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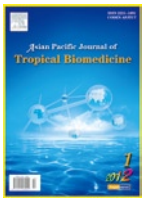

Document heading

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* Mill. **Methods:** Petroleum ether, ethyl acetate and methanolic extract of *Cassia 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 *Cassia uniflora* showed significant ($P<0.05$) and dose dependant increase in paw licking time in Eddy's hot plate method. In writhing test, extracts 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 was supported by the results of body weight, biochemical parameters and radiological analysis in complete Freund's adjuvant arthritis model. **Conclusions:** 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 (Mill.) is an herb belonging to family Caesalpiniaceae[1]. Young leaves of the plant was eaten as vegetables. 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. Roots are used to combat dropsy. *Cassia 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 *Cassia uniflora* are found useful as anti-inflammatory and in treating wound healing[3]. Hence present work was undertaken to check potential of *Cassia uniflora* leaves in the treatment of inflammation and arthritis.

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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). Leaves of *Cassia 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. These 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). These animals were maintained under standard laboratory conditions (room temperature 24-27℃ and humidity 60%-65%) with 12 h light and dark cycle. These 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. Animals were divided into six groups (n=6). These 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 days 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 animals of control and test groups were recorded by putting them on the hot plate maintained at (55±0.5)℃. 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 *Cassia uniflora* at a dose of 50 and 100 mg/kg body weight each and pentazocine lactate injection (20 mg/kg body weight) was administered by intra peritoneal route. The first group 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. These animals were removed from hot plate soon after they exhibited jumping. Cut off time was 20 seconds[5].

2.4.2. Acetic acid induced writhing

These albino mice were divided into eight groups. Petroleum ether extract or ethyl acetate extract or methanolic extract of *Cassia uniflora* at a dose of 50 and100 mg/kg body weight, *i.p.* each) and diclofenac sodium (20 mg/kg body weight, *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 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 weight each) and diclofenac sodium (20 mg/kg body weight) was administered by intraperitoneal route. Control rats 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 hours after the carrageenan injection. The difference between '0' readings and readings after 1, 2, and 3 hours, 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 3rd, 4th, 5th, 6th, 7th, 8th groups receive petroleum ether, ethyl acetate, methanolic extracts (50 and 100mg/kg, each), respectively by oral route. After 30 min, 0.1 mL complete Freund's adjuvant (Sigma, U.S.A) was injected 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 21st day[8]. The anti-arthritic effect of the extracts as well as diclofenac sodium was evaluate by measuring paw volume of inject paw on 4th, 8th, 14th and 21st 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 was calculated. Changes in body weight was recorded daily. On the day 22nd blood was withdrawn from each animal through retro-orbital vein puncture by anaesthetizing animals with ketamine. The blood was collected into vials containing EDTA and the biochemical parameters like haemoglobin content, total WBC count, differential WBC count, ESR and RBC were analysed[9].

2.7. Radiological analysis

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 second exposure time and a 60 cm tube-to film distance for anterior-posterior projection.

2.8. Statistical analysis

The data were expressed as Mean±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_{50} cut-off dose for extracts was found to be 1 000 mg/kg body weight. Hence,

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

Treatment	Latency to lick the paws (sec)					
	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±1.2**	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

*P<0.05, significant as compared to control. PE- Petroleum ether extract, EA- Ethyl acetate extract, ME- Methanolic extract.

the therapeutic doses were taken as 50 mg/kg and 100 mg/kg body weight.

3.2 Analgesic activity

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

Treatment	Mean ± SEM	% Inhibition
Control	31.75±3.40	-
Diclofenac sodium	3.00±0.71**	90.55%
PE (50 mg/kg)	13.25±0.85**	58.26%
PE (100 mg/kg)	6.00±0.91**	81.10%
EA (50 mg/kg)	13.00±1.29**	59.05%
EA (100 mg/kg)	7.25±1.65**	77.16%
ME (50 mg/kg)	10.75±0.85**	62.48%
ME (100 mg/kg)	4.75±0.48**	85.03%

*P<0.05, significant as compared to control. PE- Petroleum ether extract, EA- Ethyl acetate extract, ME- Methanolic extract.

