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ORIGINAL ARTICLE

Antiarthritic Potential of Aqueous and Ethanolic Fruit Extracts of "*Momordica charantia*" Using Different Screening Models

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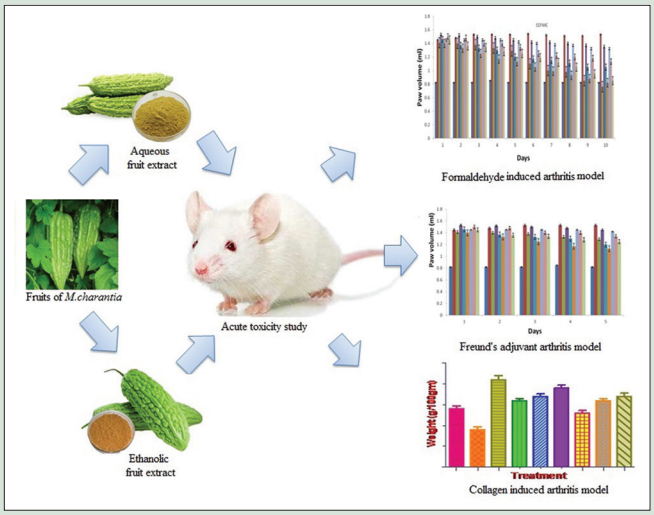
ABSTRACT

Background: *Momordica charantia (Cucurbitaceae)* is a plant, reported for its variety of ethnic medicinal uses and widely grown in Asia, Africa, and the Caribbean for its edible fruit. **Objective:** The present work has been planned to screen antiarthritic activity of fruit of the plant with the ethanolic and aqueous extracts. **Materials and Methods:** Fruit powder was successively extracted with ethanol (95%) and water using soxhlet extraction and subjected to phytochemical screening to identify different phytoconstituents. Ld_{50} studies for both (ethanolic and aqueous) extracts were conducted up to the dose level of 2 g/kg by following OECD up and down method of guidelines No. 425. Antiarthritic activity was performed using formaldehyde, Freund's adjuvant-induced arthritis in rats, and Collagen-induced arthritis model in mice. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's *t*-test. $P < 0.05$ was considered statistically significant. **Results:** Preliminary phytochemical studies revealed the presence of saponins, sterols, mucilage, glycosides, alkaloids, steroidal saponins in both the ethanolic and aqueous extracts of *M. charantia*. No mortality was observed with aqueous and ethanolic extracts up to the maximum dose level of 2 g/kg. In Formaldehyde induced arthritis model the percentage reduction in paw volume was 30.69% and 42.81% for aqueous extract whereas for ethanolic extract it was 25.23% and 39.5%. In Freund's adjuvant model, the percentage of reduction in paw volume was 56.1% and 66.51% for ethanolic extract and 52.6% and 63.83% for aqueous extract, respectively. In collagen-induced arthritis models, the arthritis index was found 6.02 and 3.68 for ethanolic extract at medium and high dosage. The arthritis index of aqueous extract was found 5.66 and 4.03 at medium and high dosage. **Conclusion:** From the present experimental findings of both pharmacological and biochemical parameters observed from the current investigation, it is concluded that at the doses of 200 and 400 mg/kg aqueous extract of *M. charantia* possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in adjuvant-induced arthritic and collagen-induced arthritis model in rats and mice

Key words: Anti-arthritic, collagen, formaldehyde, Freund's adjuvant, *Momordica. charantia*

SUMMARY

- An aqueous and ethanolic extracts of M.charantia fruit was prepared by soxhlation method. The extracts were subjected to phytochemical screening followed by oral acute toxicity study to obtain LD-50. Both extracts were thus investigated for anti-arthritic activity using Formaldehyde, Freund's adjuvant and collagen induced models. Various biochemical parameter and organ weight variation study was also conducted in Freund's adjuvant and collagen induced models. The significant anti arthritic potential was found for both the extracts.



Abbreviations Used: LD50: Lethal dose 50%, OECD: Organization for Economic and Co-operation Development, CMC: Carboxy Methyl Cellulose.

