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Original Article

ANTIARTHRITIC EFFECT OF GALANGIN ISOLATED FROM RHIZOMES OF *ALPINIA OFFICINARUM* IN COMPLETE FREUND'S ADJUVANT-INDUCED ARTHRITIS IN RATS.

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ABSTRACT

Objective: The objective of the present study was to isolate galangin from methanolic extract of *Alpinia officinarum* (MEAO) by bioassay guided fractionation using hexane–ethyl acetate as a solvent and evaluated the anti-arthritic effect of galangin and MEAO in complete Freund's adjuvant (CFA) induced arthritis in wistar rats.

Methods: Arthritis was induced by intradermal injection of CFA into the right hind paw of wistar rats. The paw volume was measured by a plethysmometer, thermal hyperalgesia was tested using tail flick analgesic meter, mechanical hyperalgesia was tested using Von Frey meter and paw thickness was measured by vernier caliper. The haematological, serum biochemical and antioxidant parameters were determined by using standard methods. The histology of right hind paw was carried out.

Results: In rats with adjuvant-induced arthritis, administration of galangin (5, 10, 20 mg/kg) and MEAO (100, 200, and 400mg/kg) significantly ((P<0.001) suppressed the paw swelling, increased the paw withdrawal latency, and reduced the paw thickness. Diclofenac was taken as standard. Galangin (20mg/kg) and MEAO (400mg/kg) inhibited the increased level of serum lysosomal enzyme activity *viz*. aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phophatase (ALP). Galangin (10 and 20 mg/kg), MEAO (200 and 400 mg/kg) exhibit anti-arthritic activity by improving the altered haematological parameters (WBC, RBC, platelets and Hb). Histopathological examination showed reduced cartilage destruction, influx of inflammatory cells, pannus formation, fibrin deposition, synovitis and chronic inflammation in galangin and MEAO treated arthritic rats.

Conclusion: It is concluded that galangin and MEAO has significant anti-arthritic activity in CFA induced arthritis in rats.

Keywords: *Alpinia officinarum*, Anti-arthritic, Complete Freund's Adjuvant

INTRODUCTION

The inflammatory process in rheumatoid arthritis provokes intense bone resorption, evidenced as bone erosions, juxta-articular osteopenia and generalized osteoporosis, these types of bone loss share a common pathogenesis, and the role of osteoclasts in focal bone erosion was verified in elegant animal studies [1]. However, chronic use of NSAIDs is associated with gastrointestinal toxicity, which has limited their use and led to the search for safer alternative agents [2].

It seems for that reason of relevance to develop new strategies for treating pain in muscle and joints, therefore need to develop medication that should be affordable and associated with minimum side effects [3].

Galangin is a naturally occurring flavonoid in *Alpinia officinarum* and which possessed mast cell-mediated allergic inflammation [4], prevents oxidative damage and has a down regulatory effect on the inflammatory pathway in liver of fructose-fed rats [5]. Also galangin was evaluated for antibacterial activity [6], *in vitro* acetylcholinesterase activity [7], anticlastogenic effect [8], antiviral [9], osteoclastic bone destruction and osteoclastogenesis effect [10]. Smaller galanga (*Alpinia officinarum Hance*) was traditionally employed in the treatment of rheumatism and whooping cough in children [11].

Pharmacologically 80% ethanolic extract of *Alpinia officinarum* was evaluated for anti-inflammatory, anti-nociceptive, and anti-psychiatric effects in CFA induced arthritis in rats [12]. However, the anti-arthritic activity of galangin isolated from methanolic extract of *Alpinia officinarum* has not been reported in CFA induced arthritis in rats. The objective of the study was to investigate the effect of isolated galangin and methanolic extract of *Alpinia officinarum* (MEAO) in complete freund's adjuvant-induced arthritis in rats.

MATERIALS AND METHODS

Plant material

The rhizomes of *Alpinia officinarum* were purchased from local market in Pune, in August 2012 and were identified by Dr. A.S.Upadhya, Scientist, Plant drug authentication service (Botany group) plant science division, Agharkar Research Institute, Pune Maharashtra, India. The voucher specimen is deposited in the same institute.

