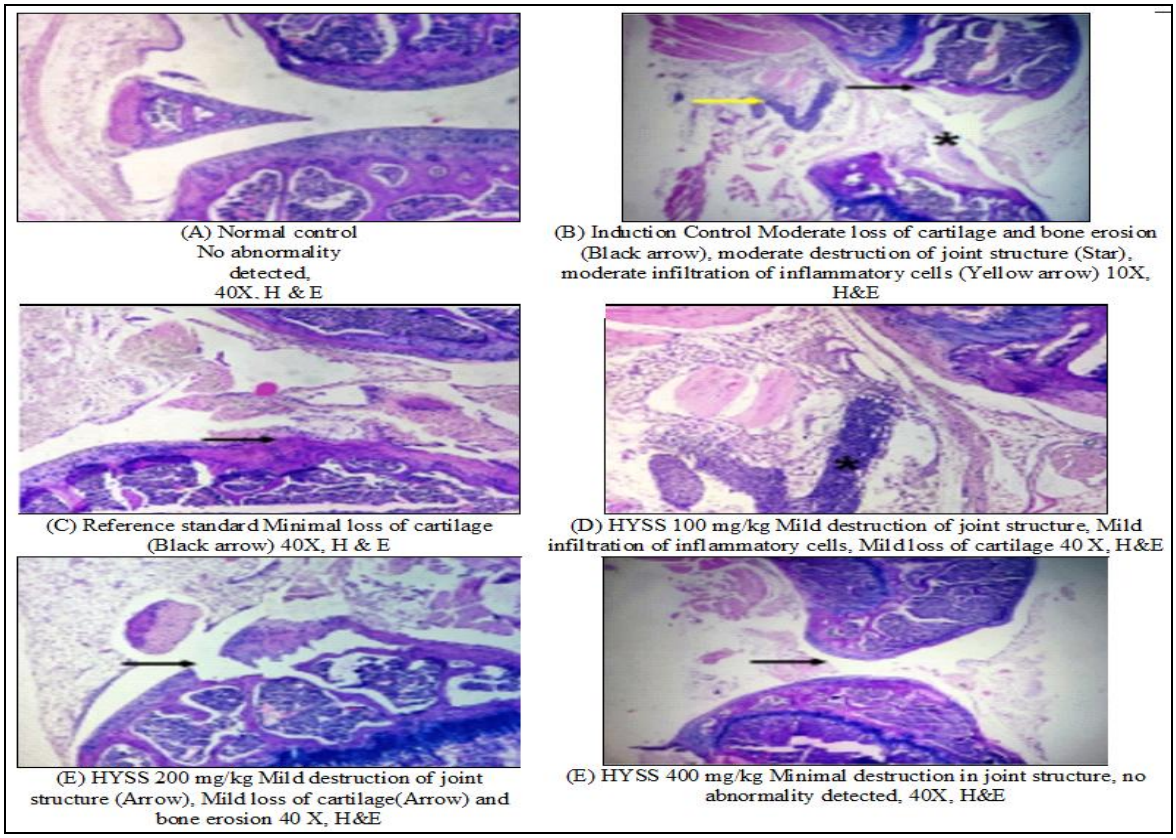


FIG. 2: HISTOPATHOLOGICAL RESULT

The effects of HYSS on synovitis within joints were suppressed in comparison to antigen-induced arthritic control animals, and this was further supported by histological analysis. The phytochemical screening includes extraction of the whole parts of *Sida spinosa* powder with 60% water and 40% alcohol by employing maceration technique. The screening revealed the presence of most the active phytoconstituents in the extract like Alkaloids, glycosides, flavanoids, tannins and phenols, saponins, terpenoids, carbohydrates, polyphenols and proteins. Three different doses of the hydroalcoholic extract of *Sida spinosa* (HYSS) at 100 mg, 200 mg, and 400 mg/kg body weight/day were given orally to arthritic mice induced with Bovine Serum Albumin in Imject Alum for 21 days. Changes biochemical parameters, and inflammatory by using real-time qPCR, the expression of genes linked to inflammation, such as TNF- α and NF-kβ, were also investigated.