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INTRODUCTION

Arthritis, a term used to describe a number of painful conditions of the joints and bones, often associated with older people, but can also affect children. About 1:1000 children develop arthritis, often called as juvenile idiopathic arthritis. The incidence of rheumatoid arthritis is 3:10,000 population per year.[1] Onset is uncommon under the age of 15 and onward the incidence rises with age until the age of 80. The risk of developing the disease (the disease incidence) appears to be greatest in women between 40 and 50 years of age. The prevalence rate is 1% with women affected 3–5 times more than men. It is 4 times more common in smokers than nonsmokers.[2] Disease-modifying antirheumatic drugs are a category or otherwise unrelated drugs defined by their use in rheumatoid arthritis (RA) to slow down the progression of the disease.[3]

Many Ayurvedic practitioners in India are using various native plants for the treatment of different types of arthritic conditions. According to the Indian system of medicine, the medicaments using by the various Ayurvedic practitioners have a profound tradition and a rational

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background. Hence, it is essential to investigate the rationality of their use in modern scientific terms.[4] *Momordica Charantia* (*Cucurbitaceae*) plant has huge traditional importance. The fruit has a distinct warty looking exterior and oblong shape, hallow in cross-section, with relatively a thin layer of flush surrounding a central seed cavity with large flat seeds and pith, appear white in unripe fruits, and red on ripening. It is used in diabetes, antipyretic, anthelmintic, appetizer, cures biliousness, kapha, blood diseases, anemia, urinary discharges, ulcer, as a carminative, aphrodisiac and astringent to the bowels also used in rheumatism.[5] Along with various synthetic molecules, there are few plants has been reported to have antiarthritic activity. For example, whole plant of *Achyranthes aspera* (*Amaranthaceae*),[6] bark of *Hippocratea excels* (*Hippocrateaceae*),[7] bark of *Thespesia populnea* (*Malvaceae*),[8] leave of *Aspilia africana* (Compositae).[9]

M. Charantia fruit extracts were previously tested for ulcer,[10] diabetes,[11] inflammation,[12] diarrhea,[13], etc., However, the fruit extract of *M. charantia has* not tested for arthritis. The scientific investigation is essential to prove the potency and to extent their scope for future use. Hence, the aim of the present study is to prove the therapeutic efficacy of the fruit as an anti-arthritic agent against, formaldehyde-induced arthritis, Freund's complete adjuvant-induced arthritis in rats and collagen-induced arthritis in mice.

MATERIALS AND METHODS

Plant material

Fruit of *M. charantia* was collected in June from The Alva Pharmacy, Mangalore, and were dried in the shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Preparation of ethanolic extract

The fruit powder (750 gm) was packed in a Soxhlet apparatus and extracted with 1 L ethanol (95%) for 18 h at >78°C. The appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at <50°C. The ethanolic extract of the fruit of *M. charantia* (EEFMC) was appeared dark brown amorphous in nature with percentage yield of 1%.

Preparation of aqueous extract

About 100 g of fruit powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water for 24 h with occasional shaking in a closed vessel. A volume of 10 ml of chloroform was added as a preservative. Then, the marc was removed by filtering the extract and then concentrated on a water bath maintained at 50°C. The extract was finally air dried thoroughly to remove all traces of the solvent. The aqueous extract of the fruit of *M. charantia* (AQEFMC) appeared dark brown sticky in nature with percentage yield of 1%.

The two extracts were examined for their color and consistency. Their percentage yield was calculated with reference to the air-dried sample used for extraction then stored in an air tight containers in a refrigerator below −4°C.

Experimental animals

Albino rats (Wistar strain) of both sex weighing between 150 and 200 g and albino mice of either sex weighting between 16 and 25 g were procured from the National Centre for Laboratory Animal Sciences, C/O Sri Venkateswara Enterprises, Bengaluru, for experimental purpose. After procuring, all the animals were acclimatized for 7 days under standard husbandry condition as, 26°C ± 2°C room temperature, with relative humidity 45%–55% and kept light/dark cycle for 12:12 h. The animals were fed with synthetic standard diet Amrut Laboratories (Pranava Agro Industries Ltd. Sangli.) Water was allowed *ad libitum,* and strict hygienic conditions were maintained. After obtaining prior permission from the Institutional Animal Ethical Committee of V. L. College of Pharmacy Raichur (Karnataka), all animal studies were performed as per rules and regulations in accordance with the guidelines of CPCSEA (Registration Number 557/02/c/CPCSEA February 18, 2016.)

Chemicals and drugs

The chemicals used for antiarthritic study were Freund's adjuvant (GeNei™ Mumbai), distilled water (Mysore petrochemicals, Raichur, India), collagen (Sigma Aldrich, Bengaluru), formaldehyde (Karnataka fine chemicals, Bengaluru, Ibuprofen (S.D. Fine chemicals, Bengaluru), anesthetic ether (Sigma Solvents and Pharmaceuticals – Mumbai). All the drugs and chemicals used were of pharmaceutical grade.