Animals

Female wistar rats (150-200 g) and female Swiss albino mice (20-25 g) were procured from National Institute of Biosciences, Pune. Animals were housed at 24 ± 1°C and relative humidity of 65 ± 10% and at standard environmental conditions (12 h light and 12 h dark cycle) in the animal house of the college. The animals were fed with standard pellet rodent diet and water was provided *ad libitum*. All the experimental protocols used in this study were approved by Institutional Animal Ethical Committee.

Preparation of methanolic extract of Alpinia officinarum (MEAO) rhizomes and isolation of galangin

The rhizomes were shade dried and powdered in hand mixer. The dried powder of the rhizomes of *Alpinia officinarum* (500 g) was extracted with 1.5L methanol as a solvent by cold percolation for 12 h in a 5L flat bottom flask at room temperature. The process of extraction was repeated three times with methanol. Each time the filtrate was concentrated *in vacuo* at 40°C using a rotary evaporator (Eqitron, Roteva), and pooled together to obtain 23.60 g of extract. The methanol extract (20.0 g) was redissolved in methanol: water (80:20) in 1 lit and partitioned with n-hexane followed by ethyl acetate in silica-gel column with (60-120 mesh). The material was eluted stepwise with a gradient of n-hexane-ethyl acetate. The ethyl

acetate fraction (9.5 g) was subjected to column chromatography over silica gel (100-200 mesh) by employing hexane-ethyl acetate gradient (0 -100%) as mobile phase with the increasing polarity of ethyl acetate. Four fractions were obtained (fraction I, II, III, IV). Fraction I on further purification by repeated column chromatography yield compound (1) as a yellow crystal (galangin), identification of which was performed by comparison of the spectral data of mass and nuclear magnetic resonance to that of reported compound [13].

Acute oral toxicity study

Healthy female Swiss albino mice were subjected to acute toxicity studies as per OECD guideline-425. The animals were fasted overnight and divided into group of 5 animals. Galangin (20mg/kg) and MEAO 2000 mg/kg were administered orally and the mice were observed continuously for behavioral and autonomic profiles for 2 hrs and for any sign of toxicity or mortality up to 48 hrs [14].

Complete Freund's adjuvant (CFA) induced arthritis in rats

Arthritis was induced by the intradermal injection of 0.1 ml of CFA (CFA, Sigma) in the right hind paw of female wistar rats of 150 to 200 g body weight. The animals were divided into nine groups of six animals each as, group 1 non-arthritic, group 2 arthritic control, group 3 arthritic animals treated with standard, diclofenac 5 mg/kg, p.o, group 4, 5, 6 were arthritic animals treated with test extract, MEAO 100, 200, 400 mg/kg, p.o, group 7, 8, 9 were arthritic animals treated with test compound, galangin 5, 10, 20 mg/kg, p.o. The dosing of all the groups was started from 12th day post CFA injection, once day [15]. The following parameters were measured at regular intervals on day 0, 1, 4, 8, 12, 16, 20, 24 and 28. Body weight, paw volume, joint diameter, thermal hyperalgesia, mechanical hyperalgesia and scored for arthritis severity according to a previously reported method of [16]. On 28th day, blood was withdrawn by retro-orbital puncture for biochemical assays. The animals were sacrificed on 28th day to study the antioxidant parameters and radiological and histological analysis of synovial joint.

Body weight

Body weight was recorded at every four days up to 28 days.

Paw volume

The development of arthritis was quantified by measuring volume of hind paws using a plethysmometer (UGO Basile, Italy). Paw volume (ml) was measured on days 0, 1, 4, 8, 12, 16, 20, 24 and 28 after arthritis induction. Data were expressed as the increase in volume on compounding day with respect to paw volume on day 0.

Joint diameter

Joint diameter was measured using a digital vernier caliper (Mitutoyo digimatic caliper, Japan) before adjuvant administration. The joint diameter was measured again on day (1, 4, 8, 12, 16, 20, 24 and 28).

