Original Article

Assessment Of Antiarthritic Potential Of Carica Papaya L. Leaves Extract And Fractions: Histopathological Analysis

Man Singh[1]*, Danish Ahmed[2], Himanshu Pandey[3], Rahul Deo Yadav[4], Shradhanjali Singh[5], Shanti Bhushan Mishra[6]

*[1,4]Department of Pharmacy, MLN Medical College, Prayagraj 211001, India
*[1, 2, 4] Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj 211008, India
[3]Centre for Teacher Education, Central Institute of Higher Tibetan Studies, Sarnath, Varanasi, 221007, India
[5,6]United Institute of Pharmacy, Prayagraj 211010, India

***Corresponding Author:** Man Singh,

*Department of Pharmacy, MLN Medical College, Prayagraj 211001, India, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj 211008, India, Email: mansinghmpharm@gmail.com
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Abstract

Carica papaya L has been traditionally claimed for curing inflammation hence the present work exemplifies the anti-arthritic potential of crude leaves extract and its fractions in arthritic models. Screening on this plant has established presence of kaempferol which might possess antiarthritic properties. Kaempferol was quantitatively estimated by high performance thin layer chromatographic method in chloroform fraction and was found to be 0.08% w/w. Arthritis was induced by intraplantar administration considering formaldehyde (2%v/v), turpentine oil emulsion (10%v/v) and complete Freund's adjuvant (1%v/v) in left hind paw of rats. Paw oedema, body weight, haematological parameters, level of serum enzyme markers, biochemical parameters, interleukin-6 and histopathological analysis were observed in all groups. Present experimental work justifies that in chronic arthritis, iinvolvement of interleukin-6 is associated with non-articular manifestations considering joint inflammation including anaemia. The plant extract and chloroform fraction possess dose dependent antiarthritic effect in acute and complete Freund's adjuvant induced arthritic model by ameliorating the level of serum enzyme biomarkers, haematological and biochemical parameters, body weight abnormalities, interleukin-6. The experimental explanation established that extract and its fraction of *Carica papaya* L. revealed anti-arthritic potential.

Keywords: Antiarthritic, Kaempferol, Complete Freund's adjuvant, *Carica papaya*, Fraction

INTRODUCTION

Rheumatoid arthritis (RA) is a symmetric, provocative, peripheral polyarthritis of unidentified etiology. Early detection and management with disease-modifying antirheumatic drugs (DMARDs) is imperative to attain control on disease and deterrence of joint injury and disability [1]. In view of safer medication for RA, fascinatingly peoples were concerned using diversified herbs and other natural products which suggest a vast resource for anti-arthritic agents. Indians used *Carica papaya* traditionally to treat various diseases. Traditionally leaves of *Carica papaya* L. are used in severe jaundice[2], to expel guinea worm[3] , as a poultice 8[4], in fracture healing[5], constipation and indigestion[6]. Scientific exploration of this plant revealed its abortifacient[7] hypoglycaemic[8], hypolipidemic[9], gastroprotective[10], antimicrobial[11], antimalarial[12], and wound healing properties[13]. *C. papaya* leaf has been accounted to include phytochemical compounds suggesting alkaloids, flavonoids, quinines, tannins and steroids which may together add on the way to its biological actions [14, 15]. Adding together to the profusion of phenolic compounds along with antioxidant actions acknowledged in a methanol extract of *C. papaya* leaf [16], the alkaloid carpaine, pseudocarpain, alkaloids, dehydrocarpaine I and II, choline, vitamine C and E, carposide were considered to foremost provider on the way to the leaf's therapeutic properties [17]. The efficacy of *C. papaya* L leaf resides in its consideration in thrombocytopaenia management through dengue infection. [18]

Complete Freund's Adjuvant induced arthritic animal model resembles human rheumatoid arthritis and hence arthritic manifestations need to be scientifically explored. The present study explored phytoconstituents in CPEE and kaempferol in CPCF along with antiarthritic activity of plant.

MATERIAL AND METHODS

Plant collection

The leaves of *Carica papaya L* were collected from herbal garden of United institute of Pharmacy, Naini, Prayagraj in the month of March 2019 and air-dried at 40^0 C. The plant was authenticated by taxonomist Dr. Arti Garg from Botanical Survey of India, Prayagraj and voucher specimen has been submitted in departmental herbarium of BSI with Accession No. 104699.

