IJPSR (2021), Volume 12, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 August 2020; received in revised form, 01 February 2021; accepted, 11 February 2021; published 01 August 2021

ASSESSMENT OF *POUZOLZIA ZEYLANICA* ROLE ON BIOLOGICAL MARKERS IN ARTHRITIS INDUCED RATS

Dwibyendu Chutia[1], Dev Jyoti Kalita [*1, 2], Kangkan Deka [1] and Bibhuti B. Kakoti [1]

Department of Pharmaceutical Sciences [1], Dibrugarh University, Dibrugarh – 786004, Assam, India.
Department of Medicine [2], Assam Medical College and Hospital, Dibrugarh – 786002, Assam, India.

Keywords:

Pouzolzia Zeylanica (L.) Benn., Rheumatoid arthritis, Arthritic score, Rheumatoid factor, Radiographic score, Histopathology

Correspondence to Author:
Mr. Dev Jyoti Kalita

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh – 786004, Assam, India.

E-mail: devkalita@gmail.com

ABSTRACT: *Pouzolzia zeylanica* (L.) Benn. is a medicinal plant widely found in the Asian continent, including the North Eastern (NE) region of India. The plant is being used for its various medicinal properties against wide range of disease conditions as folk medicine in Assam and various places. A research was designed to carry out an evaluation of anti-arthritic activity of the leaves of *Pouzolzia zeylanica* (L.) Benn. with special reference to its protective effect against arthritis-induced different pathological manifestations. Anti-arthritic activity of methanol extract of *Pouzolzia Zeylanica* (L.) Benn. leaf was investigated in two dose levels and further various studies were carried out to ascertain the degree of reversing the arthritic manifestations induced by formalin. LD_{50} values of the extract were found to be safe up to 2000 mg. The in vivo biological studies on male Wister rats at the doses of 250 mg and 500 mg/kg body weight respectively was carried out taking Aceclofenac as standard. The methanol extract of *Pouzolzia zeylanica* (L.) Benn. leaf, significantly improved the arthritic parameters such as arthritic index, paw volume, various hematological and biochemical parameters along with spleen index and radiographic score in respect to Aceclofenac group. The test extract at the dose 500 mg/kg body weight was found to be more effective than 250 mg/Kg body weight. The radiological and his to pathological studies further augment the protective effect of *Pouzolzia zeylanica* (L.) Benn. Leaf extract against arthritis induces tissue damages to extract thereby validates the anti-arthritic effect of the plant.

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease of joints mainly affecting synovial tissue. The inflammatory reactions are triggered by immunologically mediated responses of unknown mechanisms [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(8).4541-48

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(8).4541-48

Joint inflammation and pain, articular tissue erosion, restriction of joint movements are some of the major clinical features of the disease.

As the disease progresses, RA causes disability, other morbidities and affects the quality of life to a great extent [2]. Globally, RA affects about 0.5% of the adult population [3]. Non-steroidal anti inflammatory drugs (NSAID), disease-modifying anti-rheumatoid drugs (DMARD), steroids and physiotherapy are the commonly used therapeutic modalities in RA. The chronic use of these agents is associated with various adverse reactions like

gastrointestinal complications, renal toxicity and immune suppression, *etc.*, which further increases more clinical interventions [4, 5, 6]. Thus, medicinal herbs draw the scientific ccommunity to explore and develop agents to use in chronic inflammatory diseases with less toxicity and for long-term use. As medicinal plants are gaining more popularity globally, scientific validation of indigenous herbs plays an important role in the development of new therapeutic agents.

Pouzolzia zeylanica (L.) Benn. is an perennial herb belonging to the family of Urticaceae, commonly known as *Graceful pouzolzbush*. It is widely distributed in Asian and Australian regions and mostly grows in monsoon season. The plant is used in various folkloric medicinal preparations. Leaf decoction is used as anthelmintic [7]. Shoots are applied as a poultice in skin diseases [8]. Leaf and stems are used in dysentery, stomach pain, gangrenous ulcers, syphilis, and gonorrhea, as galactagogue [9]. The plant is also used in hematemesis and traumatic hemorrhage [10]. The presence of various types of phytoconstituents is reported in the plant, such as flavones, flavonoids, tannin, carotenoids, ascorbic acid [11]. Phenolic compounds such as L-epicatechin, celereoin, quercetin, kaempferol are identified, and antioxidant activity is reported [12, 13]. Therefore, the present study was carried out to explore in vivo anti-arthritic potential of *Pouzolzia zeylanica* (L.) Benn. leaf and assessment of biological parameters in Wistar rats.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The leaves of the plant *Puzolzia zeylanica* (L.) Benn. were collected from Lakhimpur district, Assam, India. The plant was identified by Dr. A.A. Mao, Botanical survey of India, Shillong vide letter No. BSI/ERC/Tech./Iden./2016/ Dt.25/07/2016. A voucher specimen of the plant sample was preserved in the department for future reference.

