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Research Article

IN VIVO ANTIARTHRITIC ACTIVITY OF THE METHANOLIC AND N-HEXANE EXTRACTS OF LEAVES OF *PENTAS LANCEOLATA* (FORSSK.) DEFLERS IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS

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ABSTRACT

Arthritis is a chronic immobilizing, skeletal and muscular disorder having quite similar symptoms as that of rheumatoid arthritis (RA) for which currently there is no medicine available for permanent cure. All modern drugs provide symptomatic temporary relief, but produce severe side effects.***Pentas lanceolata***having some ethnomedicinal record to treat the symptoms of arthritis. So, this study has been aimed at the validation of its traditional claim about anti-arthritic efficacy. In present study the methanolic and n-hexane extracts of ***Pentas lanceolate***leaves were evaluated in **CFA** (Complete Freund's Adjuvant) induced arthritis in male wistar rats for assessment in oedema on **day 28**.CFA-induced inflammatory paw oedema, total arthritic scores, arthritic index, complete haematological & radiologicalparameters were all evaluatedfor the assessment of disease progression. The analysis of various arthritic assessment parameters used in this study revealedthat *P. lanceolata* extracts have a considerable effect in preventing development or

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amelioratearthritis disease severityin both normal and CFA-induced arthritis rats in adose-dependent manner.

Keywords: Arthritis,*Pentas lanceolata*, MEPL& HEPL (Methanolic& n-Hexane extracts of *P. lanceolata*), Indomethacin, Freund's complete adjuvant, Paw volume

INTRODUCTION

Arthritis is a broad term that covers more than hundred diseases. It may be related with joints, the site of bones connection, such as wrists, knees, hips, or fingers etc. Some types of arthritis can also affect some connective tissues and organs, including skin[1].There are some evidences suggest that abnormalities in components of the immune system that direct to the body developing and inflammatory reactions, particularly in jointsare the major causes of arthritis[2]. These features result in quick loss of muscle around an affected joint with the pain and swelling lead to loss of joint function. There are some more causes that can raise chances of getting arthritis like Age, gender, genes as well as injuries [3].Symptoms of arthritis includes pain around the joints, swellingof joints, redness or feel warm to the touch of joints and difficulty in moving etc. The most common types of arthritis are Osteoarthritis , Rheumatoid arthritis andGout[4].

The prevalence of rheumatoid arthritis is about 1.5 % of the world population andthe epidemiology of arthritis in male to female ratio is 3:1[5].Since RA is a chronic disease, the treatment mainly focuses on improving pain, avert or limit joint damage, improve or preserve function of the joints and optimize the quality of life[6]. Cytokines play a major role ininflammation and damage the joint leading to tissue destructionduring the development of RA which includes the tumour necrosisfactor-a, interleukin-1b and IL-6[7]. NSAID and diseasealtering anti-rheumatoid drugshave many applicationsin treating diseases but are accompanying with side effects like GI complications, ulcers and cardiovascularproblems[8-10].Major problems of the currently available drugs for RA include poor efficacy, potential side effects and highcost of biological agents. Thus,acompetent and safe alternatefrom plants has drawn special attention from scientists worldwide.

Pentas lanceolata(Family-Rubiaceae),commonly known as Egyptian Star, is native to tropical Africa and is commonly used as herbal medicine in Ethiopia, Uganda, Rwanda and Kenya. This is erect evergreen perennial shrub, 3 to 4 feet tall and is tinted all over most of the year with 3-inch-wide, dense clusters of long-tubed, star-shaped flowers available in white, pink, red, and lavender colour. The plants grow commonly during summer season. Leaves and stems are enclosed with fine hairs, and leaves have prominent veins on the

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undersides.Traditionallythe plant is most popular andvarious parts of the plant are used asin the treatment of diseases like Lymphadenitis, Diarrhoea, Malaria, Ascariasis, Snake bite etc[11-14].

The preceding phytochemical investigation of *Pentas lanceolata* leaves with Methanol and n-Hexane extract reveals the presence of glycosides (3.5-6.0 %), terpenoids (0.5-1.0 %), sterols (0.2-0.5 %) and traces of flavonoids and carbohydrates. Anthraquinone glycosides were also isolated from the leaves of the plants named as Rubiadin-1-methyl ether,Damnacanthol, Rubiadin, Lucidin-ω-methyl ether etc[15]. Some studies reported about the medicinal importance of these anthraquinones as in the treatment of malaria and lymphadenitis[12-13].Thus, this research was conducted to find more evidence about the use of natural resources of *P. lanceolata*leaves as anti-arthritis.

MATERIALS AND METHODS

Plant Material

The fresh **leaves** of ***Pentas lanceolata*** (Forssk.) Deflers. were collected from fully grown plant from Kanpur Dist. Uttar Pradesh, India. The plant as well as plant material was authenticated by Botany department, Rajasthan University, Jaipur Rajasthan. A voucher specimen (RUBL/16/20856) has been kept in herbarium in Department of Botany, University of Rajasthan, Jaipur, Rajasthan.

Chemicals and Reagents

All the chemicals and reagents used were of extra pure and analytical Grade, procured fromSigma Chemical Pvt Ltd, USA. All solvents were obtained from Fischer Scientific Ltd, India.

Phytochemical Screening

The phytochemical screeningwasdone as per standard procedures to analyse secondary plantmetabolite. Phytochemical testing usually analyses the content of plant secondary metabolites including glycosides, flavonoids, terpenoids, alkaloids, steroids and saponins[16-17].

Preparation of Extracts

The extraction of well dried coarsely powdered leaves of *Pentas lanceolata*was done by using ***Soxhlet extraction***method. In this method of extraction, the pre-weighted plant material (500gm) was placed in ***siphon tube***and the selected solvents (900 ml each) as Methanol andn-Hexane individually was taken in round bottom flask connected with siphon tube via condenser. The whole process takes up to 4-6 hrs for each solvent. After complete extraction

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cool the extracts and were collected in concentrated form.The percentage yields of extracts for individual solventswere calculated in reference to material taken initially. The plant extracts were dissolved in distilled water for *in vivo* experiments [18].

Experimental Animals

Male wistar rats having body weight **180–280 gm**were used for the experiments. The animals were in housed under standard ecological conditions and were fed with standard pelleted diet and water *ad libitum.* All the experimental protocols were followed by as per suggestion of **CPCSEA** guidelines.

