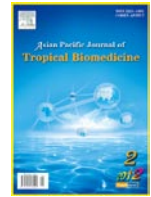




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# Analgesic, anti-inflammatory and anti-arthritic activity of *Cassia uniflora* Mill.

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### ABSTRACT

**Objective:** To evaluate the analgesic, anti-inflammatory and anti-arthritic activity of leaves of *Cassia uniflora* (*C. uniflora*) Mill. **Methods:** Petroleum ether, ethyl acetate and methanolic extract of *C. uniflora* (100 and 200 mg/kg, body weight) was screened for analgesic (Eddy's hot plate and acetic acid induced writhing), anti-inflammatory (Carrageenan induced paw edema) and anti-arthritic (Complete Freund's Adjuvant induced arthritis). In Complete Freund's Adjuvant arthritis model degree of inflammation was evaluated by hind paw swelling, body weight, and biochemical parameters and supported by radiological analysis. **Results:** Treatment with extracts of *C. uniflora* showed significant ( $P < 0.05$ ) and dose dependant increase in paw licking time in Eddy's hot plate method. In writhing test, extracts were significantly reduced the number of writhes. A dose dependant and significant inhibition of edema was observed in carrageenan induced paw edema. Petroleum ether extract at a dose of 100 mg/kg body weight showed most potent and significant activity which is supported by the results of body weight, biochemical parameters and radiological analysis in complete Freund's Adjuvant arthritis model. **Conclusion:** The extract possesses analgesic, anti-inflammatory and anti-arthritic activity which may be mediated through the phytochemical constituents of the plant.

## 1. Introduction

*Cassia uniflora* (*C. uniflora*) (Mill.) is an herb belonging to family Caesalpiniaceae[1]. The young leaves of the plant eaten as vegetables. The dried stems can be used as fuel. A poultice of the leaves is applied to wounds and the extract is reported to heal specific types of eczema. The roots are used to combat dropsy. The *C. uniflora* has not been evaluated for its chemical composition but it was reported to contain protein, polyphenols and alpha galactosidase[2]. Traditionally it is reported that leaves of *C. uniflora* are found useful as anti-inflammatory and in wound healing[3]. Hence present work was undertaken to check potential of *C. uniflora* leaves in the treatment of inflammation and

arthritis.

## 2. Materials and methods

### 2.1 Plant material and preparation of extracts

*Cassia uniflora* (Mill.) plant was collected from Gunjalwadi region of Sangamner; district Ahmednagar in the month of Aug 2010 and authenticated by Dr. P. G. Diwakar, Scientist 'E' and Head, Botanical Survey of India (BSI), Koregaon Road, Pune. A voucher specimen was deposited in BSI under reference number S. S. C-1. (BSI/WRC/Tech/2010/439). The leaves of *C. uniflora* (Mill.) were shade dried and coarsely powdered. The powdered leaves (500 g) were successively extracted with petroleum ether, ethyl acetate and methanol using Soxhlet apparatus. The extracts were concentrated under reduced pressure.

### 2.2 Animals

All experimental work was carried out using Swiss albino mice (20–25 g) and Wistar rats (180–200 g). The animals

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were maintained under standard laboratory conditions (room temperature 24–27 °C and humidity 60%–65%) with 12 h light and dark cycle. The animals were given a standard laboratory diet *ad libitum* and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (AVCOP/32/2010–11).

### 2.3 Acute toxicity study

Acute toxicity study was carried out as per the guideline set by the Organization for Economic Co-operation Development (OECD guideline 425) received from the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA). Animals were divided into six groups ( $n=6$ ). The animals were fasted for overnight with free access to food and water. The petroleum ether extract, ethyl acetate extract and methanolic extract was administered orally in doses of 500 mg/kg and 1 000 mg/kg of body weight to different groups of mice and observed over 14 d for mortality and physical or behavioral changes[4].