Numerous studies have shown that a wide range of effector molecules, including pro-inflammatory cytokines (including IL-6, TNF-α), chemokines, and metabolic proteins, mediate chronic inflammatory diseases. According to Muller-Ladner [22], Luster [23] and Esch [24], these interact with one another in a complex way that is thought to set off a vicious cycle of pro-inflammatory signals that leads to chronic and persistent inflammation. Nuclear factors, such as nuclear factor kappa B (NF-kβ), are now known to regulate the genes that encode TNF-αand many of the other factors listed above [20], suggesting that NF-kβ may be one of the master regulators of pro-inflammatory cytokines and other mediators of inflammation in chronic inflammatory conditions like arthritis.

Mentioned all the facts, serum levels of TNF-α and IL-6 were determined in the present work to evaluate the intensity of RA. The observations revealed the remarkable increased levels of these parameters ($p<0.001$) in the serum from antigen induced arthritis mice, showing severe inflammation induced by antigen mBSA with imject alum. As shown in Graph No- 1 and 2, there was significant decrease ($p<0.001$) in serum level TNF-α, NF-kβ occurred by oral administration of HYSS 400 mg/kg than other two doses i.e. 100 and 200 mg/kg as compared to antigen induced arthritis mice. Myeloperoxidase (MPO) is implicated in many chronic inflammatory illnesses as a local mediator of tissue destruction when secreted extracellularly. MPO has been linked to the development of RA as a local mediator of joint injury. Activated neutrophils in RA release MPO into the synovial fluid, where elevated levels of enzymatically active MPO are associated with the presence of HOCI-modified proteins [25]. Furthermore, mice's inflamed joints had higher levels of active MPO. In present study, as shown in Graph No- 3, there is significant elevation in (($p<0.001$) of MPO in antigen induced arthritis mice as compared to normal control group mice. Treatment of antigen induced arthritis mice with HYSS 400 mg/kg showed significant reduction (($p<0.001$) in MPO level. Radiographic study of arthritic animals was done to assess the disease's condition and whether it has subsided in the presence of the HYSS extract.

As shown in Figure No.01 it was observed that, the HYSS extract significantly decreased bone fusion and bone loss in the arthritic animals, which was shown by radiographs to be a reduction of joint spaces. The suppressive effects of HYSS (400 mg/ kg) on synovitis inside joints in comparison to antigen-induced arthritic control mice were further validated by histological investigation as shown in **Fig. 2.**

CONCLUSION: The purpose of this study was to examine the pre-clinical evaluation of Hydroalcoholic extract of *Sida spinosa* for its anti-arthritic activity on antigen induced arthritis in mice. The rationale behind this report was to demonstrate the traditional claim of the anti-arthritic potential of *Sida spinosa.* From the review of literature of the plant, various activities associated with the plant with its different parts, brief discussion on arthritis disease and profile of the plant *Sida spinosa.* In the present study, we have carried out the pre-clinical evaluation of hydroalcoholic extract mainly due to it showed presence of most of the active Phyto constituents. Mice treated with hydroalcoholic extract of *Sida spinosa* (HYSS) at high dose 400 mg/kg body weight displayed marked reduction in biochemical parameters and inflammation. Gross pathological findings showed that, in comparison to the positive control drug Indomethacin, HYSS at high dose of

400 mg/kg body weight significantly reduced the synovitis and infiltration of inflammatory cells whereas, moderate reduction was observed with low dose (100 and 200 mg/kg body weight) of HYSS. It can be concluded from the study that the HYSS extract of whole plant of *Sida spinosa* has potential as anti-arthritic due to the presence of flavonoids and glycosides mainly.

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CONFLICTS OF INTEREST: The Authors declare no Conflicts of Interest.

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