Before the study theethical clearance was taken from Institutional Animal Ethics Committee (IACE).Procedures adopted in the animal study by using different models for toxicity profiling as well as Anti-arthritis activity was approved by InstitutionalAnimal Ethical committee, NIMS University, JaipurRajasthan(**NIMSUR/IAEC/CERT/2014/09/02)(Registration No. 302/ac/CPCSEA).**

Acute Oral ToxicityStudy

Acute oral toxicity study for methanol and n-Hexane extract was conducted asper OECD guidelines 425.For the study healthy wistarrats of either sex having weight 190-300 gm were selected.The housing condition for the animals was maintained in polypropylene cagesat the room temperature (25±2^{o}c), humidity (55±5%) and 12hrs of light and dark cycle[19].For experimental protocol the animals were fasted before the study for whole day. On next day, the fasted animals were divided into six groups given theextracts at dose 100, 250, 500, 1000 and 2000 mg/kg body weight in diluted form with distilled water via oral route of drug administration. After 24 hrs the mortality counted and LD50 was determined.

Complete Freund's Adjuvant (CFA)-Induced Arthritis in Rats: -

Experimental Setup

Total forty-two animals were divided into seven groups containing six animals each. The study period was directed from day 0 to day 28 intwo different intervals as 0 – 14 days (developing phase) and 14–28 days (developed phase)[20].Indomethacin, as standard drug and methanolic extract and n-hexane extract of *P. lanceolata*with 1% solution of sodium carboxy methyl cellulose (SCMC)wasadministered immediatelyas follows: -

Group I: Normal control: Received aqueous solution of 1% **SCMC** (Sodium Carboxy Methyl cellulose)

Group II: Negative control: (arthritis induced rats without anytreatment)

Group III: Positive control: received 10 mg/kg per oral (p.o.)of Indomethacin

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Group IV: Methanolic extract of *P. lanceolata* **(MEPL)**received 250 mg/kg p.o. for 28 days fromthe day of induction of arthritis (developing phase)

Group V: n-Hexane extract of *P. lanceolata* **(HEPL)**250 mg/kg p.o. for 28 days from theday of induction of arthritis(developing phase)

Group VI:MEPL received 250 mg/kg p.o for 14 days from the14th day of induction of arthritis (developed phase)

Group VII:HEPL received 250 mg/kg p.o for 14 days from the 14th day of induction of arthritis (developed phase)

Induction of Arthritis

The rats were injected with 0.1ml of the CFA(each ml CFA composed of 1mg *Mycobacterium tuberculosis* heat kill and dried + 0.25 ml mineral oil + 0.15ml mannide mono oleate) by sub plantar region in the left hind paw[21].After the injection of FCA,the total paw volume ofleft hind paw of all the animals was measured using plethysmometerat 0, 5,9,14, 19, 23 and 28 days.

Total Arthritic Score

The degree and score of arthritis was observed daily by means of a scale from 0 to4 for each paw, targeting for a maximum score of 8 per rat. After induction of arthritis, thejoint diameters of the right hind paw were measured using an electronic Vernier calliper. The grading criteria are mentioned below:

Standard paw =0, Gentle inflammation and erythema of digits =1, Normal inflammation and erythema of the digits = 2, Rigorous inflammation and erythema = 3, Net distortion and failure to use the limb =4 on particular days. Thus, the highest possible score for both hind paws was 8[22].

Total Paw Volume: -

The total paw volume of left hind paw of all experimental drug treated animals were considered just prior to **Freund's complete adjuvant** injection on **day 0** and after that at various time durations till **day 28** using a plethysmometer. The changed paw volume observed was calculated as the distinction of the final and early paw volumes[23].

Study of Haematological Parameters

On 28thday the blood sample was collected by ocular puncture to investigate haematological parameters. The blood sample was stored in EDTA treated sample bottles. The standard method prescribed by *Chesbrough and McArthur* was adopted to count out the red blood cells and White blood cells individually[24]. To find out the level of blood haemoglobin the

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standard method suggested by *Drabkin and Austin* was adopted[25].To estimate ESR the standard method suggested by *Westergren and Wintrobe* was applied[26].

Study of Biochemical Parameters

For the estimation biochemical parameters like Anti-oxidants (SOD, Catalase and GSH), Total proteins, Serum bilirubin, liver function tests (SGOT and SGPT), Alkaline phosphate, Hydroxyproline and Hexosamine, the standard procedures were adopted [27-32].

Radiological Study

Radiographs were taken for the hind paw of all the experimentalanimals and inspected for soft tissue inflammation, bone destructions andcontraction of spaces between the joints[33].

Histopathological studies

The interphalangeal joints were removed from the hind paw,washed with saline and fixed for 24hrs. in formalin (10%). Afterdecalcification, the sections obtained were stained with eosinhaematoxylin stain and viewed under magnification of 100x.

Statistical Analysis

The results were expressed as mean ± SEM. The statistical comparisonwas made between arthritic control and the treatedgroups. They were analysed by one-way ANOVA followed byDunnett's& Turkey'smultiple comparison test. The level of significance wasset at **$p < 0.05$**.

RESULTS

Preliminary Phytochemical Screening

The percentage yield of the Methanolic extracts and n-hexane extractsobtained were calculated as 31.19% and26.07% w/w respectively.Preliminary phytochemical examinationsrevealed the presence of glycosides, alkaloids, amino acids/protein, steroids and terpenoids in methanolic extract, whereas n-hexane extract showed the presenceof glycosides, tannins, flavonoids, alkaloids, sterol and terpenoids.

Acute Oral Toxicity Study

Methanolic extract and n-Hexane extractdid not show any toxic or harmful effects upto 2000 mg/kg oral dose which indicates non-toxicityeven at higher doses. The LD50 of MEPL % HEPLwas found to be greater than2000 mg/kg. As a result,250 mg/kg was selected as the dose in order to evaluate comparative in vivoantiarthritic activity in Freund's adjuvant induced arthritic ratmodel.

Total ArthriticScore

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All the groups of animals administered with Freund's completeadjuvant raised symptoms of clinical inflammation in one ormore hind paws, assumed a biphasic response**(Table 1)**. The initialsymptom of ailment was **erythema** of one or more ankle joints. There was a primary growth in the signs of inflammation from **day 1** of drug treatment to **day 7,** followed by aminute decrements symptom of inflammation from day 8 to 14**(Figure 1)**.