### 2.4 Analgesic Activity

#### 2.4.1 Eddy's hot plate method

The initial reaction time of all the animals of control and test groups were recorded by putting them on the hot plate maintained at  $(55.0\pm 0.5)$  °C. Licking of paw or jumping was taken as the index of reaction to heat. The albino mice were divided into eight groups. Petroleum ether extract or ethyl acetate extract or methanolic extract of *C. uniflora* at a dose of (50 and 100 mg/kg body wt. each) and pentazocine lactate injection (20 mg/kg body wt.) was administered by intra peritoneal route. The first group was served as control and received only vehicle (1% dimethyl formamide in water for injection). The post treatment reaction time of each animal was recorded at 30, 60, 90, 120 and 180 min. The animals were removed from hot plate soon after they exhibited jumping. Cut off time was 20 s[5].

#### 2.4.2 Acetic acid induced writhing

The albino mice were divided into eight groups. Petroleum ether extract or ethyl acetate extract or methanolic extract of *C. uniflora* at a dose of (50 and 100 mg/kg body wt., i.p. each) and diclofenac sodium (20 mg/Kg body wt., i.p.) was administered one hour prior to intra peritoneal injection of 0.1 mL of 0.6% v/v acetic acid. Five minutes after the intra peritoneal injection of acetic acid, the number of writhing during the following 20 min. was counted. Control mice were received only vehicle (1% dimethyl formamide in water for injection). Finally the percent analgesic effect was determined. The number of writhing and stretching's was recorded and the percentage was calculated[6].

### 2.5 Anti-inflammatory activity

Albino rats of wistar strain of either sex were divided into eight groups. Acute inflammation was produced by sub plantar injection of 0.1 mL of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of rats, one hour after oral administration of petroleum ether extract, ethyl acetate extract and methanolic extract (50 and 100 mg/kg body wt. each) and diclofenac sodium (20 mg/kg body wt.) was administered by intraperitoneal route. Control rats were received only vehicle (1% dimethyl formamide in water for injection).

The paw volume was measured plethysmometrically (Medicaid Digital Volume Meter) at 0, 1, 2, and 3 h after the

carrageenan injection. The difference between '0' readings and readings after 1, 2, and 3 h respectively was taken as the volume of edema[7]. Percentage inhibition of edema was calculated.

### 2.6 Anti arthritic activity

Albino rats of wistar strain of either sex were divided into eight groups. The first group represented control rats. The second group received the standard drug diclofenac sodium at a dose of 10 mg/kg. The 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup> groups receive petroleum ether, ethyl acetate, methanolic extracts (50 and 100 mg/kg, each) respectively by oral route. After 30 min. injecting 0.1 mL Complete Freund's Adjuvant (Sigma, U.S.A) into the sub plantar region of left hind paw on day '0'. Saline or extracts were administered orally once daily, from the initial day i.e. from the day of adjuvant injection (0 day) and continued till 21<sup>st</sup> day[8].

The anti-arthritic effect of the extracts as well as diclofenac sodium evaluate by measuring paw volume of inject paw on 4<sup>th</sup>, 8<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of study by using digital plethysmometer. The mean changes in injected paw volume with respect to initial paw volume are calculated on respective days and % inhibition of paw volume with respect to control group is calculated. The changes in body weight record daily. On the day 22<sup>nd</sup> blood is withdrawn from the each animal through retro-orbital vein puncture by anaesthetizing the animals with ketamine. The blood is collect into vials containing EDTA and the biochemical parameters like haemoglobin content, total WBC count, differential WBC count, ESR and RBC analysed[9].

### 2.7 Radiological analysis

The animals X-rays were taken at the joints of the hind paw of the animals for evaluating the bone damage. Radiographs were taken using X-ray apparatus (Siemen–60 MA, Germany) and industrial X-ray film (Fuji photo film, Japan).

The X-ray apparatus was operated at 220 V with a 40 V peak, 0.2 s exposure time and a 60 cm tube-to film distance for anterior–posterior projection.