Thermal hyperalgesia

Thermal hyperalgesia was induced using tail flick analgesy meter (UGO Basile, Italy). The animals were randomly divided in various groups and pretreated with test substance for 1 h. Radiant heat was applied to the planter surface of the hind paw until the rat lifted its paw. A photoelectric cell automatically tuned the heat surface off when the reflected light beam was interrupted (i.e. when the animal withdrew its paw) and the time at which this occurred was recorded as the paw withdrawal latency (PWL). The cut-off time was 15s.

Mechanical hyperalgesia

Mechanical hyperalgesia of hind paws was evaluated by Von Frey electronic meter (IITC, life science USA). Von Frey hairs (0.6 to 12.6g) were applied to planter surface to hind paws. A series of three stimuli were applied to each paw with each hair within a period 2-3 s. The lowest weight of Von Frey hair to evoke a withdrawal from the three consecutive applications was considered to indicate the threshold.

Biochemical assays

The blood was withdrawal from retro-orbital puncture to study the haematological parameters like haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and platelets were determined by using (Sysmax KX-21). The biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), and alkaline phophatase (ALP) were also determined as per the reported method [17].

Antioxidant parameters

At the last day of treatment rats were sacrificed and liver of individual rats were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris-HCl buffer (pH 7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA). SOD, GSH and malondialdehyde assay was performed as per the method [18-20].

Histological analysis

From sacrificed animals the right hind paws were removed and fixed in 10% buffered formalin. The paws were decalcified in 5% formic acid, processed for paraffin embedding, sectioned at 5µm thickness, and subsequently stained with haematoxylin-eosin [21, 22] for examination under a light microscope with 10x magnifications. Sections were observed for the presence of hyperplasia of the synovium, pannus formation and destruction of the joint space.

Statistical analysis

The data were analyzed by one way ANOVA followed by Dunnett's test, two way ANOVA followed by Bonferroni's post hoc test. All statistical analyses were performed using Graph Pad Prism software (San Diego, CA). Data was considered statistical significant at $P<0.05$.

RESULTS

The spectrometric identification of compound 1 was 3, 5, 7-Trihydroxy flavone (Galangin)

Yellow solid, mp: 213-215^{0}C [Lit.mp: 213-214^{0}C] FT-IR (KBr) cm^{-1}: 3410 (s), 1656 (m), 1450 (m), 1026 (s). ^{1}H-NMR (400 MHz), in CD_3OD: δ (ppm) 8.20 (2H, dd, H-2', 6'), 7.62 (2H, dd, H-3', 5'), 7.53 (1H, m, H-4'), 6.43 (*J*H8/H6= 1.5 Hz, d, 1H, H-8), 6.21 (1H, d, H-6). ^{13}C-NMR (100 MHz), in CD_3OD : δ (ppm) 149.5 (C-2), 141 (C-3), 180.2 (C-4), 168.5 (C-5), 101.9 (C-6), 165.2 (C-7), 97 (C-8), 160.9 (C-9), 107.2 (C-10), 135.2 (C-1'), 131.3 (C-2', 6'), 133.4 (C-3', 5'), 132 (C-4'). MS: [M+1$^+$] peak = 271.

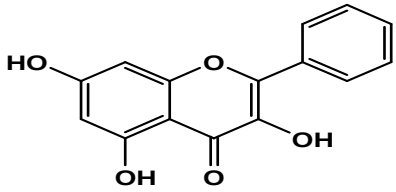


Fig 1: It shows chemical structure of 3, 5, 7-Trihydroxy flavones (galangin)

Acute oral toxicity

galangin 20 mg/kg p.o and MEAO at 2000 mg/kg did not produce any behavioral abnormalities and mortality. So the doses selected for further study were 5, 10 and 20 mg/kg for galangin and 100, 200 and 400 mg/kg for the MEAO.