Table 1.Effect of Methanolic and n-Hexane extracts on Arthritic Index–

Experimental Groups	Arthritic Index (on Days)					
	5thDay	**9th Day**	**14th Day**	**19th Day**	**23rd Day**	**28th Day**
Group I	2.63±0.9	2.75±0.60	2.71±0.50	2.62±0.87	2.53±0.56	2.54±0.23
Group II	3.77±1.03	4.87±1.01	5.54±1.04	5.95±1.00	6.23±1.10	6.19±1.09
Group III	3.73±1.04 b*	4.77±1.03 b*	3.68±1.06 b*	3.02±0.78 b*	2.73±0.58 b*	2.28±0.38 b*
Group IV	3.67±1.06 b*c*	4.57±0.79 b*c*	3.54±0.90 b*c*	2.89±0.67 b*c*	2.56±0.67 b*c*	2.21±0.33 b*c*
Group V	3.96±1.02 b*c*	4.89±1.01 b*c*	3.71±0.89 b*c*	2.99±0.55 b*c*	2.45±0.43 b*c*	2.11±0.61 b*c*
Group VI	4.09±0.09 b*c*	3.67±0.88 b*c*	3.04±0.76 b*c*	2.48±0.51 b*c*	2.23±0.45 b*c*	2.15±0.54 b*c*
Group VII	4.26±1.00 b*c*	3.86±0.08 b*c*	3.23±0.65 b*c*	2.67±0.43 b*c*	2.26±0.52 b*c*	2.18±0.44 b*c*

Group I: Normal control, Group II: Negative control, Group III: Positive control,Group IV: MEPL (28 days drug treatment), Group V: HEPL (28 days drug treatment),Group VI: MEPL (14 days drug treatment), Group VII: HEPL (14 days drugtreatment).Values are expressed as mean ± SEM, n=6 animals in each group.Comparisons were made between: **b:**Group II vs. groups III, IV, V, VI and VII.**c:**Group IIIvs. groups IV, V, VI and VII.

*Represents the statistical significance at **p < .05.**

Figure 1. Effect of Methanolic and n-Hexane extracts on Arthritic Index–

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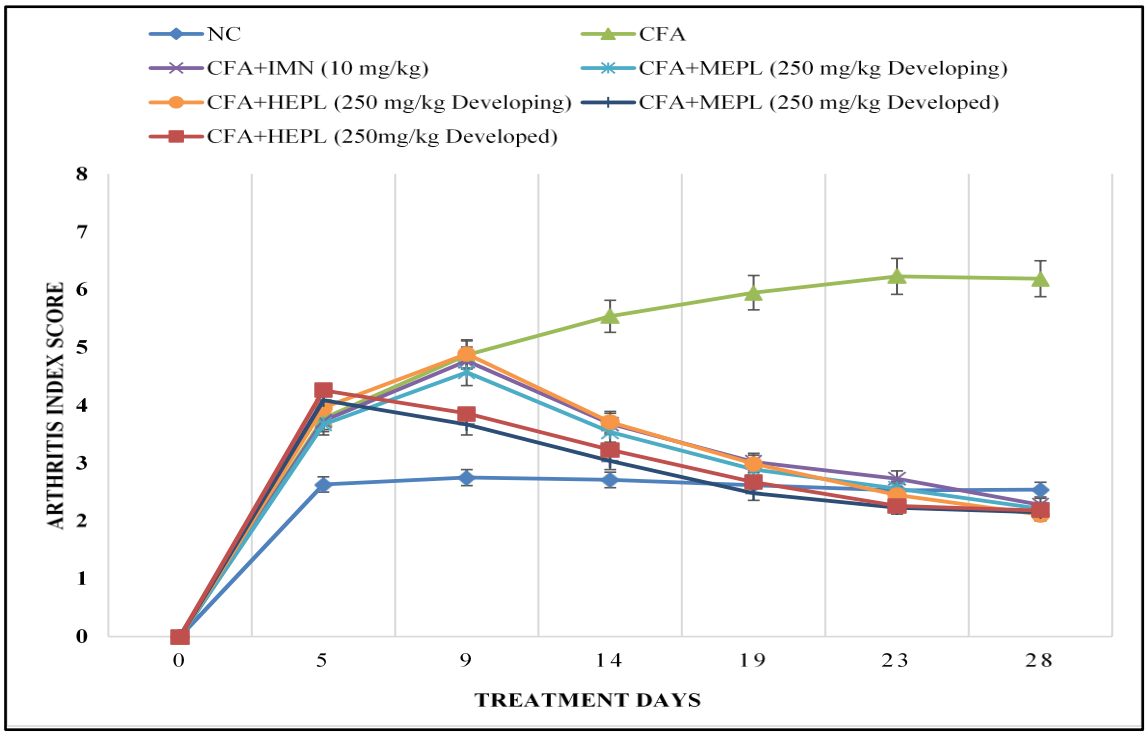

Values are expressed as **Mean ± SEM of n=6 animals.** Statistically significant when **P < 0.05.**

Total Paw Volume

There was a considerable improvement in paw volume in all Complete Freund's adjuvant **(CFA)** groups when compared to vehicle control but this development in paw volume was a biphasic response, i.e. there was a negligible decrease in paw volume from **day 09 to 23** but. The paw volume was furthermost on **day 5th**in all Complete Freund's adjuvant administered rats. On treatment with MEPL & HEPL (250 mg/kg) significantly **(P < 0.01)** diminish the paw volume, analysed till **28thday**. **(Table 2) (Figure2)**.

Table 2.Effect of Methanolic and n-Hexane extracts on Paw Volume –

Groups/ Days	1st	5th	9th	14th	19th	23rd	28th
Group I	1.44± 0.076	1.72± 0.11	1.43± 0.06	1.46± 0.08	1.45± 0.10	1.56± 0.09	1.27± 0.06
Group II	1.26± 0.04*	3.49± 0.13*	2.95± 0.09*	2.68± 0.15*	2.44± 0.25*	2.34± 0.13*	2.17± 0.06*
Group III	1.12± 0.09 b*	2.29± 0.80 b*	2.40± 0.82b*	1.82± 0.16 b*	1.70± 0.13 b*	1.63± 0.14 b*	1.21± 0.11b*
Group IV	1.17± 0.11 b*c*	2.45± 0.09b*c*	2.24± 0.08b*c*	1.92± 0.13b*c*	1.66± 0.14b*c*	1.63± 0.11b*c*	1.36± 0.11b*c*