### 2.8 Statistical analysis

The data were expressed as Mean $\pm$ SEM. Results were analyzed statistically by one way ANOVA followed by Dunnett's test.

## 3. Results

### 3.1 Acute toxicity

From the acute toxicity study, the LD<sub>50</sub> cut-off dose for extracts was found to be 1 000 mg/kg body weight. Hence, the therapeutic doses were taken as 50 mg/kg and 100 mg/kg body weight.

### 3.2 Analgesic activity

#### 3.2.1 Eddy's hot plate method

The effect of petroleum ether extract, ethyl acetate extract, methanolic extract of *C. uniflora* at (50 and 100 mg/kg, i.p.) and pentazocine lactate injection (20 mg/kg, i. p) were evaluated for central analgesic activity. Table 1 shows the results on thermal stimulus induced pain (Eddy's hot plate) in mice. Pretreatment with Pentazocine or extracts did not

**Table 1**Effect of different extracts of leaves of *C. uniflora* Mill. On thermal stimulus induce pain in mice (Hot plate test).

Treatment	Latency to lick the paws (Sec.) S.E.M					
	0 min	30 min	60 min	90 min	120 min	180 min.
Control	1.19±0.14	2.99±0.07	4.74±0.07	5.86±0.70	6.48±0.27	5.63±0.38
Pentazocin	2.33±0.05	3.24±0.13	9.76±0.08**	10.66±0.09**	12.43±0.24**	9.64±0.09**
PE(50 mg/kg)	3.35±0.34	6.93±0.26**	10.62±0.20**	9.03±0.89*	9.60±0.62*	6.61±0.05
PE (100 mg/kg)	2.82±0.12	8.09±0.28**	12.33±0.24**	12.43±0.22**	14.66±0.20**	7.75±0.22
EA (50mg/kg)	2.74±0.14	8.60±0.98*	9.13±0.96**	10.13±1.09**	12.79±1.20**	7.74±1.08
EA (100 mg/kg)	3.39±0.17	5.45±0.39	8.20±0.40**	10.86±0.68**	13.50±0.37**	5.17±1.57
ME(50 mg/kg)	3.63±0.05	4.50±0.12	7.55±0.56*	8.06±0.45*	10.17±1.57**	6.54±1.55
ME(100 mg/kg)	3.49±0.16	4.38±0.37	5.95±0.94	10.79±0.34**	11.50±1.83**	6.60±1.18

Values are mean ± S. E. M,  $n=6$ , \* $P<0.05$ , significant as compared to control. PE– Petroleum ether extract, EA– Ethyl acetate extract, ME– Methanolic extract.

produce any significant changes in paw licking time in the early phase of pain. However in the late phase, a dose dependent and significant ( $P<0.01$ ) increase in licking time was observed in mice treated with extracts and Pentazocine. The maximum activity was observed with petroleum ether (100 mg/kg) and ethyl acetate extract (100 mg/kg) at the 120 min. time interval when compared to the standard pentazocine. The maximum analgesia induced by methanolic extract (100 mg/kg) was at the 60 min. time interval.

### 3.2.2 Writhing test method

The peripheral analgesic activity of *C. uniflora* was determined by acetic acid induced writhing method (Table 2). Pretreatment with petroleum ether extract, ethyl acetate extract and methanolic extract at the dose of (50 and 100 mg/kg, i.p.) and standard diclofenac sodium injection (20 mg/kg, i.p.) significantly reduced the number of writhes.