Preparation of Extract: The collected leaves of the plant were cleaned, shade dried, and grinded into coarse powder, and stored in an airtight container for further use. About 300 gms of powdered leaves of *Pouzolzia zeylanica* (L.) Benn was initially extracted with petroleum ether (40-60 °C) in a Soxhlet apparatus until the powder becomes completely exhausted. The defatted plant material was then extracted with methanol in a Soxhlet apparatus. The resulting methanol extract (MEPZ) was filtered, concentrated and evaporated to dryness under vacuum and used for the study.

Animals: Adult Wistar rats of either sex (90-120 gm) were used in the present experimental study. Protocol of the study was approved by the Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences, Dibrugarh University (Regd. No 1576/GO/ERe/S/11/CPCSEA Date: 30/03/2015). The rats were acclimatized to experimental conditions in polypropylene cages and housed under standard environmental conditions (22 ± 3 °C; 12/12 h light/dark cycle) and fed with a standard pellet diet and water *ad libitum*.

Acute Toxicity Study: Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals-425 was followed for evaluation of Oral acute toxicity of MPEZ [14]. Oral acute toxicity study was carried out on five rats by orally administering a single dose of MPEZ (2000 mg/kg body weight). The rats were then observed for 14 days for mortality and morbidity conditions if any.

Experimental Design for Anti-arthritic Activity: Thirty Wister rats are divided into 5 groups of 6 animals each. On the first day of the experiment, the basal paw volume of the left hind paw of each animal was measured using the Plethysmo meter. 0.1 ml of 2% v/v formaldehyde in normal saline was injected into the sub plantar region of the left hind paw of all the animals except group I (normal control). Group I and group II (disease control) received vehicles for 21 days. Group III (reference standard) receives Aceclofenac 10 mg/kg b.w./day for 21 days p.o. Group IV (MPEZ250) and Group V (MPEZ500) received the plant extract at a dose of 250 and 500 mg/Kg b.w./day for 21 days p.o. respectively. On the third day, again second dose of2% v/v formaldehyde (0.1 mL) was injected into the same paw of all the animals except group I [15, 16].

Assessment of Arthritis: Arthritis score: Arthritis score are the morphological features of arthritis-like swelling, redness, deformity and erythema, was monitored by the visual criteria as a rat of each

group were measured daily for arthritis score using macro scoping scoring as follows [17].

0 = Normal paw or no sign of arthritis or no swelling.

1 = mild swelling and redness in paw/ joint.

2 = Swelling and redness in paw/joints.

3 = Severe swelling and redness in the paw.

4 = deformity and inability to use the limb.

Paw Volume: Paw volume was measured on every alternate day from the beginning of day one when arthritis was first visible. The left paw volume was measured with digital plethysmometer on the day 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21. The change in volume of the affected paw was calculated by the difference between initial and final paw volumes. In before induction of arthritis and after induction of arthritis [15].

Hematological and Biochemical Parameters: On the 22nd day, blood was withdrawn through retro-orbital plexus puncture from all the rats under light ether anesthesia, and serum was separated for biochemical estimations.

The hematological parameters like hemoglobin content (Hb), red blood cell count (RBC), total white blood cell count (WBC), erythrocyte sedimentation rate (ESR) were measured by standard methods (Hematology cell counter). Estimation of rheumatoid factor (RF), serum alkaline phosphatase (ALP), serum Glutamate Pyruvate Transaminase (SGPT), serum Glutamate Oxaloacetate Transaminase (SGOT), total bilirubin (TB) and total protein (TP) was done using standard diagnostic kits with blood auto-analyzer.

Index of Immune Organ (Spleen Index): The rats were sacrificed by cervical dislocation on 22nd day, and the spleen was promptly excised and weighed. The spleen index (SI) is expressed as the percentage of spleen weight to the bodyweight of rats18.