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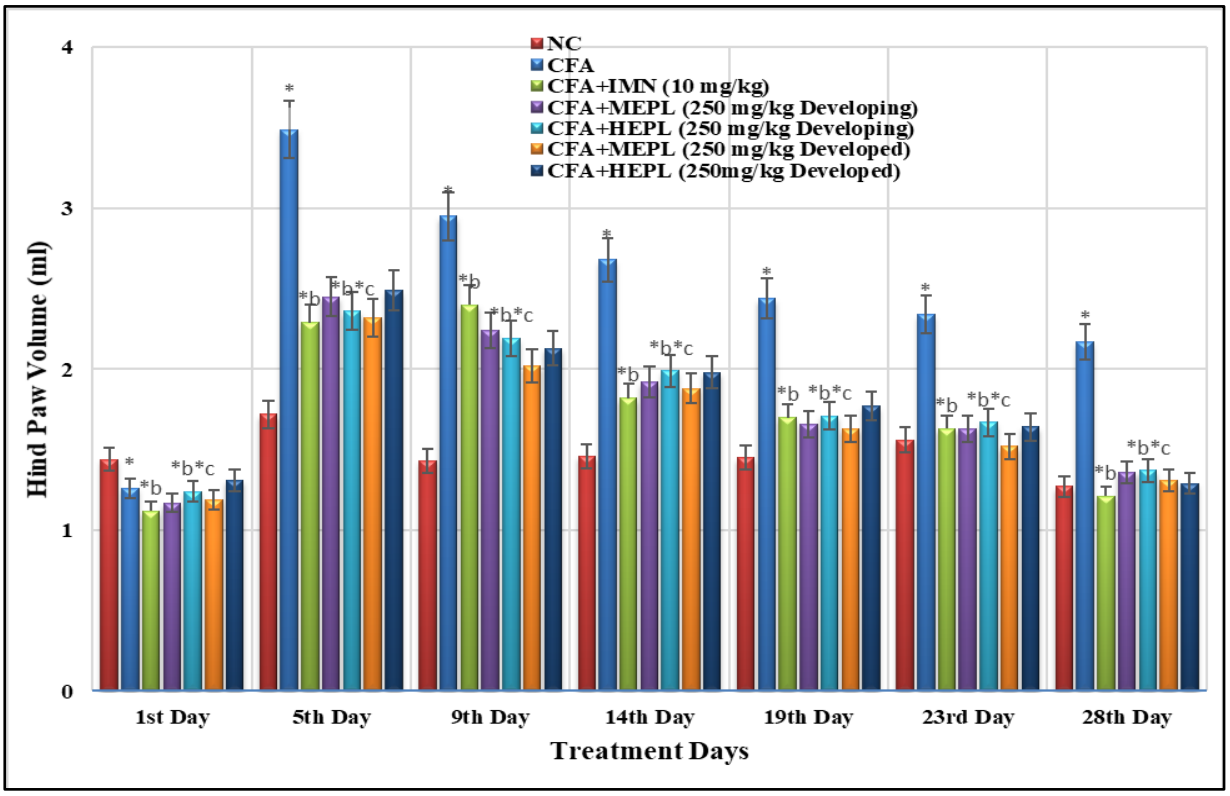
Group V	1.24± 0.09b*c*	2.36± 0.11b*c*	2.19± 0.09b*c*	1.99± 0.08b*c*	1.71± 0.12b*c*	1.67± 0.09b*c*	1.37± 0.11b*c*
Group VI	1.19± 0.08b*c*	2.32± 0.10b*c*	2.02± 0.09b*c*	1.88± 0.11b*c*	1.63± 0.10b*c*	1.52± 0.07b*c*	1.31± 0.11b*c*
Group VII	1.31± 0.11b*c*	2.49±0.12 b*c*	2.13±0.11 b*c*	1.98±0.09 b*c*	1.77±0.10 b*c*	1.64±0.09 b*c*	1.29±0.09 b*c*

Figure2. Effect of Methanolic and n-Hexane extracts on Paw Volume–



Group I: Normal control, Group II: Negative control, Group III: Positive control, Group IV: MEPL (28 days drug treatment), Group V: HEPL (28 days drug treatment), Group VI: MEPL (14 days drug treatment), Group VII: HEPL (14 days drug treatment).Values are expressed as Mean ± SEM, n =6 animals in eachgroup.Comparisons were made between: **b:** Group II vs. groups III, IV, V, VI and VII; **c:** Group III vs. groups IV, V, VI and VII.*Represents the statistical significance at **p<.01.**

Haematological Parameters

A considerable decline in the levels of **Hb** and **RBCs** was resulted out in control group when compared to Normal group. On treatments of **MEPL** & **HEPL** (250mg/kg) to Test Groups **(Group IV, V, VI and VII)** improved the levels of RBC and Hb to comparison with normal

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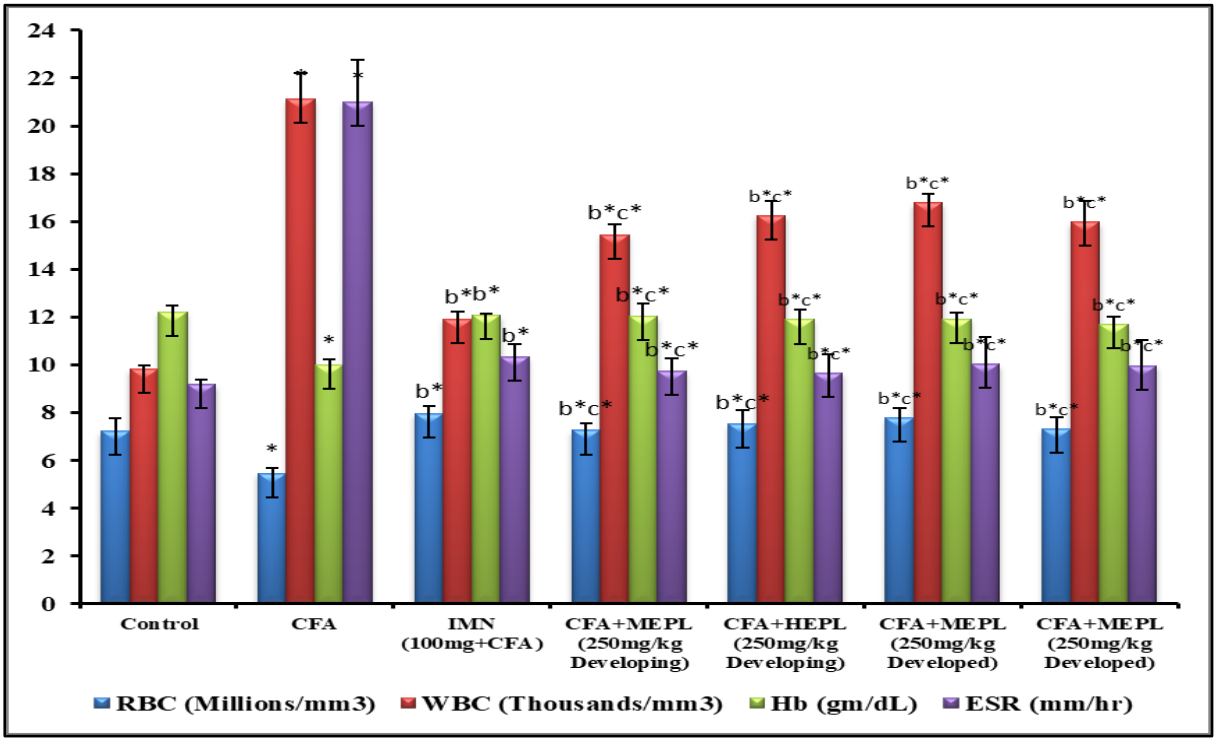
levels. The improvement in **WBC** count and **ESR** were significantly increased in the Test Groups **(Group IV, V, VI and VII)(Table 3) (Figure 3)**.