Ant-arthritic activity

Effects of galangin and MEAO on body weight paw edema, joint diameter, thermal hyperalgesia and mechanical hyperalgesia in CFA induced arthritic rats

The overall reduction in body weight of all groups was non-significant (fig 2-A). Oral administration of galangin (5, 10 and 20 mg/kg), MEAO (100, 200 and 400 mg/kg) and diclofenac (5 mg/kg)

showed significant (P<0.001) inhibition of right hind paw edema on 28th day (fig 2-B). Diclofenac (5 mg/kg), MEAO (100, 200 and 400 mg/kg) and galangin (5, 10 and 20 mg/kg) showed significant (P<0.001) reduction of the right hind limb joint diameter compared with that of arthritic rat on 28th day (fig 2-C). Treatment with diclofenac (5 mg/kg), MEAO (200 and 400 mg/kg) and galangin (5, 10 and 20 mg/kg) showed significant (P<0.001) increase in paw withdrawal latency on 28th day, while the lower doses of MEAO showed moderate significant (P<0.01) reduction of the paw withdrawal latency (Figure 2-D). The oral treatment of diclofenac (5 mg/kg), MEAO (200 and 400 mg/kg) and galangin (10 and 20 mg/kg) significantly (P<0.001) suppressed the mechanical withdrawal threshold on 28th day, while the lower doses of MEAO and galangin showed lesser suppression of the mechanical withdrawal threshold on 28th day (fig 2-E).

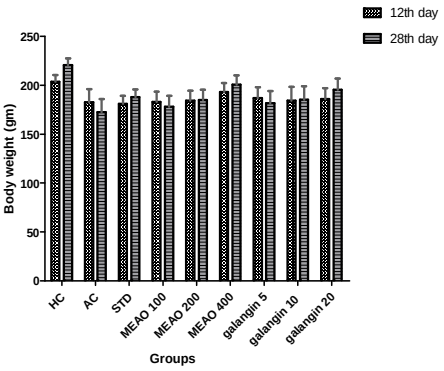


Fig. 2: It shows effect of galangin and MEAO on body weight (A).

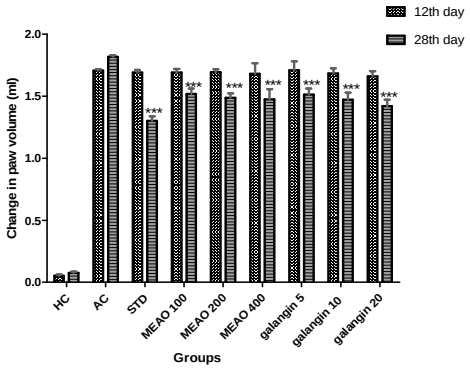


Fig. 2: It shows effect of galangin and MEAO on Right hind paw edema (B).

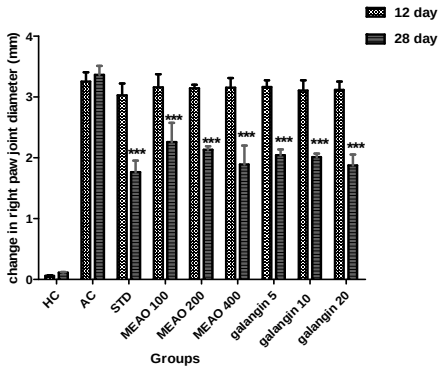


Fig. 2: It shows effect of galangin and MEAO on Right hind paw joint diameter (C).

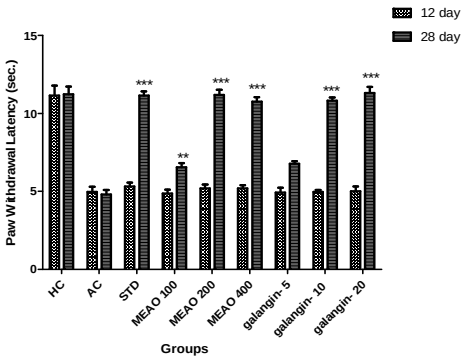


Fig. 2: It shows effect of galangin and MEAO on Right hind paw withdrawal latency (D).

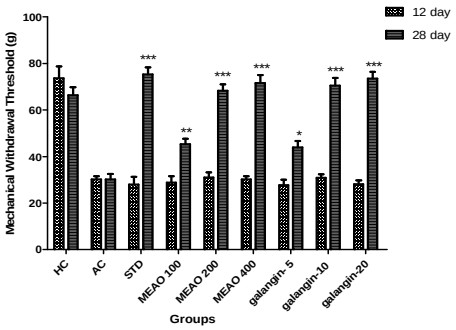


Fig. 2: It shows effect of galangin and MEAO on Right hind paw mechanical withdrawal threshold (E).

Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.

Effects of Galangin and MEAO on biochemical parameters in CFA-induced arthritic rats

The markers for cellular toxicity (serum ALT, AST, and ALP level) were significantly increased (P<0.001), while TP level significantly (P<0.001) decreased in arthritic rats compared with the non arthritic rats. All of these changes were completely modulated in arthritic rats that received Galangin and MEAO from day 12th onwards. Table-1 shows treatment with galangin and MEAO on biochemical parameters in arthritic rats. Galangin (20 mg/kg) showed significant (P<0.001) decrease in the serum AST, ALT, and ALP levels; whereas the TP level was less significantly (P<0.001) increased as compared to arthritis control, while MEAO (100, 200, 400 mg/kg) showed dose dependent modulation in serum AST, ALT, ALP and TP levels. Diclofenac (5mg/kg) showed insignificant reduction in the serum AST, ALT and ALP levels; whereas TP level showed insignificant increase compared to arthritis control.

Effects of galangin and MEAO on haematological parameters of CFA-induced arthritic rats

Arthritic rats showed significant (P<0.001) reduction in hemoglobin and RBC, on the other hand WBC and platelets count were significantly (P<0.01) increased compared to nonarthritic rats. Diclofenac (5 mg/kg), MEAO (400 mg/kg) and galangin (20 mg/kg) significantly (P<0.001) increased the hemoglobin and RBC count. The WBC and platelet counts were significantly (P<0.001) reduced by high doses of galangin (20 mg/kg).

Effects of galangin and MEAO on antioxidant parameters of CFA-induced arthritic rats

Arthritic rats showed significant (P<0.001) reduction in liver SOD and GSH level and increased MDA level compared to that in liver of

healthy control. Oral administration of galangin (20 mg/kg) showed significant (P<0.001 and P<0.01) increase in SOD and GSH level respectively, while MDA level was significantly (P<0.001) decreased in the liver of arthritic rats. Treatment of MEAO (200 and 400mg/kg) showed dose dependent increase in SOD and GSH level respectively, while MDA level was decreased in the liver of arthritic rats. Diclofenac 5 mg/kg also produced a significant (P<0.001) increase in SOD activity and a reduction of MDA level in the liver of arthritic rats.

Histopathology of Synovial joint

Fig- 3 shows histopathology of synovial joint of normal rats has intact morphology of synovium. No inflammation and influx of inflammatory cells was observed. Arthritic rats showed cartilage destruction, influx of inflammatory cells, pannus formation, fibrin deposition, synovitis and chronic inflammation. Diclofenac treated rats showed protection against cartilage destruction, vascular proliferation and synovial space thickening, low influx of inflammatory cells and no pannus formation. MEAO (400 mg/kg) treated rats showed lesser cartilage destruction, synovial space thickening, vascular proliferation, low influx of inflammatory cells and no pannus formation. MEAO (200 mg/kg) treated rats showed moderate cartilage destruction and synovial space thickening and influx of few inflammatory cells. MEAO (100 mg/kg) treated rats showed minimal inflammation, influx of few inflammatory cells in synovium with evidence of disturbed synovial lining or pannus formation.

Galangin (5 mg/kg) treated rats showed moderate cartilage destruction and synovial space thickening, influx of few inflammatory cells. Galangin (10 mg/kg) treated rats showed lesser cartilage destruction, synovial space thickening, vascular proliferation, low influx of inflammatory cells and no pannus formation. Galangin (20 mg/kg) treated rats showed protection against cartilage destruction, vascular proliferation and synovial space thickening, low influx of inflammatory cells and no pannus formation.