Radiographic Analysis: Animals were sacrificed on the 22nd day, and leg were dissected out and placed on plastic bags containing formalin solution. This plastic bag were kept a distance of 90 cm from the X-ray source. The X-ray images were obtained with digital X-ray machine with 300-mA explosion for 0.01 s. Radiographs were examined with a stereomicroscope and abnormalities were graded as radiographic scores (RC) for bone necrosis. An investigator blinded for the treatment regimen performed the radiograph score as follows [19].

- Periosteaic reaction, 0 – 3 (None, Slight, Moderate, Many, Large)
- Erosion, 0 – 3 (None, Few, Small, Many, Moderate, Marked);
- Joint space narrowing, 0 – 3 (None, Minimal, Moderate, Marked,);
- Joint space Destruction, 0 – 3 (None, Minimal, Extensive, Ankylosis)

Histopathological Analysis: The animals were sacrificed on day 22 by cervical dislocation. Ankle joints were separated from the left hind paw and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioning and stained with haematoxylin and eosin dye.

The sections of the paw (5 μm thickness) were made into sagittal plane and joint articulations were examined under a light microscope for the presence of inflammatory cell infiltrations, synovium hyperplasia, pannus formation and destruction of joint space.

RESULTS AND DISCUSSION: Arthritis Score: The first manifestation of the disease was erythema of ankle joints was the first manifestation of disease followed by involvement of the metatarsal and interphalangeal joints. Sub plantar administration of the formaldehyde results in an insignificant increased ($P< 0.05$) in arthritis score in all formaldehyde-treated rats (DC) as compared to normal rats (arthritic score=0). Standard group rats treated with Aceclofenac (10 mg/kg) showed significantly decreased ($P<0.05$) arthritic score as compared to disease control (formaldehyde) rats. The rats treated with MPEZ (250 and 500 mg/kg) showed a significant and dose-dependent decrease in the arthritic score ($P < 0.05$) till the end of the experiment. A decrease in the arthritic index indicates decreased swelling, erythema and reversal of inflammatory responses. The results are shown in **Fig. 1**.

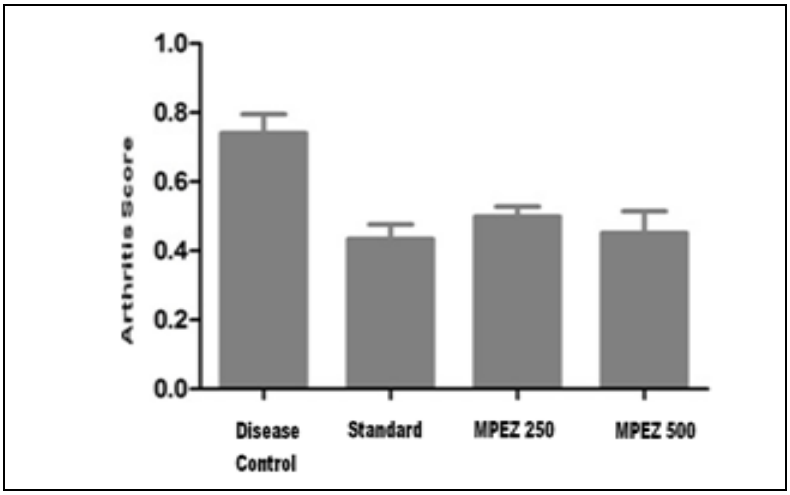

FIG. 1: EFFECT OF MPEZ ON ARTHRITIS SCORE OF RATS. Values are mean ± SD (n=6). P < 0.05 vs. Disease Control