Extraction and purification
The pulverized dried leaves of plant (500g) were meticulously extracted with 50% ethanol by continuous extraction process in Soxhlet assembly at 50-60^{0}C[19]. The obtained semi solid plant extract have been subjected for drying on water bath under controlled conditions and then store in air tight container in a desiccator. Thus 70.8 g of solid remains (yield 41.8 % w/w) was obtained. Purification of extract was done by fractionation. The yield of chloroform fraction was 22% w/w.

Preliminary phytochemical screening and TLC
Carica papaya ethanolic extract and *Carica papaya* chloroform fraction were examined phytochemically considering standard procedures [20]. Experimentation of spots was done considering Ethyl acetate: formic acid: acetic acid: water (8.3:0.9:0.9:2.3v/v/v/v). CPEE showed (04) spots in UV light and subjected for further fractionation. In CPCF, four spots were observed in UV light hence considered for anti-arthritic activity.

Quantitation of kaempferol from purified fraction of the plant
The standard solution of kaempferol was all set by mixing 10.52mg in 20 ml methanol. The sample solutions were equipped by mixing 198.7 mg of CPCF in 5 ml methanol. 10 µl of the standard and sample liquid were taken and spotted on the HPTLC plates (20X20cm) and developed at 254nm.

Experimental animals
Male albino wistar rats 180-210g were considered for the experiment. The animals were kept at 25±2 °C in the animal facility of United Institute of Pharmacy, Prayagraj. The experimental protocol was approved by the Institutional Animal Ethical Committee of United Institute of Pharmacy with approval number UIP/IAEC/Nov.-2020/08**.**

Toxicity study of the plant
The lethal median dose (LD_{50}) assessment was performed in rats by OECD guidelines [21]. The animals were observed every hour all through 12 h right through the study period (14 days) for any anomalous changes.

Acute arthritic models
The present study incorporates (n=6) rats in which Group I suggested as positive rats, provided vehicle, Group II was well thought-out as arthritic rats provided with 0.1 ml of 2% formaldehyde/ 0.1ml turpentine oil suspension (10%v/v) intraplanterally, Group III as in rats indulgence with aspirin at 100 mg/kg body weight, Group IV as in rats suggested with extract (CPEE) 200 mg/kg body weight, Group V was well thought-out as rats suggested with extract (CPEE) 400 mg/kg body weight, Group VI as in rats treated with fraction (CPCF) 200 mg/kg body weight, Group VII as in rats indulged with fraction (CPCF) 400 mg/kg body weight.

Formaldehyde tempts arthritic model on day 0, 2, 4, 6, 8, and 10, was used to assess anti-arthritic potential of CPEE and CPCF on the paw suggesting paw volume and joint diameter.
CPEE and CPCF were measured for anti-arthritic potential for turpentine tempt arthritic model up to 6 hrs by appraising paw oedema.

Chronic arthritic model
Complete Freund's adjuvant arthritic model
Following parameters as paw oedema, body weight on day 0, 1, 4, 8, 12, 16, 20, 24, and day 28 were considerably assessed for anti-arthritic activity of CPEE and CPCF. Implications of different parameters were well thought-out by withdrawing the blood by retro-orbital puncture on 28th day.

Hematological and serum parameters
Serum parameters of the rats were estimated by a modified method[22].

Histopathological analysis
The treated and control group rats were sacrificed. The joint sections were then deparaffinized in xylene and stained with eosin hematoxylin stain and viewed under 40X magnifications. The snaps of histopathological slides were captured with a Nikon E400 microscope (Chiyoda, Tokyo, Japan).

Statistical analysis
Data was expressed as mean ± SD and statistical analysis was carried out by using GraphPad Prism 9.1.2 software by applying two-way ANOVA with Newman-Keuls method. $P<0.001$ was considered to be significant.

RESULT
Phytochemical analysis
Carbohydrates, phenolic compounds, alkaloid, flavonoids, phytosterols, proteins and amino acids were present in 50% *Carica papaya* ethanolic extract (CPEE) and *Carica papaya* chloroform fraction (CPCF).

Toxicity of the plant extract

CPEE and CPCF did not demonstrate any mortality up to 5000 mg/kg. Hence, two treatment doses were managed and selected i.e., 200mg/kg and 400mg/kg b.w.