Table 1: It shows effect of oral administration of galngin and MEAO on biochemical parameters in arthritic rats

Treatment Groups	Dose.Mg/kg	Ast (u/l)	Alt (u/l)	Alp (u/l)	Tp (gm/dl)
Healthy control		**41.1 ± 1.89**	**53.5 ± 1.76**	**74.7 ± 1.88**	**7.25 ± 0.225**
Arthritic control		124 ± 2.83#	187 ± 1.99#	448 ± 2.99#	5.63 ± 0.229#
Diclofenac	5	117 ± 3.33	181 ± 2.21	438 ± 3.68	6.15 ± 0.263
MEAO	100	117 ± 3.65	180 ± 1.54	442 ± 1.36	5.83 ± 0.180
	200	113 ± 1.88*	175 ± 2.72**	436 ± 2.24*	6.05 ± 0.132
	400	111 ± 1.64**	165 ± 2.17***	433 ± 1.95**	6.60 ± 0.280**
galangin	5	113 ± 1.52*	177 ± 2.11*	436 ± 3.02*	5.85 ± 0.144
	10	110 ± 4.09**	173 ± 1.51***	434 ± 4.01*	6.33 ± 0.170
	20	87 ± 2.73***	157 ± 1.85***	400 ± 1.86***	7.20 ± 0.129***

Data are expressed as mean ± S.E.M.; n=6 rats per group. One way ANOVA followed by Dunnett's test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001 and when compared with healthy control group #P<0.001.

Table 2: It shows effect of oral administration of galngin and MEAO on haematological parameters in arthritic rats

Treatment Groups	Dose Mg/kg	Hb (gm/100ml)	Rbc (million/µl)	Wbc (thousands/µl)	Platelet (lacks/µl)
Healthy control		14.4 ± 0.322	6.85 ± 0.113	7.63 ± 0.212	9.24 ± 0.132
Arthritic control		8.90 ± 0.182#	3.27 ± 0.074#	15.4 ± 0.255#	18 ± 0.127#
Diclofenac	5	13.2 ± 0.152***	6.03 ± 0.119***	14.3 ± 0.196**	16.9 ± 0.287*
MEAO	100	9 ± 0.180	3.16 ± 0.121	15.3 ± 0.058	18 ± 0.118
	200	10.2 ± 0.527*	4.25 ± 0.294*	14.5 ± 0.114*	17 ± 0.315*
	400	13.2 ± 0.327***	4.89 ± 0.404***	14.3 ± 0.327**	16.7 ± 0.271**
Galangin	5	9.43 ± 0.206	3.66 ± 0.189	14.5 ± 0.244*	17.4 ± 0.274
	10	10.3 ± 0.146**	4.36 ± 0.242**	14.3 ± 0.195**	17.1 ± 0.164
	20	11.5 ± 0.246***	5.30 ± 0.147***	13 ± 0.129***	16.1 ± 0.089***

Data are expressed as mean ± S.E.M.; n=6 rats per group. One way ANOVA followed by Dunnett's test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001 and when compared with healthy control group #P<0.001.

DISCUSSION

Freund's adjuvant (a mixture of heat killed *Mycobacterium tuberculosis* with liquid paraffin) formed inflamed lesions in areas of the body [23]. CFA is commonly used for preclinical studies of NSAIDs and anti-rheumatic drugs and this model is most appropriate as like human arthritis [24].

In arthritis different inflamantory mediators were involved which are the products of arachadonic acid metabolism, histamine, 5-HT, bradykinin, cytokines, and nitric acid.

CFA produces a characteristic inflammation and associated hyperalgesia, which can be used to quantify the anti-inflammatory or anti-hyperalgesic actions of drugs [25].

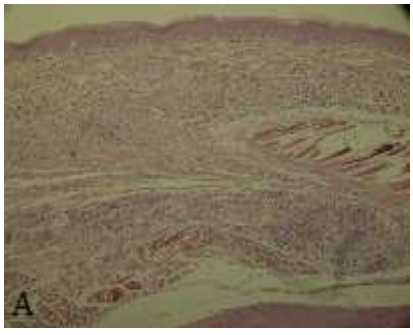
Mediators, like bradykinin, which are released from injured tissue directly stimulate nociceptors, and stimulate tumour necrosis factor-alpha (TNF-α) release. The TNF-α in turn, stimulates the release of interleukin-1beta (IL-1β) and interleukin-6 (IL-6), promoting the initiation of cycloxygenase enzymes, which convert arachidonic acid to prostaglandins [26]. Tumour necrosis factor-alpha (TNF-α) also stimulates the release of cytokine-induced neutrophil chemoattractant (CINC-1) in rats or interleukin-8 (IL-8) in humans.