Determination of acute oral toxicity (LD_{50})

The acute oral toxicity study[14] of fruit extracts of *M. charantia* was determined in female albino mice (16–25 g) maintained under standard husbandry conditions. The animals were fasted 4 h before the experiment and up and down procedure (OECD Guidelines No. 425) method of CPCSEA were adopted for acute toxicity studies. Animals were administered with single doses of each extract and observed for their mortality during 48 h study period (short-term toxicity). Based on the short-term profile of extracts, the doses for the next animals were determined. All the animals were observed for long-term toxicity (7 days). The LD_{50} studies of the test extracts were conducted up to the maximum dose level of 2000 mg/kg body wt. 1/20, 1/10, and $1/5^{th}$ doses of the LD_{50} dose of the individual extracts were selected for the study as low, medium, and high doses.

Formaldehyde-induced arthritis

Male albino rats weighing between (150 and 200 g) were divided into nine groups of 6 rats in each, i.e., normal control (1% CMC, 1 ml/1 kg body weight), toxicant control, standard (Ibuprofen), and six drug-treated groups of (ethanolic and aqueous extracts) low, medium, and high All the groups administered with 2% v/v formaldehyde except normal control.[15] Afterward daily, the paw volume was measured for 10 days. The values were expressed as the mean ± standard deviation (SD) from 6 animals.

Freund's adjuvant-induced arthritis

Male albino rats weighing between (150 and 200 g) were divided into nine groups of six rats in each,[16], i.e., normal control (1% CMC, 1 ml/1 kg body weight), toxicant control, standard (Ibuprofen), and six drug-treated groups of (ethanolic and aqueous extracts) low, medium, and high. All the groups administered with 2% v/v formaldehyde except normal control were injected with single dose of 0.1 ml of Freund's adjuvant and were treated with standard/extract for 12 consecutive days. Paw volumes of both paws were measured plethismographically, and body weights are recorded on the 1^{st} and 21^{st} day of injection. On days 3, 5, 9, 13, and 21 the volume of injected paw is measured again plethismographically to note the primary lesion and to study the influence of standard and extracts on this phase. The severity of adjuvant-induced disease is followed by measurement of noninjected paw (secondary lesions) with a plethysmometer. Purposely from day 13^{th} to 21^{st}, the animals are not dosed with the standard/extract. On day 21, the animals were anesthetized with ether. Blood was collected from the retro-orbital puncture later sacrificed by overdose of ether separated serum was subjected to serum analysis of biochemical parameters. Weight of organs was also noted simultaneously. The volume of edema was measured at prefixed time interval, i.e., 3, 5, 9, 13, and 21 days. The

difference between paw volumes of the treated animals was measured, and the mean edema volume was calculated. Percentage reduction in edema volume was calculated using the formula,

Percentage reduction= $(V_c - V_t/V_c) \times 100$

$V_{c\ =}$ Mean volume of paw edema in control Group A.

V_t = Mean volume of paw edema in drug-treated group of animals.

Collagen-induced arthritis

Mice weighing between (16 and 25 g) were divided into nine groups of 6 mice in each i.e, normal control (1% CMC, 1 ml/1 kg body weight), toxicant control, standard (Ibuprofen), and six drug-treated groups of (ethanolic and aqueous extracts) low, medium, and high. All the groups administered with 0.1 ml of collagen + 0.1 ml of Freund's adjuvant into base of the tail intradermally for 14 days.[17,18] Purposely from day 13th to 21st, the animals are not dosed with the standard/extracts, mice were observed daily for clinical signs of arthritis, and each paw was scored on a scale of 0–4 (arthritis index) as follows: 0 = unaffected, 1 = 1 type of joint affected, 2 = 2 types of joints affected, 3 = 3 types of joints affected, 4 = 3 types of joints affected and maximal erythema and swelling. The total score for each mice was calculated as an arthritis index.[19] On the 21st day, all animals were anesthetized, and blood was withdrawn by retro-orbital puncture and collected in plain and ethylenediaminetetraacetic acid containing tubes, respectively, for serum separation. The homogenized samples were subjected to biochemical examination.

Data analysis

The obtained values in all three models were expressed as mean ± SD from 6 animals, subjected to statistical analysis using one-way analysis of variance followed by Dunnett's-*t*-test to verify significant difference if any among the groups. $P < 0.05$*, 0.01**, and 0.001*** was considered statistically significant.