**Table 2**Effect of different extracts of leaves of *C. uniflora* Mill. on acetic acid induced writhing test in mice.

Treatment	Mean ± S. E. M	Inhibition (%)
Control	31.750 0 ± 3.400 0	–
Diclofenac sodium	3.000 0 ± 0.707 1**	90.55
PE (50 mg/kg)	13.250 0 ± 0.853 9**	58.26
PE (100 mg/kg)	6.000 0 ± 0.912 9**	81.10
EA (50 mg/kg)	13.000 0 ± 1.291 0**	59.05
EA (100 mg/kg)	7.250 0 ± 1.652 0**	77.16
ME (50 mg/kg)	10.750 0 ± 0.853 9**	62.48
ME (100 mg/kg)	4.750 0 ± 0.478 7**	85.03

**Table 3**Effect of different extracts of leaves of *C. uniflora* Mill. on carrageenan induced rat paw edema.

Treatment	Paw volume (mL) % inhibition at 3 h				
	0 h	1 h	2 h	3 h	
Control	0.180 ± 0.009	0.160 ± 0.006	0.290 ± 0.009	0.180 ± 0.009	–
Diclofenac sodium	0.350 ± 0.076	0.410 ± 0.041	0.260 ± 0.049	0.076 ± 0.006**	68.19%
PE (50 mg/kg)	0.280 ± 0.081	0.240 ± 0.056	0.055 ± 0.016**	0.076 ± 0.016**	58.91%
PE (100 mg/kg)	0.190 ± 0.056	0.300 ± 0.030	0.160 ± 0.027	0.065 ± 0.011**	64.86%
EA (50 mg/kg)	0.330 ± 0.034	0.360 ± 0.060*	0.320 ± 0.087	0.078 ± 0.003**	57.83%
EA (100 mg/kg)	0.310 ± 0.032	0.300 ± 0.040	0.190 ± 0.048	0.073 ± 0.003**	62.16%
ME (50 mg/kg)	0.410 ± 0.051*	0.360 ± 0.043*	0.160 ± 0.018	0.071 ± 0.010**	61.62%
ME (100 mg/kg)	0.380 ± 0.078	0.290 ± 0.044	0.180 ± 0.053	0.130 ± 0.052	29.18%

Values are mean ± S. E. M,  $n=6$ , \* $P<0.05$ , significant as compared to control. PE– Petroleum ether extract, EA– Ethyl acetate extract, ME– Methanolic extract.

Values are mean ± S. E. M,  $n=6$ , \* $P<0.05$ , significant as compared to control. PE– Petroleum ether extract, EA– Ethyl acetate extract, ME– Methanolic extract

### 3.3 Anti-inflammatory activity

The result (Table 3) obtained with extract and standard diclofenac showed significant ( $P<0.01$ ) inhibition of inflammatory edema at the first and second hours after the carrageenan treatment. The effect of petroleum ether at a dose 100 mg/kg, ethyl acetate at 50 mg/kg was comparable to standard diclofenac. The dose dependent inhibition of edema was observed with petroleum ether and ethyl acetate treatment.

### 3.4 Anti-arthritis activity

After inoculation with the CFA suspension, the vehicle treated rats developed visible clinical signs of arthritis characterized by edema and/or erythema in paws around day 7. The arthritis continued to grow until day 21 after CFA injection (Table 4). In the animals treated with the different extracts of *Cassia uniflora* leaves, the inflammatory response was clearly reduced. Administration of 50 and 100 mg/kg of the petroleum ether, ethyl acetate and methanolic extract lead to a significant ( $P<0.05$ ) decrease in the paw volume on days 8, 14 and 21. The decrease in the WBC count and ESR was observed with the animals treated with petroleum ether extract (50 & 100 mg/kg) and ethyl acetate extract (50 & 100 mg/kg) respectively, while increase in RBC count and haemoglobin was observed with petroleum ether and ethyl acetate extract, when compared with the control (Table 5).