3.2.1 Eddy's hot plate method

The effect of petroleum ether extract, ethyl acetate extract, methanolic extract of *Cassia 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 results of thermal stimulus induced pain (Eddy's hot plate) in mice. Pretreatment with pentazocine or extracts did not 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.

Table 3.
Effect of different extracts of leaves of *Cassia 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%

n=6. *P<0.05, significant as compared to control. PE- Petroleum ether extract, EA- Ethyl acetate extract, ME- Methanolic extract.

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

Treatment	Paw volume (mL)				
	0^{th} day	4^{th} day	8^{th} day	14^{th} day	21^{st} 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 ± 0.09 **	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**

*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 (cu.mm)	RBC (million/cu.mm)	Haemoglobin (g/dL)	ESR (mm/hr)
Control	14 700.00 ± 1.20	6.66 ± 0.01	12.00 ± 0.79	11.10 ± 0.76
Diclofenac sodium	6 160.00 ± 1.05	8.83 ± 0.02	15.50 ± 0.55	4.50 ± 0.22
PE (50mg/kg)	6 760.00 ± 1.07	9.18 ± 0.02	12.30 ± 0.35	8.30 ± 0.75
PE (100mg/kg)	6 900.00 ± 2.56	7.28 ± 0.04	12.10 ± 0.13	6.00 ± 0.48
EA (50mg/kg)	9 980.00 ± 3.00	7.36 ± 0.07	13.30 ± 0.45	3.20 ± 0.22
EA (100mg/kg)	9 220.00 ± 0.76	8.20 ± 0.01	13.40 ± 0.63	4.32 ± 0.37
ME (50mg/kg)	10 540.00 ± 0.88	7.51 ± 0.10	10.90 ± 0.35	6.60 ± 0.74
ME (100mg/kg)	6 630.00 ± 0.75	7.44 ± 0.03	12.90 ± 0.53	5.00 ± 0.37

PE- Petroleum ether extract, EA- Ethyl acetate extract, ME- Methanolic extract.