RESULTS

Ethanolic and aqueous extracts of fruit of *M. charantia* were subjected to phytochemical screening for all types of phytoconstituents, for example, alkaloid, glycoside, terpenoids, tannins, saponins, and flavonoids. The ethanolic extract was found positive for lead acetate test and ferric chloride test, which confirms the presence of flavonoids. Positive Salkowski test confirms the presence of triterpenes. Liebermann-Burchard test was found positive which confirms the presence of sterols. Foam and froth test confirms the presence of saponins. The phytochemical study of aqueous extract confirms the presence of triterpenes and flavonoids.

Ethanolic and aqueous extracts of *M. charantia* fruit were administered orally to different groups of mice at different dose levels. It was found that even up to the dose level of 2000 mg/kg body weight either of the extracts did not produce any behavioral symptoms or mortality.

Effect of formaldehyde on paw volume in rats

Formaldehyde-treated group is noted with a significant increase in paw volume from 1st to 10th day of the experimental study in comparison to the control group (a). The percent increase in paw volume is noted as 42.85% and 50% on 1st and 10th days of the study, respectively. In Ibuprofen treatment (10 mg/kg) before formaldehyde challenge, the percent reduction in paw volume (minimum and maximum) is noted as 3.3% and 48.2%, respectively, on 1st and 10th day of the study in comparison to the control group (b). In EEFMC with three different doses, i.e.,100, 200, and 400 mg/kg body weight, the minimum and maximum percent reduction in paw volume recorded with the three different doses at 1st and 10th day of the study are 5.57%, 10.4%, 3.49%, 30.69%, and 5.71%, 42.81%, respectively. Similar type of results are also noted with the AQEFMC and the minimum and maximum percent

Table 1: Percentage change in edema volume at different time intervals (days)

Treatment	Dose (mg/kg) p.o	Edema volume (mean±SD)									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
Toxicant (formaldehyde)	0.1	42.85±1.85	42.85±1.85	46.66±2.04	46.66±2.04	46.66±2.04	46.66±2.04	46.66±2.04	46.66±2.04	46.66±2.04	50±2.27
Standard (ibuprofen)	30	3.3±3.41	6.5±3.22*	10.73±3.22*	11.91±2.40**	15.72±5.47**	28.56±9.8**	34.38±2.74**	39.02±4.49**	43.43±2.84**	48.2±5.92**
EEFMC	100	5.57±4.82	2.85±3.39	4.78±3.25	4.28±3.22	4.84±4.79*	7.46±4.17*	6.49±3.96*	7.63±3.81*	7.63±4.36*	10.41±4.33*
EEFMC	200	3.49±3.82	12.22±8.86*	13.41±6.41*	16.70±5.40**	22.62±6.12**	23.65±6.44**	23.90±7.38**	26.27±5.63**	25.13±13.7**	30.69±5.92**
EEFMC	400	5.71±2.80	10.11±2.99*	18.34±3.13**	24.54±3.67**	26.09±2.15**	31.8±2.14**	34.97±1.53**	38.4±3.61**	41.73±1.90**	42.81±2.57**
AQEFMC	100	4.70±3.23	4.51±3.50	3.42±2.98	2.88±2.52	6.52±4.04*	8.06±2.90*	9.26±2.15*	9.66±3.51*	10.9±4.58*	12.56±4.03*
AQEFMC	200	4.08±3.43	3.41±3.09	8.19±2.49*	8.19±2.49*	12.91±4.19**	18.4±4.94**	19.72±3.71**	20.8±2.93**	21.90±3.32**	25.23±2.15**
AQEFMC	400	4.6±3.57	7.77±5.01*	12.86±5.02*	17.31±4.59**	17.82±5.23**	22.6±4.50**	26.17±5.54**	30.2±4.24**	38.80±3.00**	39.51±2.64**

n=6, Significant at *$P<0.05$, **$P<0.01$ and NS. Statistical analysis by one-way ANOVA followed by Dunnett's *t*-test. EEFMC: Ethanolic extract of fruit of *M. charanti*; AQEFMC: Aqueous extract of fruit of *M. charantia*; NS: Not significant; *M. charantia*: *Momordica charantia*; SD: Standard deviation

Table 2: Effect of Ethanolic extract of the fruit of *Momordica charantia* and aqueous extract of the fruit of *Momordica charantia* on Freund's adjuvant-induced arthritis in rats at different time intervals