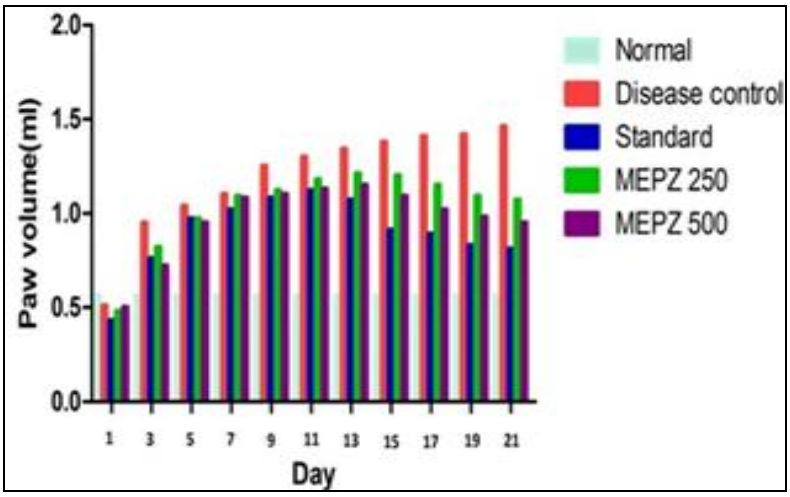

FIG. 2: EFFECT OF MPEZ ON PAW VOLUME OF RATS. Values are mean ± SD (n=6). p<0.05 vs Disease Control

Paw Volume: All the animals show a significant increase in paw volume from day one of the intra-plantar administration of formaldehyde except the normal control group. After the primary phase (day 0 to 11) of arthritis, from day 12 to 21, Aceclofenac (standard) and MPEZ treated rats show a significant decrease (P < 0.05) of paw volume as compared to the disease control group. The MPEZ (250 and 500 mg/kg) treated rats dose-dependent decrease in paw volume. This shows the MPEZ effectively inhibits the progression of arthritis in treated rats. The results are shown in **Fig. 2**.

Hematological and Biochemical Parameters: Marked changes in hematological parameters were observed with induction of arthritis. Hb and RBC is decreased in arthritic rats while there was an increase in WBC and ESR. Significant decrease in biochemical parameters *viz*, RF, ALP, SGOT, SGPT, TB and TP were observed in arthritic rats. Such marked changes in hematological and biochemical parameters were not observed in Aceclofenac, and MPEZ treated animals, as shown in **Table 1**.

After treating the test group in different dose levels, a significant reversal of elevated marker enzymes was observed. Arthritic conditions lead to changes in hematological and biochemical parameters. In RA, inflamed synovial tissues mediate various kinds of pro-inflammatory cytokines like TNF-α, IL-1, IL-6. These mediators cause functional disturbances in other organs and develop at herogenic changes, including pro-oxidative stress. Anaemia in chronic diseases like RA is immune-driven as circulating cytokines cause disbalances in iron homeostasis and the life span of RBCs, due to which a decrease in Hb and RBC is observed in disease control rats. An increase in WBC suggests stimulation of the immune system due to inflammatory responses, infection and physio-logical stress.

An increase in ESR indicates chronic inflammation and progression of RA in disease control rats. RF is a marker antibody found in blood, elevated in RA and autoimmune diseases. High level of RF antibody and other inflammatory cytokines also causes damage to body tissues, resulting in elevated serum markers like ALP, SGPT, SGOT, TB and TP.

In our study, treatment with MPEZ significantly controlled the levels of Hb, RBC, WBC, ESR, ALP, SGPT, SGOT, TB, TP and RF in a dose-dependent manner as compared to disease control and aceclofenac treated rats. Treatment with MPEZ re-establishes various hematological and biochemical parameters to normal levels in arthritic rats during treatment, as shown in **Table 1**.

TABLE 1: EFFECT OF MPEZ ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Parameters	Normal	Disease Control	Standard	MEPZ 250	MEPZ 500
Hb (gm/dl)	12.3±0.002	10.1±0.001	10.3±0.003*	12.3±0.002*	11.6±0.004*
RBC (millions/mm3)	6.5±0.001	4.8±0.002	6.78±0.001*	6.55±0.003*	6.56±0.001*
WBC (thousands/mm3)	6000±0.034	10200±0.054	7700±0.068*	9700±0.047*	8660±0.065*
ESR (mm/hr)	5±0.002	14±0.001	06±0.002*	10±0.003*	8±0.002*
RF (IU/ml)	11.42±0.42	35.60± 0.56	15.32±0.38**	27.20±0.36**	22.60±0.48**
ALP (U/L)	329±0.501	472±0.415	432±0.546**	338±0.364**	363±0.365**
SGOT (U/L)	40±0.210	120±0.541	65±0.213**	75±0.023**	70±0.035**
SGPT (U/L)	37±0.060	45±0.052	40±0.050**	40±0.086**	36±0.021**
TB (mg/dl)	1.2±0.005	1.9±0.061	1.8±0.021**	1.7±0.003**	1.2±0.021**
TP (mg/dl)	7.9±0.045	7.1±0.035	6.6±0.012**	6.5±0.010**	6.9±0.005**

Values were expressed Mean ± SD (n=6); *P < 0.01, **P < 0.05(*vs.* Disease control group).