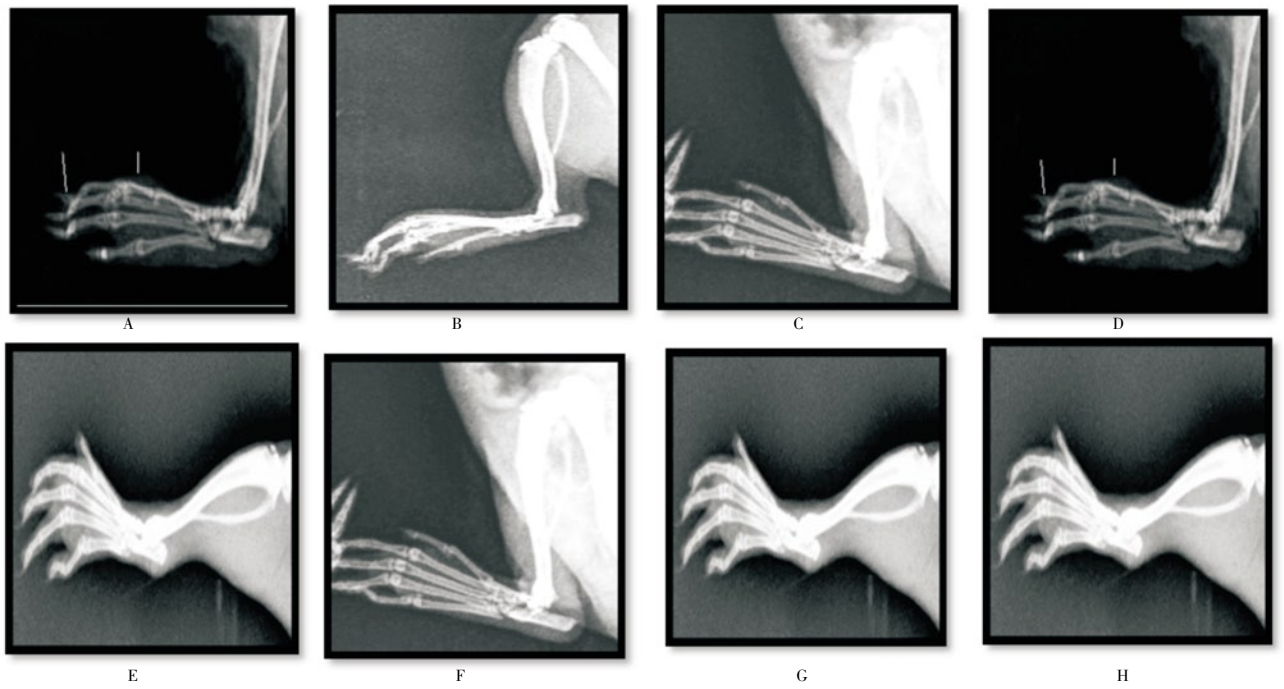

Figure 1. Radiographic analysis of the proximal interphalangeal joints of control and experimental rats.
A) Control, B) Diclofenac sodium (20 mg/kg), C) Petroleum ether extract (50 mg/kg),
D) Petroleum ether extract (100 mg/kg), E) Ethyl acetate extract (50 mg/kg),
F) Ethyl acetate extract (100 mg/kg), G) Methanolic extract (50 mg/kg),
H) Methanolic extract (100 mg/kg).

3.2.2. Writhing test method

The peripheral analgesic activity of *Cassia 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 6.
Mean changes in body weight in adjuvant-induced arthritis in rats.

Treatment	Body weight (g)		Mean changes in body weight
	0th day	21st day	
Control	175.16	182.83	8.66 ± 0.92
Diclofenac sodium	166.83	210.33	42.16 ± 1.01
PE (50 mg/kg)	165.33	192.33	27.83 ± 1.47
PE (100 mg/kg)	167.33	197.33	29.83 ± 1.99
EA (50 mg/kg)	166.33	185.33	15.66 ± 0.71
EA (100 mg/kg)	166.33	187.83	21.50 ± 0.56
ME (50 mg/kg)	165.60	194.00	29.66 ± 2.16
ME (100 mg/kg)	164.00	194.33	30.33 ± 1.12

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-arthritic activity

After inoculation with the complete Freund adjuvant (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 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 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 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). There was markedly improvement in the loss of body weight (Table 6) compared to the vehicle-treated arthritic. And the potent anti-arthritic effect of extracts is further confirmed by radiological studies (Figure 1).

4. Discussion

The hot-plate test is 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 *Cassia 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 prostaglandin-E_2 (PGE_2) and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products[12]. The significant reduction in acetic acid-induced writhes by petroleum ether extract, ethyl acetate extract and methanolic extract of *Cassia 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, polymerphonuclear cells and prostaglandins produced by tissue macrophages[13,14]. The significant (P<0.05) suppressive activity of the different extracts of *Cassia 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* (2000) 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]. PGE_2, 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 different extracts of *Cassia 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-a (TNF-a), 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 weight 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* (2010) supported the alterations in the metabolic activities of diseased rats[21]. Earlier findings suggest that absorption of 14C-glucose and 14C-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 during inflammation[22,23]. The increased body weight during the treatment with diclofenac and the extracts of *Cassia uniflora* leaves 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. 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 to as diminished joint space is the hallmark 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-α and IL-1 which stimulate the chrondocytes to produce 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.