HPTLC fingerprinting analysis

The Quantitative estimation of kaempferol was performed considering purified chloroform fraction of *Carica papaya* L. at 254 nm using ethyl acetate: formic acid: acetic acid: water (8.3:0.9:0.9:2.3v/v/v/v) as the mobile phase. The HPTLC plate and densitograms were shown in Figure 1, Figure 2a and Figure 2b respectively. The rf value of standard kaempferol and in the chloroform fraction at 254nm was found to be 0.79. The amount of kaempferol by quantitative HPTLC method was found to be 0.08 %w/w.

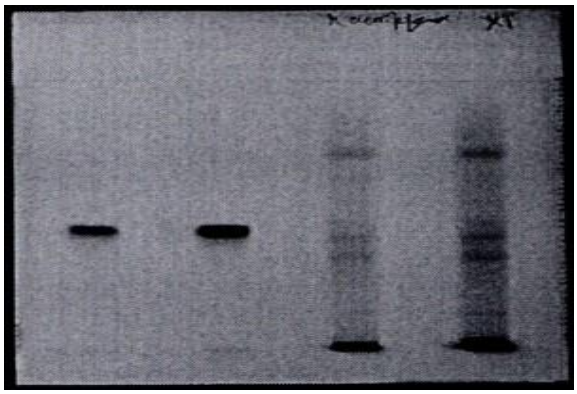

Fig. 1: HPTLC fingerprinting plate of kaempferol marker compound and chloroform fraction (CPCF) of *Carica papaya* L (X1)

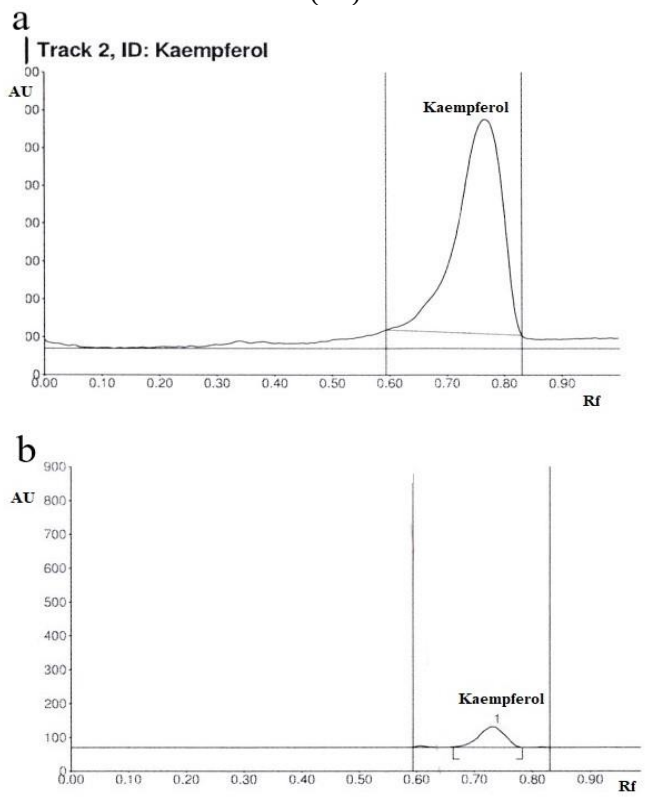

Fig. 2: a) HPTLC densitogram of kaempferol marker compound b) HPTLC fingerprinting of chloroform fraction of *Carica papaya* L.

EFFECT OF CPEE AND CPCF ON ACUTE ARTHRITIC MODELS

The result of joint odema from 0^{th} to 10^{th} day in rats was obtainable in Figure 3 along with comparing with arthritic control.

Formaldehyde induced arthritic model

Diseased control rats treated with formaldehyde had a significant ($p<0.001$) rise in paw volume and diameter as matched to healthy rats. CPCF at 400mg/kg b.w & aspirin declined paw volume and diameter considerably ($p<0.001$) from day 6 onwards matched to the disease control group. The alteration in paw volume of CPEE was (200mg/kg; 0.49±0.15; $p<0.001$ and 400mg/kg; 0.40±0.40; $p<0.001$), CPCF treated groups (200mg/kg; 0.34±0.63; $p<0.01$ and 400mg/kg; 0.33±0.52; $p<0.001$) was evident as compared to arthritic control (0.99±0.19; $p<0.001$) Figure 3a on day 10. The variation in paw

diameter of CPEE group (200mg/kg; 4.57±0.77; p<0.001 and 400mg/kg; 4.31±0.67; p<0.001) & for CPCF group (200mg/kg; 4.23±0.62; p<0.001 and 400mg/kg; 4.07±0.60; p<0.001) was obvious as compared to arthritic control (6.59±0.58; p<0.001) Figure 3b on day 10.