Table3. Effect of MEPL & HEPL on Haematological Parameters: -

Treatment Groups	RBC (Millions/mm^3)	WBC (Thousands/ mm^3)	Hb (gm/dL)	ESR (mm/hr)
Group I	7.23 ± 0.53	9.82 ± 0.17	12.21 ± 0.27	9.19 ± 0.21
Group II	5.46 ±0.24*	21.12 ± 1.09*	9.99 ± 0.23*	21.02 ± 1.76*
Group III	7.97 ± 0.49 b*	11.93 ± 0.87 b*	12.07 ± 0.33 b*	10.32 ± 1.05 b*
Group IV	7.26 ±0.32 b*c*	15.45 ± 0.32 b*c*	12.02 ±0.09 b*c*	9.73 ± 0.56 b*c*
Group V	7.53 ± 0.29 b*c*	16.25 ± 0.42 b*c*	11.89 ± 0.53 b*c*	9.67 ± 0.54 b*c*
Group VI	7.78 ± 0.56 b*c*	16.78 ± 0.59 b*c*	11.93 ± 0.41 b*c*	10.03±0.78 b*c*
Group VII	7.32 ± 0.43 b*c*	15.98 ± 0.37 b*c*	11.69 ± 0.26 b*c*	9.97 ± 1.12 b*c*

Figure 3. Effect of MEPL & HEPL on Haematological Parameters: -



RBC: Red blood cell,**WBC:** White blood cell,**Hb:** Haemoglobin, **ESR:** Erythrocyte sedimentation rate.

Group I: Normal control, Group II: Negative control, Group III: Positive control, Group IV: MEPL (28 days drug treatment), Group V: HEPL (28 days drug treatment), Group VI: MEPL (14 days drug treatment), Group VII: HEPL (14 days drugtreatment).Values are expressed as mean ± SEM, n =6 animals in each group.Comparisons were made between: **b:** Group II vs.

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groups III, IV, V, VI and VII.**c:** Group III vs. groups IV, V, VI and VII. *Represents the statistical significance at **p < .05.**

Biochemical Parameters

A. Effect on Anti-oxidant Enzymes

CFAs treated group resulted that the levels of **nitric oxide (NO)** and **Malondialdehyde** were **improved** considerably, while all the endogenous antioxidants were **reduced** significantly in arthritic Groups. The effect of **Test Compounds** significantly **reversed** the above changes. Treatment with MEPL & HEPL**enhanced** the presentation of **GSH** that of the CFAs group, while **Indomethacin (IMN)** was neutral for GSH performances. There was no regulative effect of MEPL & HEPL on the activity of SOD, whereas significantly increase the action of SOD of the test groups, and higher than the control group. **(Table 4) (Figure 4).**

Table 4. Effect of MEPL & HEPL on Anti-oxidant Enzymes:

Groups	GSH (U/mg Protein)	Catalase (U/mg Protein)	SOD (U/min/mg Protein)	Total Protein (mg/ml)
Group I	7.845 ± 0.39	1.521 ± 0.09	1.765 ± 0.18	9.71 ± 0.55
Group II	4.761 ± 0.59*	0.203 ±0.05*	0.518 ± 0.16*	5.31 ± 0.32*
Group III	7.534 ± 0.34 b*	1.073 ± 0.14 b*	1.721 ± 0.18 b*	11.03 ± 0.61 b*
Group IV	8.012 ± 0.23 b*c*	0.865 ± 0.03 b*c*	1.563 ±0.21 b*c*	9.41 ±0.24 b*c*
Group V	7.983 ± 0.26 b*c*	0.824 ± 0.04 b*c*	1.612 ±0.19 b*c*	9.12 ±0.29 b*c*
Group VI	8.022 ± 0.21 b*c*	0.835 ± 0.03 b*c*	1.592 ±0.18 b*c*	9.55 ±0.31 b*c*
Group VII	8.098 ± 0.29 b*c*	0.876 ± 0.06 b*c*	1.623 ±0.22 b*c*	9.03 ±0.27 b*c*

Figure 4. Effect of MEPL & HEPL on Anti-oxidant Enzymes:

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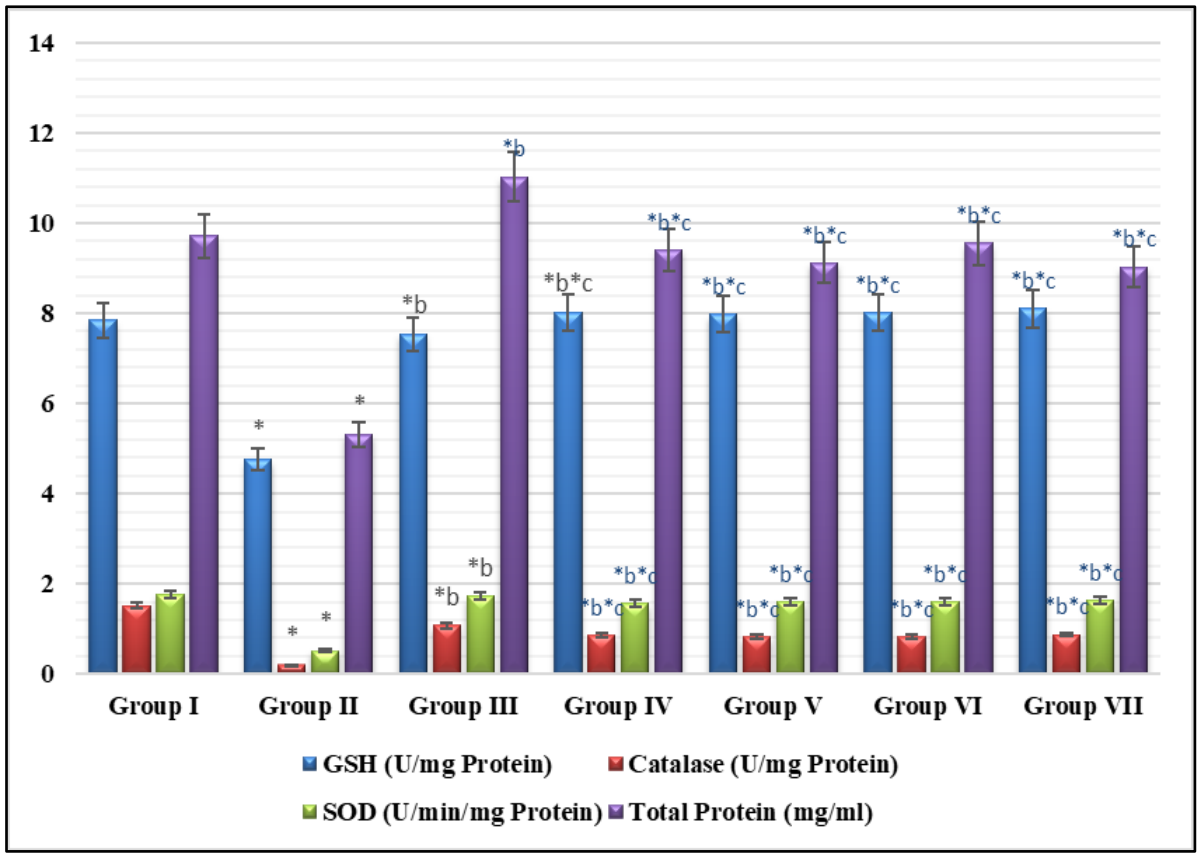