Acknowledgments

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References

[1] Singh NP. Cassia sericea, a new record for India. *Bull Bot Surv* 1981; **21**: 203-205.
[2] Muzumdar PM, Percha V, Farswan M, Upganlawar A. Cassia: a wonder gift to medical sciences. *Int J Comm Pharm* 2008; **1**: 16-38.
[3] Nadkarni AK. *Indian materia medica*. 3rd ed. Bombay: Bombay Popular Prakashan; 1991, p. 811-816.
[4] Sourabh SB, Sonal D, Prashant S, Priya S, Yogesh S. Acute toxicity study of aqueous extract of *Coccinia indica* (Roots). *Asian J Res Pharm Sci* 2011; **1**: 23-25.
[5] Nikajoo LT. Analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (forsk.) chiov. *Int J Pharm Pharm Sci* 2009; **1**: 33-37.
[6] Purnima A, Koti BC, Tikare VP, Viswanathaswamy A, Thippeswamy A, Dabadi P. Evaluation of analgesic and antipyretic activities of *Centratherum anthelminticum* (L) kuntze seed. *Ind J Pharm Sci* 2009; **71**: 461-464.
[7] Sulaiman MR, Perimal EK, Akhtar MN, Mohamad AS, Khalid MH, Tasrip NA, et al. Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. *Fitoterpia* 2010; **81**; 855-858.
[8] Prakash Babu N, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of *Albizia lebbeck* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. *J Ethnopharmacol* 2009; **125**: 356-360.
[9] Sanmugapriya E, Senthamil SP, Venkataraman S. Evaluation of antiarthritic activity of *Strychnos potatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. *BMC Compl Alter Med* 2010; **10**: 56-64.
[10] Pasero C, Paice JA, Caffery MM. *Basic mechanisms underlying the causes and effects of pain*. St. Louis: Mosby; 1999, p. 15-34.
[11] Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; **66**: 355-474.
[12] Loubna F, Amine L, Rachida A, Ahmed B, Abderrahman C. Evaluation of the analgesic effect of alkaloid extract of *Peganum harmala* L.: Possible mechanisms involved. *J Ethnopharmacol* 2008; **115**: 449-454.
[13] Chavan MJ, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activities of 18-acetoxy-ent-kaur-16-ene from *Annona squamosa* L. bark. *Inflammopharmacol* 2011; **19**: 111-115.
[14] Olivera de Melo JO, Truiti MCT, Muscara MN, Bolonheis SM, Dantas JA, Caparroz-Assef SM, et al. Antiinflammatory activity of crude extract and fractions of *Nectandra falcifolia* Leaves. *Biol Pharm Bull* 2006; **29**: 2241-2245.
[15] Ueno A, Naraba H, Ikeda Y, Ushikubi F, Murata T, Naramiya S, et al. Intrinsic prostacyclin contributes to exudation induced by bradikinin or carrageenan: A study on the paw oedema-induced in ipreceptor- deficient mice. *Life Sci* 2000; **66**: 155-160.
[16] Foyet HS, Abdou BA, Ponka R, Asongalem AE, Kamtchouing P, Nastasa V. Effects of *Hibiscus asper* leaves extracts on carrageenan induced oedema and complete Freund's adjuvant-induced arthritis in rats. *J Cell Animal Biol* 2011; **5**: 69-75.
[17] Barsante MM, Roffe E, Yokoro CM, Tafuri WL, Souza DG, Pinho V, et al. Anti inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. *Eur J Pharmacol* 2005; **516**: 282-289.
[18] Kaithwas G, Majumdar DK. Therapeutic effect of *Linum usitatissimum* (flaxseed/linseed) fixed oil on acute and chronic arthritic models in albino rats. *Inflammopharmacology* 2010; **18**: 127-136.
[19] Lee J, Ah Kim K, Jeong S, Lee S, Park HJ, Kim NJ, et al. Antiinflammatory, anti-nociceptive, and anti-psychiatric effects by the rhizomes of *Alpinia officinarum* on complete Freund's adjuvant induced arthritis in rats. *J Ethnopharmacol* 2009; **126**: 258-264.
[20] Cai X, Zhou H, Fan Wong Y, Xie Y, Qiu Liu Z, Hong Jiang Z, et al. Suppressive effects of QFGJS, a preparation from an anti-arthritic herbal formula, on rat experimental adjuvant-induced arthritis. *Biochem Biophys Res Comm* 2005; **337**: 586-594.
[21] Rekha R, Ekambaram K. Anti-arthritic activity of *Premna serratifolia* Linn., wood against adjuvant induced arthritis. *J Med Biotechnol* 2010; **2**: 101-106.
[22] Brunet-Guedj E, Brunet B, Girardier J, Moyen B. *Médecine du sport*. 7e ed. Masson; 2006, p. 263-267.
[23] Gareth RD, Wilkies ME, Rampton DS. Effects of metronidazole and misoprostol on indomethacin-induced changes in intestinal permeability. *Digest Dis Sci* 1993; **38**: 417-425.
[24] Simon G. *Principles of bone X-ray diagnosis*. Great Britain: Butler Worth & Co; 1965, p. 157-163.