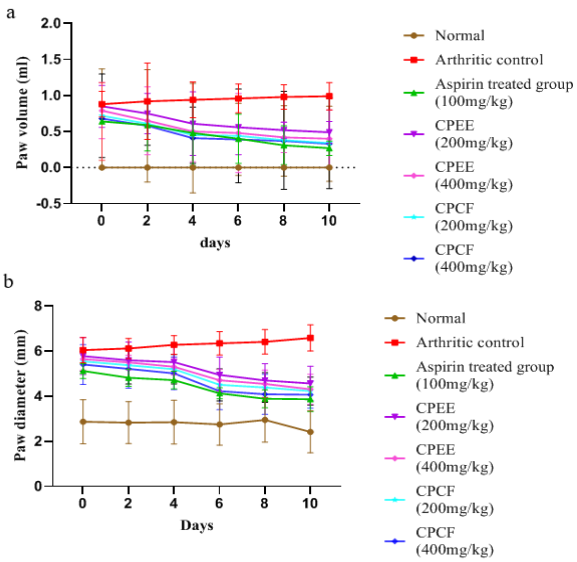

Fig. 3: a) Effect of ethanolic extract and chloroform fraction of *C. papaya* on paw volume in formaldehyde induced arthritic model b) Effect of ethanolic extract chloroform fraction of *C. papaya* on paw diameter in formaldehyde induced arthritic model

Turpentine induced arthritic model

Diseased control rats treated turpentine had a significant (p<0.001) rise in paw volume and diameter as matched to healthy rats. CPEE, CPCF and aspirin lessen the distension and diameter from 3 hour onwards as compared to diseased rats as shown in Figure 4.

The alteration in paw volume of CPEE (200mg/kg; 0.36±0.61; p<0.01 and 400mg/kg; 0.32±0.90; p<0.01 & CPCF treated groups (200mg/kg; 0.30±0.84; p<0.001 and 400mg/kg; 0.25±0.93 p<0.001) was noticeable as compared to arthritic control (0.78±0.30; p<0.001) on 6hr in Figure 4a.

The variation in paw diameter of CPEE & CPCF treated groups (200mg/kg; 3.51±0.41; p<0.001 and 400mg/kg; 4.31±0.44; p<0.001) & (200mg/kg; 4.19±0.94; p<0.001 and 400mg/kg; 3.88±0.96; p<0.001) respectively was evident as compared to arthritic control (7.89±0.69; p<0.001) on 6hr in Figure 4b.

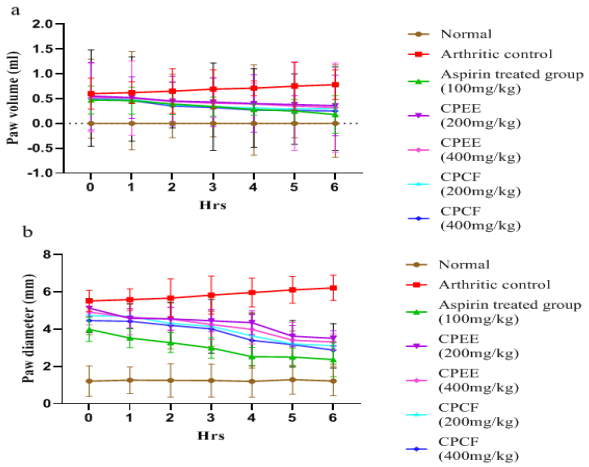

Fig. 4: a) Effect of ethanolic extract and chloroform fraction of *C. papaya* on paw volume in turpentine induced arthritic model b) Effect of ethanolic extract and chloroform fraction of *C. papaya* on paw diameter in turpentine induced arthritic model

EFFECT OF CPEE AND CPCF IN CFA INDUCED ARTHRITIC MODEL

Paw swelling

When matched to the positive control as in Figure 5, all CFA treated rats had a significant (p<0.001) rise in paw volume and diameter. When compared to the arthritic control group, rats given CPEE and CPCF, as well as aspirin, have significant (p<0.001) reduced paw volume and diameter by day 16 headlong.