Spleen Index: Spleen index (SI) is an indicator of the status of circulation and recruitment of inflammatory cells. Induction of arthritis led to a marked increase in SI when compared to normal rats arthritic rats treated with MPEZ significantly decrease in the SI value compared to the non-treated disease control group (P<0.05). as shown in **Table 2**. Spleen hyperplasia is a result of immune hyperfunction. In our study, findings suggest that the treatment with MPEZ (250 and 500 mg/kg) helps in the recovery of hyperfunctioning of immune organs without causing damage.

TABLE 2: EFFECT OF MPEZ ON SPLEEN INDEX AND RADIOLOGICAL SCORE

Parameters	Normal	Standard	Disease Control	MEPZ 250 MEPZ 500
SI	0.46±0.048	0.52 ± 0.038	0.42± 0.029*	0.32± 0.043
RC	5.24 ±0.16	9.82±0.34	.47±0.25** 7.85 ±0.34**	7.10 ±0.64**

Values were expressed Mean ± SD (n=6); *P < 0.05, **P < 0.01 (*vs.* Disease control group).

Radiographic Analysis: The radiographic score (RC) evaluation of the affected limb after 21 days is shown in **Table 2**. There is a marked / significant increase (P < 0.01) in RC in disease control rats as compared to normal group rats.

MPEZ (250 and 500 mg/KG) treated rats show a marked decrease in RC like Aceclofenac treated rats in dose dependent manner when compared with disease control rats. This reveals the inhibition of art herogenic progression by MPEZ. The radiographic images of various groups are shown in **Fig. 3**, the common clinical features of bone erosion and related abnormalities were analyzed with radiographs. A general clinical course in formaldehyde-induced models shows intertarsal joint space narrowing, diffuse edema of soft tissue include digits, diffuse bone demineralization, periostal thickening with abnormal ossification, and erosion and narrowing of joint spaces.

These features were prominently seen in images of diseases control rats. Inflammatory changes on the Tarso-Metatarsal joint due to arthritis are found partially healed in Aceclofenac and MPEZ treated groups.

Thus, the findings show that the rats treated with MPEZ like Aceclofenac treated rats attenuate abnormalities such as asymmetric soft tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws. MPEZ treatment shows the potential bone and synovial tissue-protective effects.