**Table 4**Effect of different extracts of *C. uniflora* on Freud's adjuvant induced arthritis in rats.

Treatment	Paw volume (mL)				
	0 <sup>th</sup> day	4 <sup>th</sup> day	8 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
Control	0.310 ± 0.012	0.290 ± 0.013	0.280 ± 0.013	0.270 ± 0.011	0.240 ± 0.010
Diclofenac sodium	0.290 ± 0.013	0.210 ± 0.012*	0.140 ± 0.011**	0.063 ± 0.004**	0.008 ± 0.001**
PE (50 mg/kg)	0.180 ± 0.027	0.140 ± 0.013**	0.080 ± 0.020**	0.063 ± 0.008**	0.028 ± 0.006**
PE (100mg/kg)	0.230 ± 0.017	0.180 ± 0.090**	0.140 ± 0.007**	0.085 ± 0.005**	0.043 ± 0.003**
EA (50 mg/kg)	0.280 ± 0.010	0.250 ± 0.012	0.240 ± 0.012*	0.220 ± 0.001**	0.190 ± 0.015**
EA (100 mg/kg)	0.270 ± 0.013	0.240 ± 0.011*	0.220 ± 0.009**	0.210 ± 0.0079**	0.170 ± 0.010**
ME (50 mg/kg)	0.200 ± 0.028	0.173 ± 0.021**	0.130 ± 0.018**	0.056 ± 0.005**	0.025 ± 0.003**
ME (100 mg/kg)	0.230 ± 0.020	0.180 ± 0.025**	0.140 ± 0.027**	0.085 ± 0.010**	0.061 ± 0.013**

Values are mean ± S. E. M, n=6, \*P<0.05, significant as compared to control. PE– Petroleum ether extract, EA– Ethyl acetate extract, ME– Methanolic extract.

**Table 5**

Effect of biochemical parameters on adjuvant-induced arthritis in rats.

Group	Total WBC count (mm <sup>3</sup> )	RBC (million/mm <sup>3</sup> )	Haemoglobin (g/dL)	ESR (mm/h)
Control	14 700.000 ± 1.195	6.660 ± 0.007	12.000 ± 0.785	11.100 ± 0.763
Diclofenac sodium	6 160.000 ± 1.054	8.830 ± 0.022	15.500 ± 0.545	4.500 ± 0.223
PE (50mg/kg)	6 760.000 ± 1.065	9.180 ± 0.023	12.300 ± 0.354	8.300 ± 0.753
PE (100mg/kg)	6 900.000 ± 2.556	7.280 ± 0.044	12.100 ± 0.132	6.000 ± 0.477
EA (50mg/kg)	9 980.000 ± 3.000	7.361 ± 0.067	13.300 ± 0.453	3.200 ± 0.223
EA (100mg/kg)	9 220.000 ± 0.764	8.200 ± 0.007	13.400 ± 0.634	4.320 ± 0.365
ME (50mg/kg)	10 540.000 ± 0.880	7.510 ± 0.103	10.900 ± 0.354	6.600 ± 0.743
ME (100mg/kg)	6 630.000 ± 0.754	7.440 ± 0.028	12.900 ± 0.532	5.000 ± 0.365

**Table 6**

Mean changes in body weight in adjuvant-induced arthritis in rats.

Treatment	Body weight (g)		Mean changes in body weight
	0 <sup>th</sup> day	21 <sup>st</sup> day	
Control	175.16	182.83	8.660 0 ± 0.918 9
Diclofenac sodium	166.83	210.33	42.160 0 ± 1.014 0
PE (50 mg/kg)	165.33	192.33	27.830 0 ± 1.470 0
PE (100 mg/kg)	167.33	197.33	29.830 0 ± 1.990 0
EA (50 mg/kg)	166.33	185.33	15.660 0 ± 0.714 9
EA (100 mg/kg)	166.33	187.83	21.500 0 ± 0.562 7
ME (50 mg/kg)	165.60	194.00	29.660 0 ± 2.155 0
ME (100 mg/kg)	164.00	194.33	30.330 0 ± 1.116 0

There was markedly improved the loss of body weight (Table 6) compared to the vehicle-treated arthritic.

#### 4. Discussion

The hot-plate tests are useful in elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. The significant increase in pain threshold produced by petroleum ether extract of *C. uniflora* suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in inflammation and pain[10,11].