The alteration in paw volume of CPEE & CPCF treated groups (200mg/kg; 0.42±0.82; p<0.05 and 400mg/kg; 0.34±1.23; p<0.01) & (200mg/kg; 0.31±0.33; p<0.001 and 400mg/kg; 0.29±1.07; p<0.001) respectively was evident as compared to arthritic control (1.65±0.50; p<0.001) on day 28 as shown in Figure 5a.

The variation in paw diameter of CPEE & CPCF treated groups (200mg/kg; 4.26±1.49; p<0.01 and 400mg/kg; 4.18±1.32; p<0.001) & (200mg/kg; 4.07±1.88; p<0.001 and 400mg/kg; 3.99±1.72; p<0.001) respectively was evident as compared to arthritic control (8.15±1.35; p<0.001) on day 28 as shown in Figure 5b.

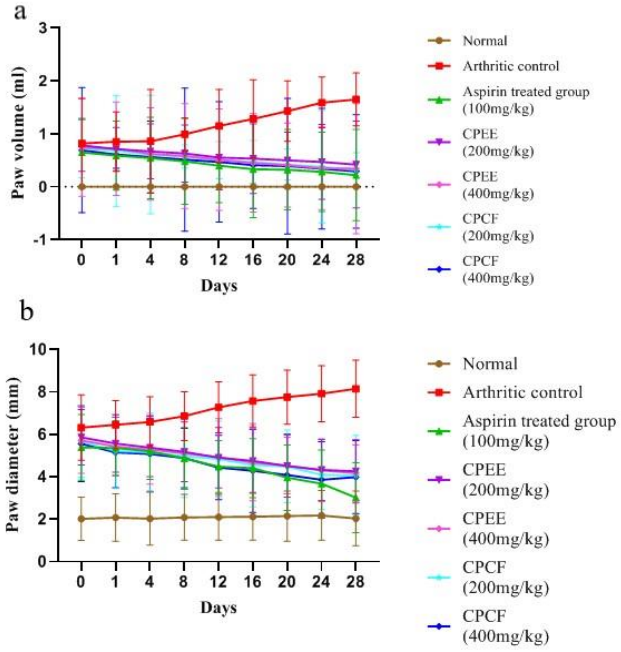

Fig. 5: a) Effect of ethanolic extract and chloroform fraction of *C. papaya* on paw volume in CFA induced arthritic model b) Effect of ethanolic extract and chloroform fraction of *C. papaya* on paw diameter in CFA induced arthritic model

Body Weight Recording

In comparison to the CPEE, CPCF and aspirin-treated groups, the rats in the arthritic control groups lost weight. The body weight of CPEE & CPCF treated groups (200mg/kg; 176.50±1.89; p<0.001, and 400mg/kg; 174.83±5.26; p<0.001 & 200mg/kg; 172.66±2.71; p<0.001 and 400mg/kg; 165.16±8.48±3.48; p<0.001) respectively was obvious as compared to arthritic control group (122.16±3.48; p<0.001) on day 28. The results were shown in Table 1.

Table 1: Effect of ethanolic extract and chloroform fraction on body weight in CFA induced arthritic model

Groups/ Days	Group-I Normal	Group-II Arthritic control	Group-III Aspirin treated group	Group-IV CPEE (200mg/kg)	Group-V CPEE (400mg/kg)	Group-VI CPCF (200mg/kg)	Group-VII CPCF (400mg/kg)
0th	165.83±6.52	145.33±8.35	160.83±7.35	157.16±4.11	155.66±3.27	152.16±4.76	150.33±2.26
1st	173.33±5.52	138.83±6.35	165.83±5.35	159.16±3.11	158.66±4.27	155.16±7.76	152.33±6.26
4th	164.83±3.52	135.50±6.14	169.33±2.54	160.83±6.96	161.66±3.58	156.33±6.16	154.33±5.11
8th	167.50±7.80	134.50±6.58	172.66±1.67	162.83±5.52	165.83±5.30	158.16±5.81	158.16±4.64
12th	169.83±7.47	130.16±5.99	176.66±3.08	163.33±3.53	168.83±6.99	161.83±3.02	160.83±6.11
16th	172.50±7.80	125.33±5.28	178.83±3.18	167.16±7.61	170.83±3.99	164.66±2.36	161.16±8.97
20th	168.50±3.80	121.66±4.44	179.16±2.18	168.16±1.97	172.16±4.65	168.50±1.93	162.66±1.88
24th	166.16±6.22	119.16±3.48	181.33±4.25	169.50±2.59	173.16±2.86	169.66±5.08	163.16±6.54
28th	177.50±6.03	115.16±4.48^{z}	182.33±2.96^{c}	176.50±1.89^{c}	174.83±5.26^{c}	172.66±2.71^{c}	165.16±8.48^{c}