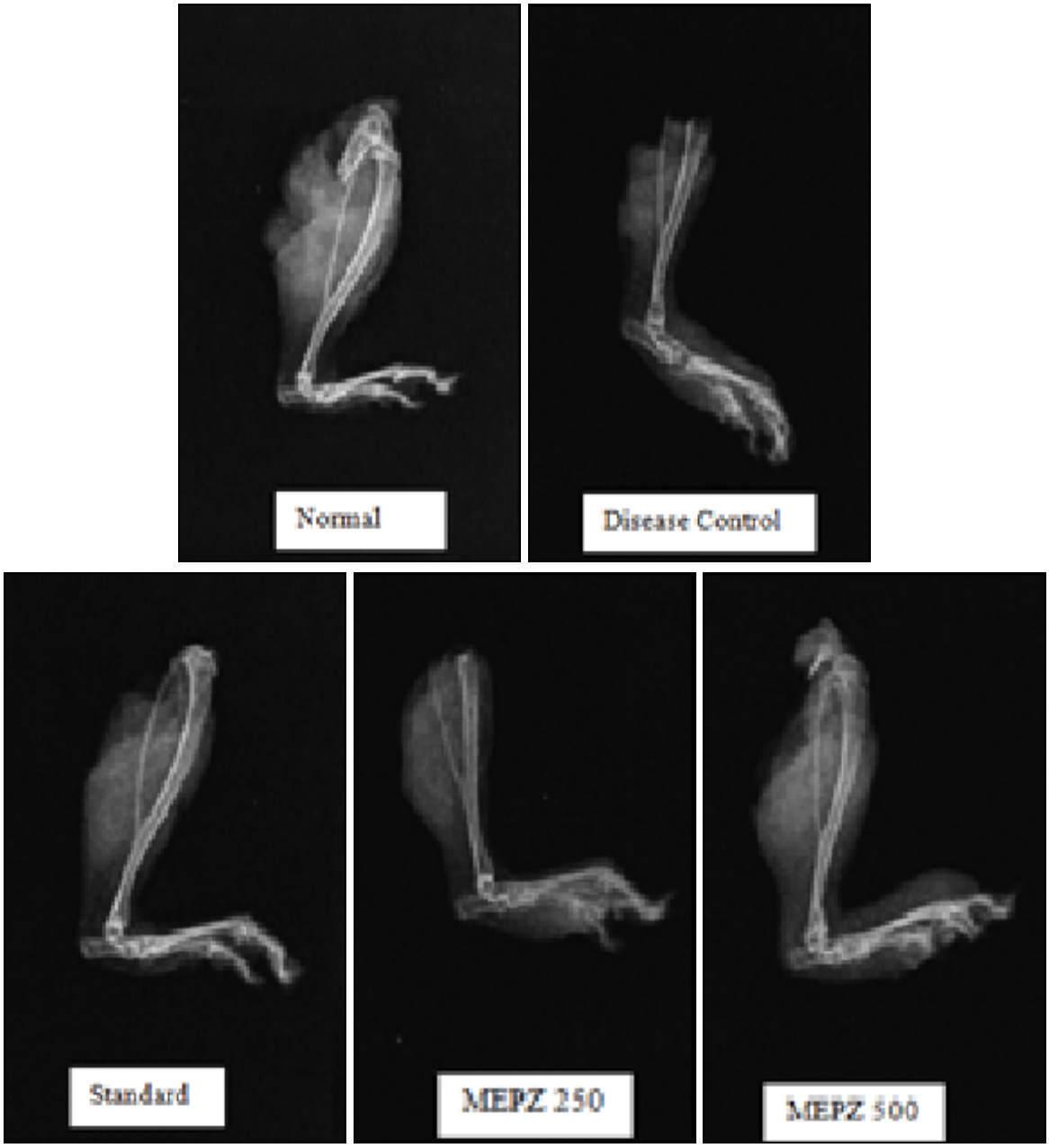

FIG. 3: RADIOGRAPHIC IMAGES SHOWING EFFECT OF MPEZ ON RATS

Histopathological analysis: The histopathology sections of the tibiotarsal joints are shown in **Fig.4**. Group, I showed normal tibiotarsal joints. Group II (Disease control) showed prominent histological abnormalities like edema formation, increased inflammatory infiltration with vasodilation, bone erosion, joints space damage, and destruction.

The standard drug and MPEZ (250 and 500 mg/kg) treated rats' joints showed normal joints and less cellular infiltrates and tissue injury. The overall drug-treated group showed anti-inflammatory activity. Inhibition of edema and cell infiltration during tissue inflammation is mainly due to a cyclooxygenase (COX) inhibition.

Anti-inflammatory agents like Aceclofenac cause inhibition of COX enzyme, and this feature is also observed in MPEZ treated rats. Degeneration of the joints was slightly observed in any of the drug-treated groups when compared with the disease control group.

Thus, the animals treated with MPEZ for the 21-day duration markedly reduced cellular infiltration and synovial tissue inflammation; also, healing of bone and cartilage was observed. These his to logical characteristics reveal the anti-inflammatory potential of MPEZ, which can be used in RA for protective effects against joint tissue destruction.