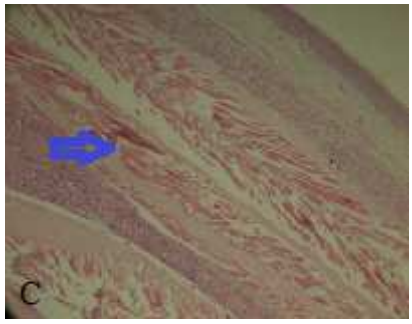
Cytokines, like IL-1β, TNF-α and IL-6, plays imp role in rhumatoid arthritis [27], these cytokines plays imp role in hyperalgesia by sensitizing peripheral nociceptors, decreasing the peripheral nociceptor threshold [28]. As per the results galangin and MEAO were significant to treat CFA induced arthritis.

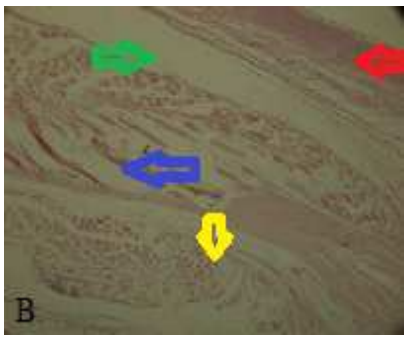
Table 3: It shows effect of oral administration of galngin and MEAO on antioxidant parameters in arthritic rats

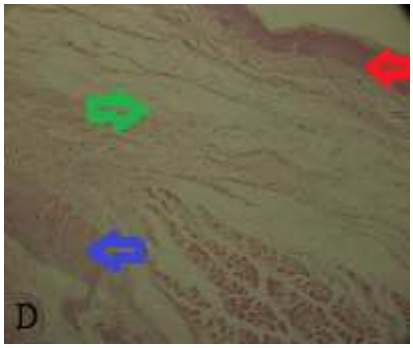
Treatment Groups	Dose Mg/kg	Mda (nmole mda/mg protein)	Sod (mu/mg protein)	Gsh (µmole/mg protein)
Healthy control		1.99 ± 0.0187	4.43 ± 0.0208	70.6 ± 0.946
Arthritic control		3.44 ± 0.0208$^{\#}$	2.41 ± 0.0165$^{\#}$	44.9 ± 1.45$^{\#}$
Diclofenac	5	2.97 ± 0.0284***	2.93 ± 0.0171***	56.2 ± 0.928***
MEAO	100	3.41 ± 0.0307	2.44 ± 0.0246	48.7 ± 1.20
	200	3.33 ± 0.0175*	2.54 ± 0.0397*	48.2 ± 2.40
	400	3.31 ± 0.0175**	2.57 ± 0.0359**	51.6 ± 1.43*
galangin	5	3.35 ± 0.0132**	2.49 ± 0.0229	45.8 ± 0.888
	10	3.32 ± 0.0165**	2.54 ± 0.0314*	50.1 ± 1.18
	20	3.31 ± 0.0189***	2.83 ± 0.0384***	52.9 ± 1.89**

Data are expressed as mean ± S.E.M.; n=6 rats per group. One way ANOVA followed by Dunnett's test when compared with arthritic control group $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ and when compared with healthy control group $^{\#}P<0.001$.