The values was signified as Mean ± SD 6 rats per groups.^{a}p<0.05,^{b}p<0.0l,and^{c}p< 0.00l when compared to arthritic control p<0.00l when compared to positive control

Haematological parameters

The effects observed by CPEE and CPCF on haematological parameters were dose dependent as shown in Figure 6.

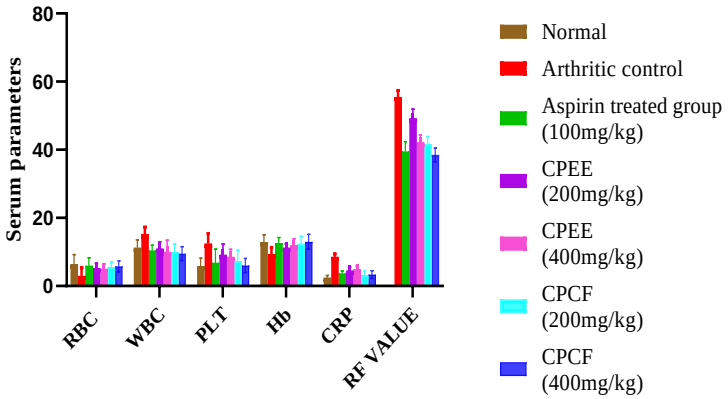

Fig. 6: Effect of CPEE and CPCF on haematological parameters in CFA induced arthritic model

Biochemical parameters

The level of enzyme AST, ALT and ALP were significantly ($p<0.001$) decreased by treatment with CPEE & CPCF 200 mg/kg, 400 mg/kg and aspirin 100 mg/kg as shown in Figure 7.

The effect of CPEE and CPCF on total protein and A/G ratio also significantly ameliorated the value as shown in Figure 8. For total protein the value for CPEE at 200mg/kg was (5.85±2.78) and at 400mg/kg (5.61±4.87) & for CPCF at 200mg/kg (4.78±2.96) & at 400mg/kg (3.85±3.23). For A/G ratio the value for CPEE at 200mg/kg was (1.49±0.78) & at 400mg/kg (1.69±0.32) & for CPCF at 200mg/kg (1.98±0.56) & at 400mg/kg (2.31±0.14).

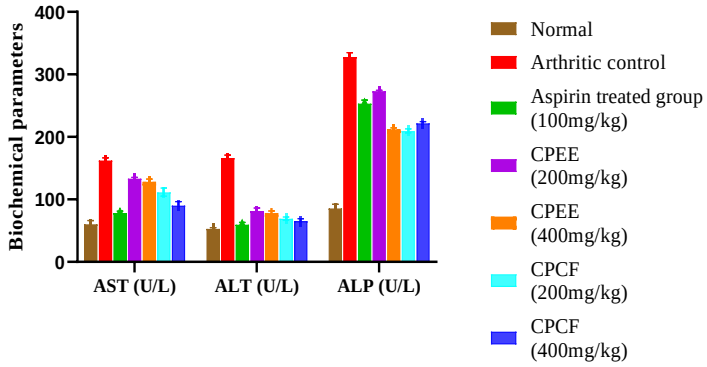

Fig. 7: Effect of CPEE and CPCF on biochemical parameters (AST, ALT, ALP) in CFA induced arthritic model