The abdominal constriction response induced by acetic

acid is a sensitive procedure to evaluate peripherally acting analgesics. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2 $\alpha$  in peritoneal fluids as well as lipoxigenase products[12]. The significant reduction in acetic acid-induced writhes by petroleum ether extract, ethyl acetate extract and methanolic extract of *C. uniflora* Mill. suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3 h)

is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages[13, 14]. The significant ( $P<0.05$ ) suppressive activity of the different extracts of *C. uniflora* leaves in late phase shows its potent anti-inflammatory effect. This result is quite similar to the one observed for diclofenac at 20 mg/kg, which inhibited the edema by 68.19%.

The results were statistically significant ( $P<0.05$ ). Ueno *et al* found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates[15]. Besides, in the carrageenan-induced rat paw oedema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism[16]. Prostaglandin-E2 (PGE2), a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation. Therefore, it is suggested that the mechanism of action of the extracts may be related to histamine and prostaglandin synthesis inhibition.

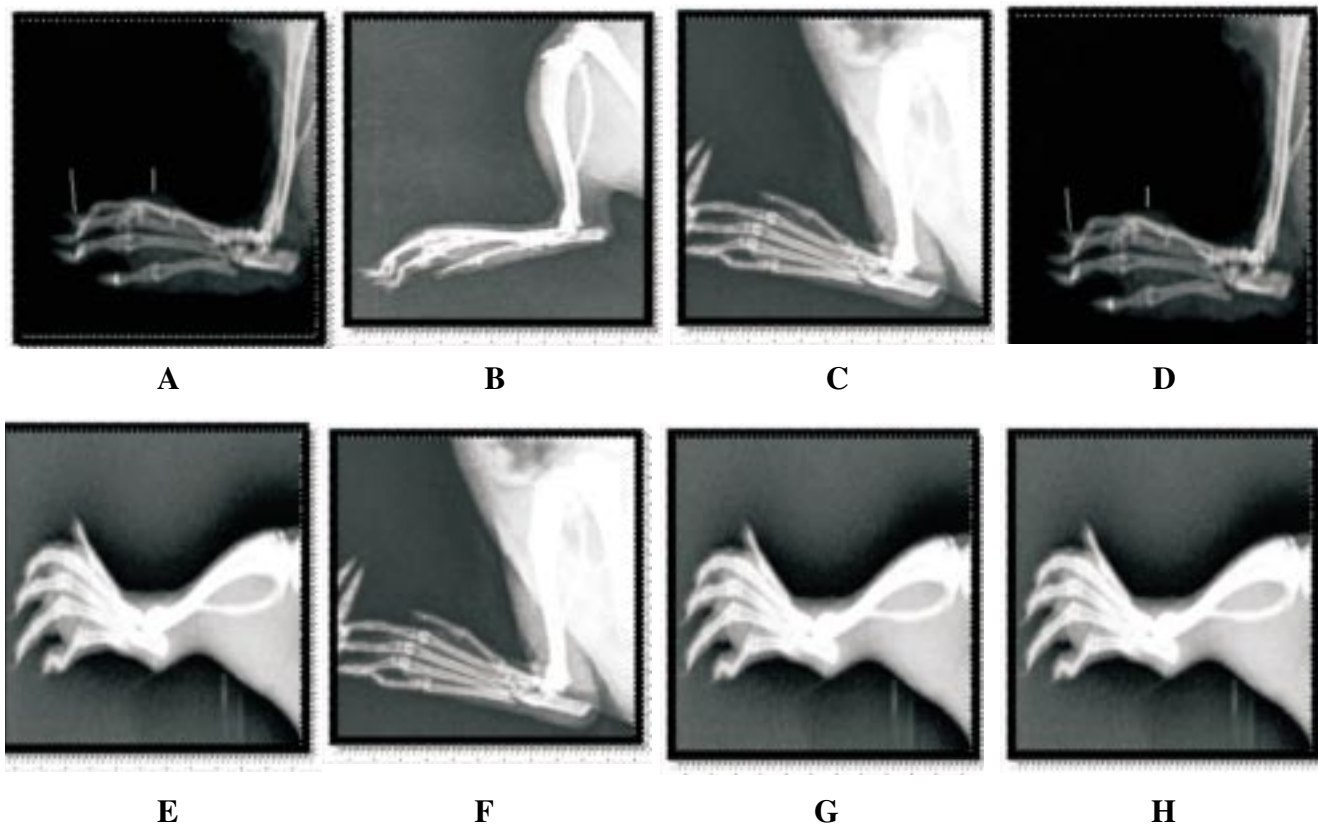
The results of the present study also indicate that the different extracts of *C. uniflora* leaves exhibits anti-arthritic effects in rats with Freund's adjuvant-induced arthritis. The model of adjuvant induced arthritis in rats has been extensively used in the study of inflammatory processes[17]. Freund Adjuvant is an antigen solution emulsified in mineral oil that is used as an immune-potentiator. The