GSH: Glutathione, **SOD:**Superoxide dismutase.Group I: Normal control, Group II: Negative control, Group III: Positive control, Group IV: MEPL (28 days drug treatment), Group V: HEPL (28 days drug treatment), Group VI: MEPL (14 days drug treatment), Group VII: HEPL (14 days drug treatment). Values are expressed as mean ± SEM, n =6 animals in each group. Comparisons were made between: **b:** Group II vs. groups III, IV, V, VI and VII. **c:** Group III vs. groups IV, V, VI and VII. *Represents the statistical significance at **p < .05.**

B. Effect on Serum Enzyme Levels:

As a result of oedema induced by **CFA**, the levels of **SGPT, SGOT** and **ALP** were enhanced in all arthritis groups as compared to control groups. After treatment with MEPL & HEPL, the levels of these enzymes were significantly reduced in Test compounds **250mg/kg** groups as compared to control group. **Indomethacin** (100mg/kg) treatment not permitted biochemical changes to a greater degree than the test doses. The **SGOT, SGPT, ALP** and **Bilirubin** levels of all the groups were determined and compared with each other.**(Figure 5).**

Figure 5. Effect of MEPL & HEPL on Serum Enzyme Levels:

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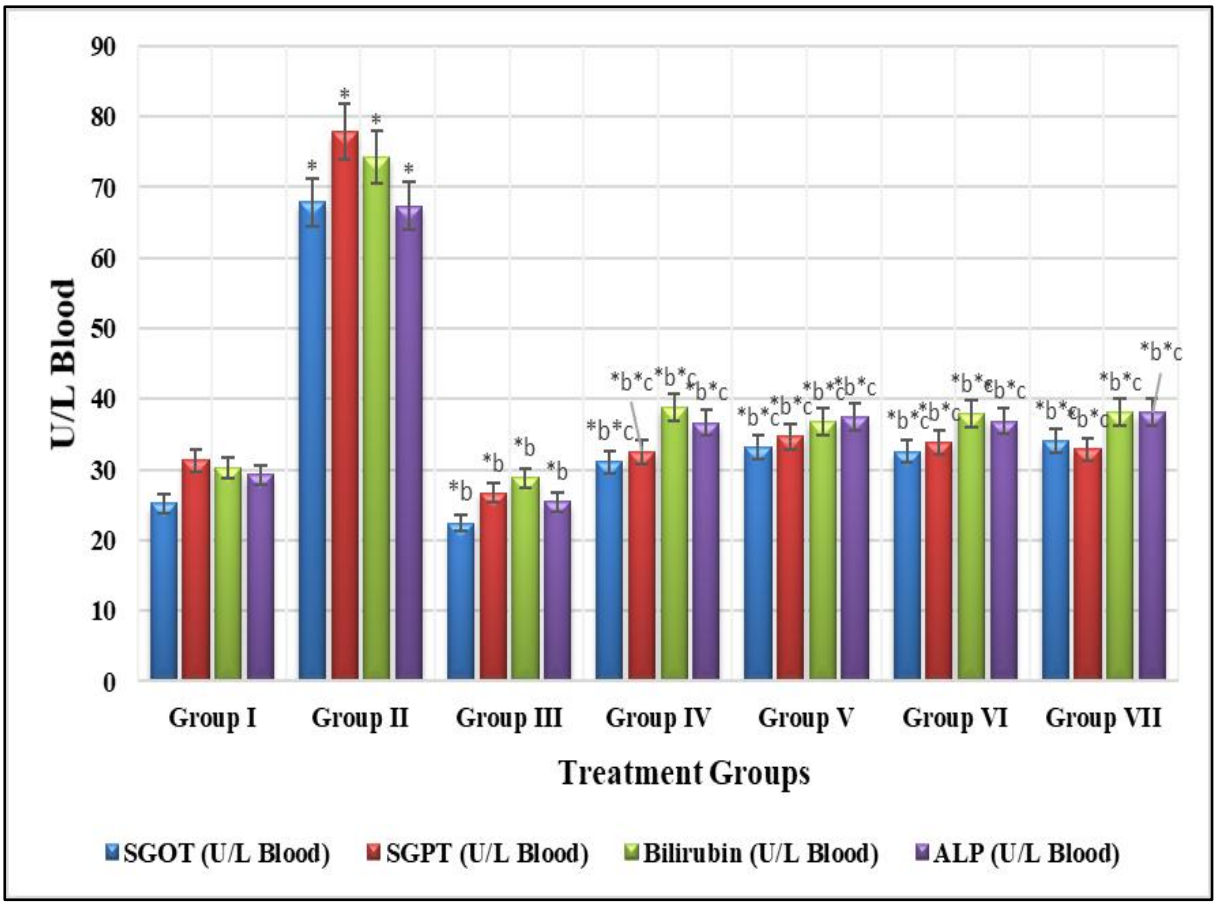

SGOT: Serum glutamic-oxaloacetic transaminase, **SGPT:**Serum glutamic pyruvic transaminase, **ALP**: Alkaline phosphatase. Group I: Normal control, Group II: Negative control, Group III: Positive control, Group IV: MEPL (28 days drug treatment), Group V: HEPL (28 days drug treatment), Group VI: MEPL (14 days drug treatment), Group VII: HEPL (14 days drug treatment). Values are expressed as mean ± SEM, n =6 animals in each group. Comparisons were made between: **b:** Group II vs. groups III, IV, V, VI and VII. **c:** Group III vs. groups IV, V, VI and VII. *Represents the statistical significance at **p < .05.**

C. Effect on Hydroxyproline and Hexosamine:

Administration of **Indomethacin** in rats resulted in a considerable elevation in the levels of **Hydroxyproline** and **hexosamine** along with enhance the paw thickness. On pre-treatment of **MEPL & HEPL** with dose of 250mg/kg the level was unchanged.**(Figure6).**