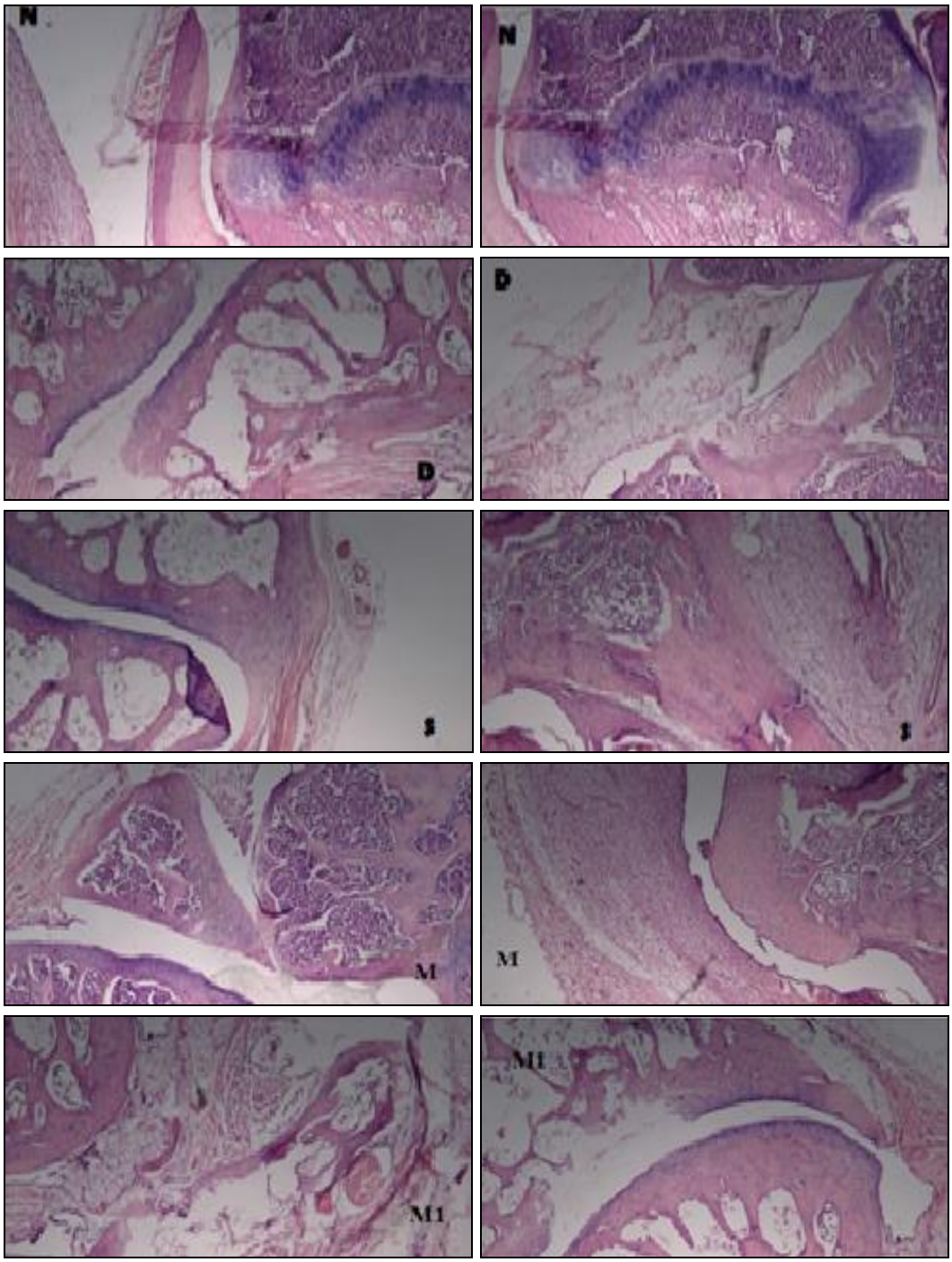

FIG. 4: PICTOMICROGHRAPH SECTIONS OF TIBIOTARSAL JOINTS SHOWING HISTOPATHOLOGICAL CHANGES IN RATS. N: NORMAL CONTROL; D: DISEASE CONTROL; S: STANDARD DRUG; M: MEPZ 250MG; M1: MEPZ 500MG

CONCLUSION: In our study, extract of *Pouzolzia zeylanica* (L.) Benn. leaf was found to have anti-arthritic activity and reverses various art herogenic biochemical and pathological abnormalities to normal state. Based on the positive result is obtained in the above study, we conclude that *Pouzolzia zeylanica* (L.) Benn. leaf has the potential to be used as an a disease-modifying agent in the treatment of RA and could be further explored for a safer alternative in the treatment of RA.

ACKNOWLEDGEMENT: The authors are grateful to the Department of Pharmaceutical Sciences, Dibrugarh University, for providing facilities to carry out the work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Chutia D, Kalita DJ, Deka K and Bibhuti BK: Assessment of *Pouzolzia zeylanica* role on biological markers in arthritis induced rats. Int J Pharm Sci & Res 2021; 12(8): 4541-48. doi: 10.13040/IJPSR.0975-8232.12(8).4541-48.