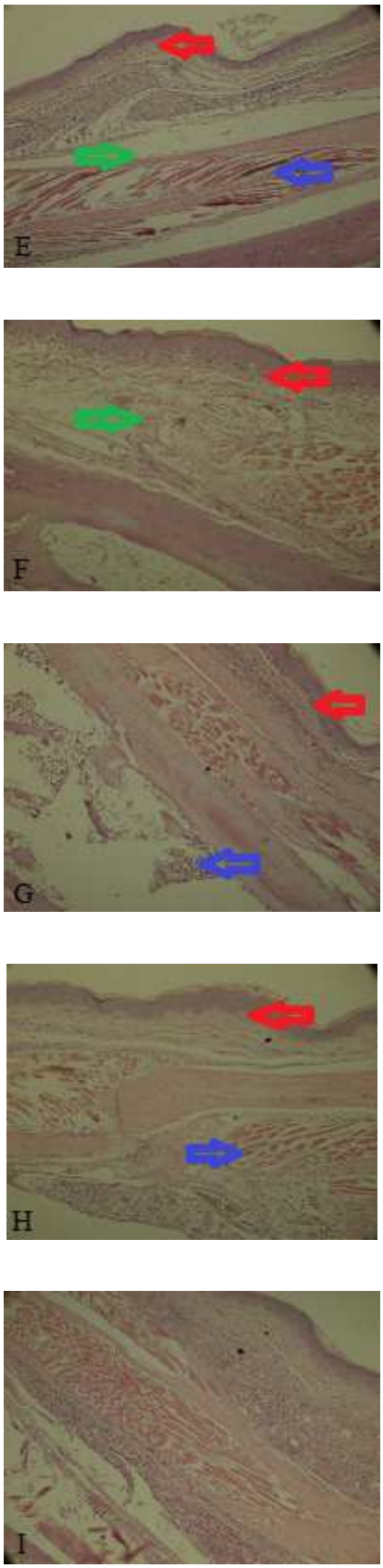


Fig. 3: It shows histopathology of synovial joint. (A) Normal non-arthritic, (B) Arthritic control, (C) Diclofenac 5 mg/kg, (D) MEAO 100 mg/kg, (E) MEAO 200 mg/kg, (F) MEAO 400 mg/kg, (G) galangin 5 mg/kg, (H) galangin 10 mg/kg, (I) galangin 20 mg/kg. (Red colour – Synovitis, Green colour – Synovial space, Blue colour – Pannus, Yellow colour – Cartiladge destruction)

Free radicals production that occurs during development of arthritis in the articular cartilage leads to decreased GSH and SOD levels, increased ROS levels in rhumatoid arthritis may result in a pro-oxidation environment, which in turn could result in increased MDA levels. As a result, lipid peroxidation may have a role in the pathogenesis of the rhumatoid arthritis [29]. Pathogenesis of arthritis is associated predominantly with the formation of free radicals at the site of inflammation. In rheumatic condition oxidative injury and inflammatory status was confirmed by increased levels of prostaglandins in serum and synovial fluid compared to controls. T cells isolated from the synovial fluid of patients with rheumatoid arthritis showed signs of decreased intracellular GSH level [30]. In the present study, the levels of SOD and GSH were increased, while the level of MDA was reduced by galangin and MEAO. From the results it is clear that reduction in RBC count and hemoglobin level represents the anemic condition in arthritic rats. More significant causes are the irregular storage of iron in the reticuloendothelial system and synovial tissue and the breakdown of bone marrow to respond to anemia [31]. Anaemia is the most common haematological deformity seen in patients with rheumatoid arthritis [32]. Inflammation causes increase in the WBC [33]. The increase in both WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism [34]. It is clear by the infiltration of inflammatory mononuclear cells in the joints of arthritic rats. In the present study, the level of Hb and RBC was significantly increased, while the level of WBC and platelets was significantly reduced by galangin and MEAO.

Lysosomal enzymes play an important role in the physiology and pathology of the joint tissues in arthritis [35]. Measurement of their level provide an excellent tool for anti-arthritic activity of drugs, the activities of aminotransferases and ALP were significantly increased in arthritic rats, since these are excellent indices of liver impairment, which are also measured as the features of adjuvant arthritis [36, 37]. Treatment with galangin and MEAO significantly ($P<0.001$) decreased the levels of ALT, AST, and ALP in arthritic cases.

CONCLUSION

It is concluded that galangin (10 and 20 mg/kg) and MEAO (200 and 400 mg/kg) possess anti-arthritic activities in animals. The possible mechanisms may be due to inhibition of release of mediators of inflammation and antioxidant activity.

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