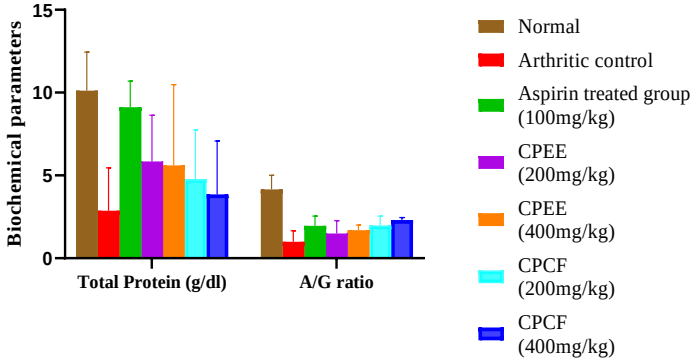

Fig. 8: Effect of CPEE and CPCF on biochemical parameters (total protein & A/G ratio) in CFA induced arthritic model

Interleukin was also estimated and the value for group II was (436.00±25.54; $p<0.001$) which was significantly decreased for CPCF treated group at 400 mg/kg (293.33±39.13; $p<0.001$) as compared to CPEE at 400mg/kg (345.33±35.31; $p<0.05$). The results were shown in Figure 9.

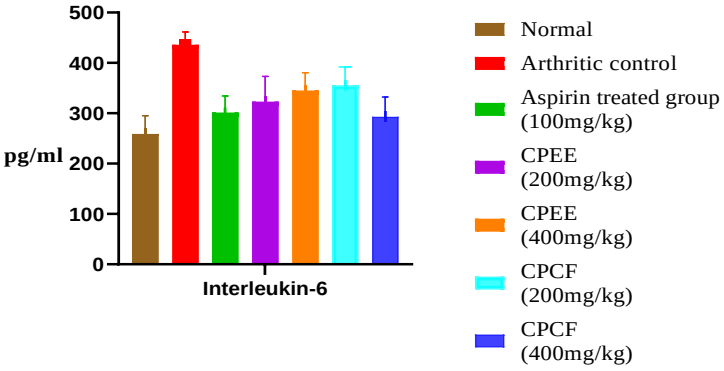

Fig. 9: Effect of CPEE and CPCF on biochemical parameters (Interleukin-6) in CFA induced arthritic model

Histopathological analysis

The histopathological analysis was shown below in Figure 10. Group I seen histological features in healthy rats paw showed no sign of inflammation. Group II exhibited foci of fibrinoid, necrosis and fibrin deposition, marked thickening of the synovial membrane due to oedema, congestion and multilayering of synoviocytes, synovial, numerous folds of large villi of synovium. Group III showed inadequate inflammatory cells and no fibrin depots. Group IV shows (200mg/kg b.w.) treated rats exposed depletion in less necrosis of bone, less declined inflammatory cells, less diminished vascular proliferation and less synovial space and slight pannus formation and minor fibrin depots. Group V CPEE (400mg/kg b.w.) exposed depletion in necrosis of bone, declined inflammatory cells, diminished vascular proliferation and less synovial space and slight pannus formation and minor fibrin depots. Group VI, CPCF (200mg/kg b.w.) showed decreased synovial incursion. Group VII showed CPCF 400mg/kg b.w.) Treated rats showed protection of synovial space lesion, fibrin depot, little penetration of cells light synovitis.