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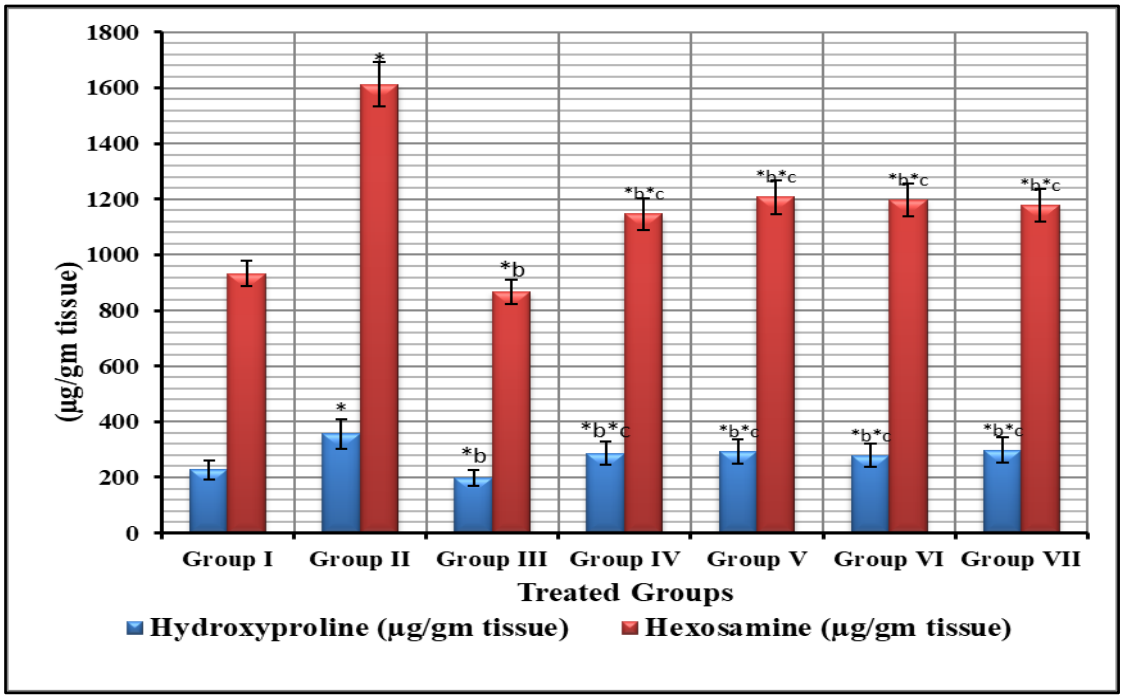
Figure 6. Effect of MEPL & HEPL on Hydroxyproline & Hexosamine:



Radiological Study

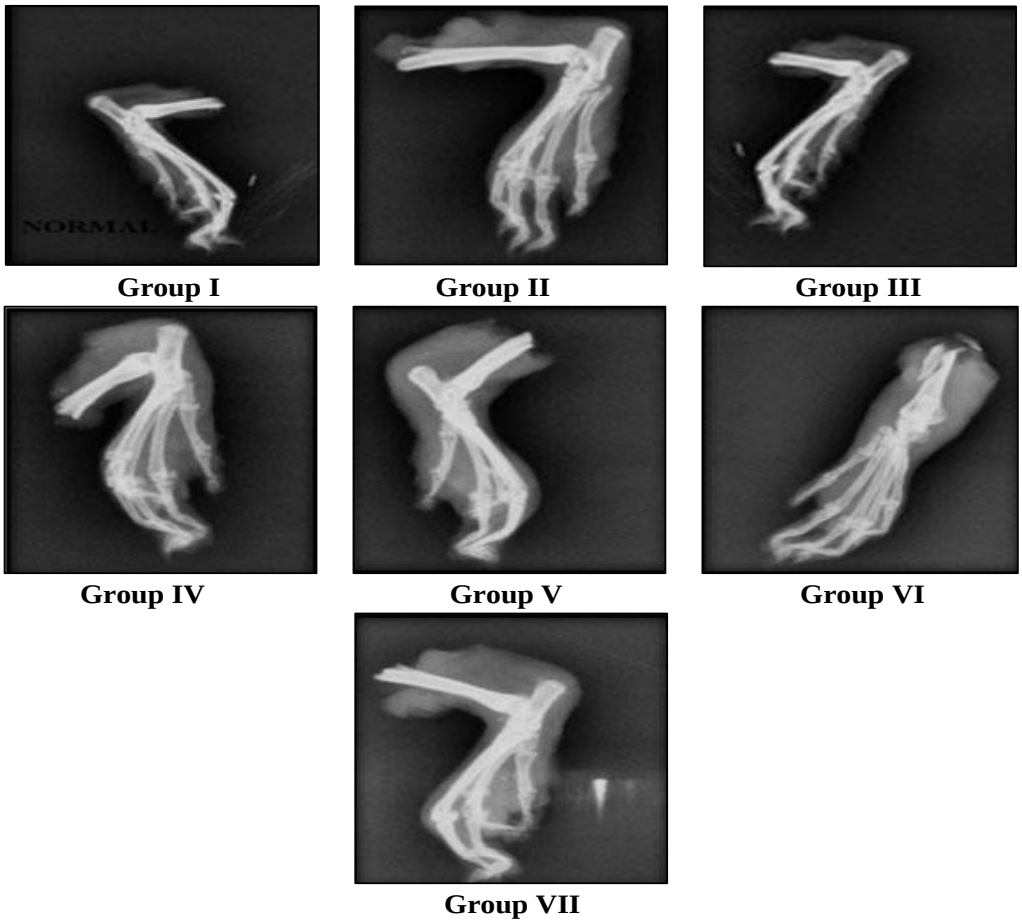


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Figure 7. Radiographs of hind legs in adjuvant-induced arthritic rats. **Group I:**Normal control,**Group II**:Negative control, **GroupIII:** positive control,**Group IV:** MEPL (28 days treatment),**Group V**:HEPL(28 days treatment),**Group VI**:MEPL (14 days treatment),**Group VI**:HEPL(14 days treatment).