complete form (CFA) is composed of inactivated and dried mycobacteria and is effective in stimulating cell mediated immunity and may lead to the potentiation of the production of certain immunoglobulins. Shortly after the administration of CFA into hind paw; pronounced swelling appears in the hind paw which persists for weeks (primary reaction). After few days, the contralateral paw as well as front paw also becomes swollen and arthritic nodules appear in ear and tail (delayed systemic response)[18,19].

Rheumatoid arthritis (RA), which is associated with systemic inflammatory disorders, is a chronic inflammatory disease involving multiple joints. It is an autoimmune disorder of unknown etiology that is characterized by progressive joint destruction, deformity, disability and premature death in most patients. Recent studies have revealed the key roles of pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1b (IL-1b), IL-6 and IL-8 in the pathogenesis of RA[20].

In the present study, we showed that petroleum ether, ethyl acetate and methanolic extract at a dose of 50 and 100 mg/kg body wt. significantly inhibit the progression of the rheumatoid arthritis in treated animals. The effect of the extracts was dose dependent and for a long period compared to the standard.

Earlier observations by Rekha *et al* supported the alterations in the metabolic activities of diseased rats[21]. Earlier findings suggest that absorption of  $^{14}\text{C}$ -glucose and  $^{14}\text{C}$ -leucine in rat's intestine was reduced in inflamed rats and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine



**Figure 1.** Radiographic analysis of the proximal interphalangeal joints of control and experimental rats.

A) Control; B) Diclofenac sodium (20 mg/kg); C) Petroleum ether extract (50 mg/kg); D) Petroleum ether extract (100 mg/kg), E) Ethyl acetate extract (50 mg/kg); F) Ethyl acetate extract (100 mg/kg), G) Methanolic extract (50 mg/kg); H) Methanolic extract (100 mg/kg).

during inflammation[22,23]. The increased body weight during the treatment with diclofenac and the extracts of *C. uniflora* leaves as observed in this work may be due to the restoration of the absorption capacity of the intestine.

The potent anti–arthritic effect of extracts is further confirmed by radiological studies (Figure 1). The diagnosis of RA is usually obvious clinically and it allows therapeutic monitoring which remains the standard method in evaluating disease progression.

The X–ray appearance, commonly referred to as diminished joint space is the hallmark of arthritis[24]. In control rats, erosion representing bony destruction were evident on bone unprotected by cartilage, since they are exposed directly to cytokines such as TNF– $\alpha$  and IL–1 which stimulate the chondrocytes to produce proteolytic enzymes such as proteolytic enzymes such as collagenases, glycohydrolases and neutral proteases degrading the cartilage. As a result, the pannus invades the joint and sub–chondral bones and eventually the joint is destroyed and undergoes fibrous fusion or ankylosis.

These changes were reverted back to near normal upon petroleum ether and methanol extracts treatment.

### Conflict of interest statement

We declare that we have no conflict of interest.

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