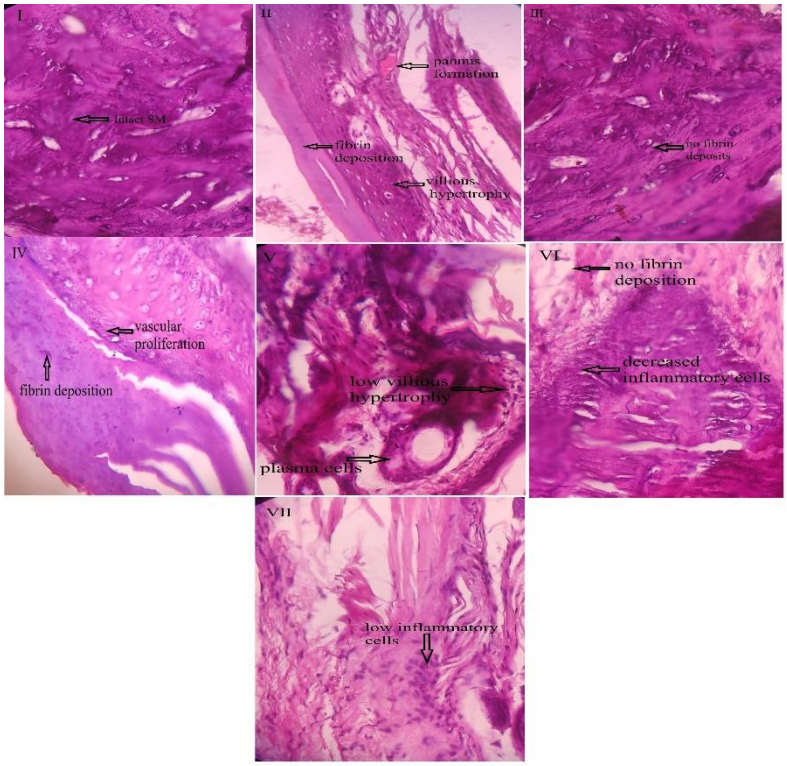

Fig. 10: Histopathological analysis of ankle joint stained with haematoxylin-eosin after 28 days Positive control. (B) Arthritic control. (C) Aspirin control 100mg/kg b.w. (D) CPEE 200mg/kg b.w. (E) CPEE 400mg/kg b.w. (F) CPCF 200mg/kg b.w. (G) CPCF 400mg/kg b.w. SM: Synovial membrane

DISCUSSION

Present study justifies the antiarthritic potential of *Carica papaya* in turpentine induced arthritic model, in which mediators including prostaglandins and histamine certainly aggravated inflammatory reaction initially and subsides gradually. Formaldehyde induced inflammatory response in rats spreads all over the joint. Results of the study justified that chloroform fraction of *C. papaya* at 400 mg/kg b.w. were the basis of significant drop in joint swelling in both acute models.

Amongst the cytokines like (TNF-α), IL-6, Interleukin-17A (IL-17A) and Interleukin-1β (IL-1β), IL-6 has been studied in persistent state of the disease [23]. In the present study, chloroform fraction of *C. papaya* diminishes the elevated interleukin (IL-6) level suggesting decreased inflammatory reactions at molecular level due to the presence of kaempferol in the fraction.

In rheumatoid arthritis, many enzymes are observed and seen to be increased in serum levels ensuing in the making of inflammatory substances [24]. In this study, the chloroform fraction shows significant alteration in the AST, ALP and ALT enzyme levels.

Report claimed that arthritis is linked with decrease in plasma albumin and increase in plasma globulin on account of amplified permeability of vascular tissues to albumin [25]. In this study high levels of globulin and low levels of albumin in extract and fraction treated group indicates recovery of inflammatory condition. High A/G ratio in this study indicates recovery of inflammatory condition in extract and fraction treated groups in dose dependent manner.

Previous report suggests that [26], excessive flow of interleukin 6 (IL6), arouses release of hepcidin from liver cells and exerts reticence effect on liberation of iron by macrophages. Rheumatoid factor RF was taken into account to get the information about immune response and so considers as marker in RA count. Depending upon the severity of rheumatoid arthritis there is an increase in platelet count. In this study an increase in Hb, RBC count, decrease in RF and restoration of CRP, IL-6 and platelets showed ameliorating inflammatory condition. Augmented levels of neutrophils allied with interleukin increases WBC and acute phase proteins along with CRP in adjuvant arthritis. In this study rise in WBC count was observed in arthritic group but in extract and fraction treated groups WBC count decreases which indicate improvement in the inflammatory condition.

Histopathology of organ implies the texture of affected tissues related to the disorder. In this study, the tissue infiltration in extract and fraction treated groups decreased hence improvement in the arthritic conditions.

CONCLUSION

Carica papaya L. has potential antiarthritic effect in this experimental work. Phytochemical studies and quantitative HPTLC analysis established the existence of diversified phytoconstituents including kaempferol in chloroform fraction of the plant (CPCF). Alkaline phosphatase and transaminases enzymes were also found to be ameliorated with chloroform fractions in experimental animals. There is a decline in the weight of rats in chronic model in arthritic control group and hence possible antiarthritic effect could probably be interrelated with the existence of kaempferol and decrease in the interleukin (IL-6) level in the chloroform fraction treated animals. Hence this study supports the pharmacological specifics to traditional claim of the plant in the management of symptoms related to arthritis.

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