Histopathological studies

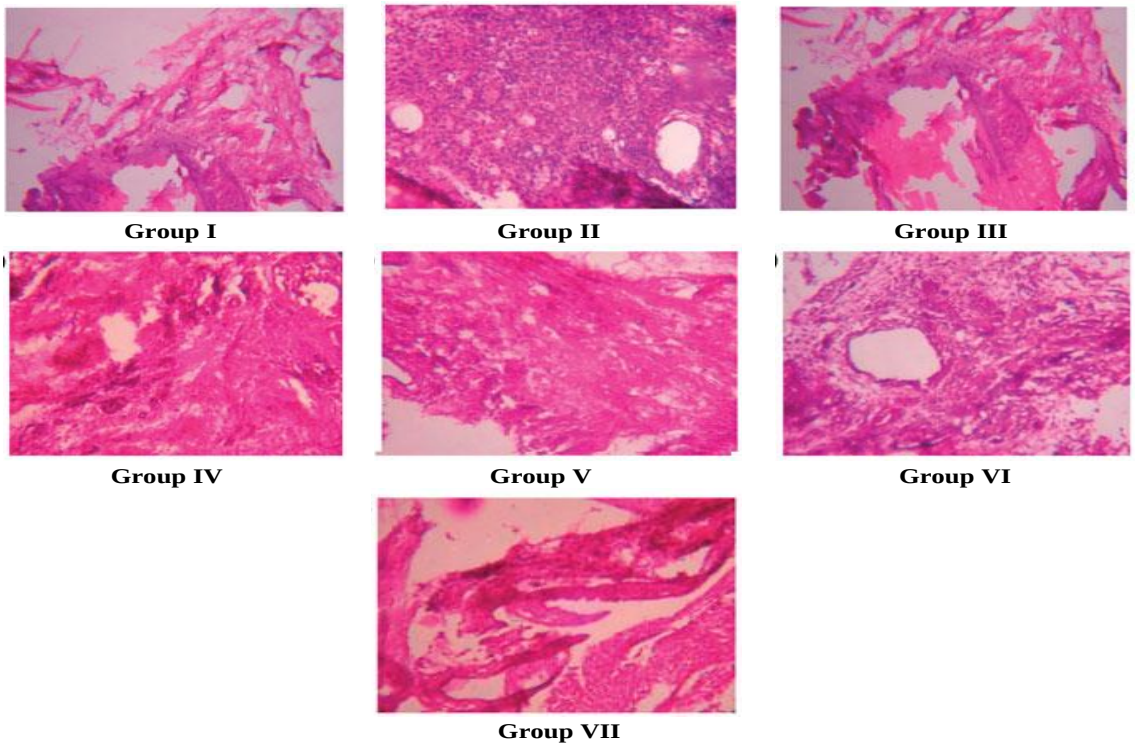

Figure 8. Histopathology of proximal interphalangeal joints on adjuvant-inducedarthritic rats.**Group I:**Normal control,**Group II**:Negative control, **GroupIII:** positive control,**Group IV:** MEPL (28 days treatment),**Group V**:HEPL(28 days treatment),**Group VI**:MEPL (14 days treatment),**Group VI**:HEPL(14 days treatment).

DISCUSSION

The % yields of the methanolic and n-hexane extracts were calculated andwere subjected to preliminary phytochemical analysis toidentify the phytoconstituents present. Acute toxicity study was conducted as per OECD guideline425. The MEPL and HEPL did not confirm any toxic effects ordeadliness at the limit dose of 2000 mg/kg p.o. for each extract, which represented that the extracts are safe to use at higher doses.

Variations in the paw volume of the adjuvant-induced arthriticrats were determined using digital plethysmometer. From theresults obtained, it was observed that both MEPL and HEPL were effective inequivalent to the standard drug Indomethacin on fallingthe increase in paw volume. Further, the effects of MEPL & HEPL were also substantial indeveloping phase relatively the developed phase of arthritis.The decline in the RBC & Haemoglobin count and

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haemoglobin level in thearthritic control group confirms the anaemic condition in arthriticrats. Anaemia is the most common extra-articular index ofrheumatoid arthritis, estimated to occur in 30% to 60% patients[34]. The two most common reasons for anaemia inarthritic patients are gastrointestinal blood loss from arthriticmedication and bone marrow changes in patients with inflammatoryarthritis which prevents the release of iron for incorporationinto the RBC's. MEPL, HEPL and Indomethacin-treated groupsshowed significant repossession from anaemia. The WBC count wasreported to be increased in arthritic control rats, because of the stimulation of the immune system against the invasiveantigens and the substantial decrease in MEPL and HEPL treatedgroups showed its immunomodulation effect[35]. The ESR reported as declined in extract treated groups significantly comparison to standard drug.

In reference to biochemical parameters, the CFA treated groups indicates the elevated levels of nitric oxide (NO) and Malondialdehydein all arthritic Groups, while the levels of all the endogenous antioxidants were reduced significantly in all arthritic Groups. These changes in the levels were vice versa in MEPL & HEPL treated groups of animals.Furthermore, HEPL & MEPL treated groups represents that the performance of GSH was increased in the model group, whereas Indomethacin (IMN)as a standard drug was neutral for the GSH performances. MEPL & HEPL had no significant effect on the activity of SOD, whereas considerably enhance the action of SOD of the test groups, and was greater than the control group.

The anti-arthritic effect of the MEPL & HEPL also supported by effect of these extracts on serum enzyme levels. The levels of SGPT, SGOT and ALP were improved in all arthritis groups in comparison to control groups of CFA treatment. MEPL & HEPL leads to these enzymes level and effective to maintain these levels of the enzymes. Both the extracts with the dose of 250mg/kg shows valuable reduction in the levels of serum enzymes in comparison control group. Indomethacin(100mg/kg) treatment showed almost neutral effect and barred biochemical variations to a larger level than the test doses.Indomethacin also resulted in a considerable enhance in the levels of **Hydroxyproline** and **hexosamine** along with the paw thickness. These changes were withdrawn by the pre-treatment of MEPL & HEPL with dose of 250mg/kg. in developing and developed phase respectively.

Histopathological study expressed the differences in the normalankle joint and adjuvant-induced arthritic rat joint. The study of the histopathological parameters of hind paw joints in arthritic control rats alsorepresented the prominent abnormalities like destruction of the bone marrowand extensive infiltration of the cells in the articular surface. MEPL and HEPL

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treatment have shown marked reduction in allthe above-mentioned pathological situations, signifying itseffective antiarthritic activity by protecting the bone fromdegeneration.

Radiographic study of the MEPL and HEPL was found to be effective in reducing the soft tissue swellingand reduction of joint spaces, especially in developing phaseof arthritis. The radiographic report confirms the effective antiarthriticactivity of MEPL and HEPL.

CONCLUSION

On assessing the *in vivo* antiarthritic activity of MEPL and HEPL, theeffects of both extracts were found to be significant, especially in the developingphase of arthritis. The antiarthritic activity of the MEPL and HEPL was significant, may be due to the presence of phytoconstituents such as saponin and anthraquinone glycosides, 3-o-β-fucoryl-quiniovic acid, Quermiside and rubiadin-1-methyl-3-o-β-primeveroside etc. Alkaloids like speciophylline and pentacyclic indole alkaloid inophyllins and triterpenoids may be also responsible for the activity. Further, it authenticates thetraditional use of *Pentas lanceolata* leaves in the treatment of rheumatism.However, further studies are necessary to recognize the active phytoconstituent

responsible for the antiarthritic activity. Themolecular mechanism involved in the antiarthritic activity of theplant extracts of *Pentas lanceolata*, especially MEPL, can be studied in future todevelop it as an alternative treatment for rheumatoid arthritis.

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AUTHORS CONTRIBUTIONS

Dr. Pranay Wal gave a substantial contribution by executingthe experimental workin the laboratories, drafted the manuscriptand extensively revised to improve the quality of the manuscript.Conception, the design of the study and supervision of the workwere done by Dr.Awani Kumar Rai.

CONFLICT OF INTERESTS

We declare that there were no conflicts of interest.

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