Group	Treatment	Dose (mg/kg) p.o	Paw volume (mean±SD)						Percentage reduction in paw volume (mean±SD)				
			0 day	3rd day	5th day	9th day	13th day	21st day	3rd day	5th day	9th day	13th day	21st day
1	Control	-	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	0.82±0.01	-	-	-	-	-
2	Toxicant control (Freund's adjuvant)	0.1 ml Sub plantar region	0.6±0.0.03	1.17±0.02	1.5±0.02	1.59±0.02	1.59±0.02	1.9±0.05**	46.87±3.42	88.54±2↑	88.54±2↑	88.54±2↑	88.54±2↑
3	Standard (indomethacin)	10	0.52±0.06	0.92±0.02**	0.93±0.02**	0.93±0.02**	0.79±0.03**	0.55±0.03**	21.21±3.41**	38.11±1.66**	41.34±2**	50.25±2.1**	70.99±2.47**
4	EEFMC	100	0.59±0.02	1.24±0.04*	1.19±0.02*	1.19±0.02**	1.08±0.15**	1.02±0.04**	5.62±2.07*	20.96±2.36*	25.13±0.32*	31.9±9.65*	46.03±2.04*
5	EEFMC	200	0.6±0.05	1.21±0.14**	1.07±0.09**	1.07±0.09**	0.98±0.1**	0.83±0.04**	12.67±6.31**	28.74±6.27**	32.42±6.58**	38.21±6.67**	56.11±2.27**
6	EEFMC	400	0.64±0.03	1.08±0.26**	1.0±0.09**	0.1±0.09**	0.83±0.02**	0.63±0.02**	16.33±3.25**	33.18±5.31**	36.58±6.47**	47.59±1.96**	66.51±1.02**
7	AQEFMC	100	0.65±0.03	1.23±0.04*	1.2±0.03*	1.2±0.03**	1.12±0.02**	1.06±0.04**	4.92±1.66*	20.41±2.95*	24.61±1.38*	29.31±1.54*	43.83±2.05*
8	AQEFMC	200	0.6±0.02	1.15±0.15**	1.1±0.1**	1.1±0.1**	1.02±0.13**	0.91±0.02**	9.23±7.46**	23.18±11.8**	30.34±6.92**	36.13±8.83**	52.65±1.93**

n=6, significant at *P<0.05, **P<0.01 and NS. Statistical analysis by one-way ANOVA followed by Dunnett's *t*-test. EEFMC: Ethanolic extract of fruit of *M. charanti*; AQEFMC: Aqueous extract of the fruit of M. charanti; *M. charantia*: *Momordica charantia*; SD: Standard deviation

Table 3: Effect of ethanolic extract of fruit of *Momordica charantia* and aqueous extract of fruit of *Momordica charantia* on biochemical parameters in Freund's adjuvant arthritis in rats (mean±standard deviation)

Groups	Treatment (p.o), mg/kg	ALT (U/L)	AST (U/L)	ALP (mg/dl)	BUN (U/L)	CHO (mg/dl)	TG (mg/dl)	TP (U/L)	GLU (mg/dl)	CRE (U/L)	ALB (U/L)
Normal control	Vehicle only 10	46.7±1.12	110±4.5	65.0±4.04	59.37±2.34	74.77±10.9	57.95±9.92	12.94±0.43	87.5±5.02	0.75±0.28	6.96±0.56
Toxicant control	Freund's adjuvant 0.1 ml	68.2±1.87**	260±5.3**	77.5±4.45**	78.66±4.70**	165.92±22.0**	165.28±5.62**	7.96±0.11**	105.03±9.6**	0.94±0.27**	3.7±0.44**
Standard	Ibuprofen 50	52.3±1**	150±33.2**	67.69±2.8**	72.06±6.63**	79.7±4.45**	62.02±1.85**	10.8±0.23**	83.4±7.03**	0.79±0.38**	5.4±0.24**
EEFMC	100	64.30±1.9 (NS)	254±4.33 (NS)	76.09±3.5 (NS)	75.49±6.22 (NS)	161.5±5.57 (NS)	160.5±5.57 (NS)	7.30±0.11 (NS)	100.33±3.03 (NS)	0.90±0.35 (NS)	4.2±0.51 (NS)
EEFMC	200	60.1±2.0*	220±4.0**	70.89±7.0**	67.76±8.23**	133.5±6.0**	100.2±10.1**	9.41±0.11*	87.3±6.45**	0.81±0.02*	5.0±0.13*
EEFMC	400	54.13±2**	170±2.50**	68.2±8.89**	69.5±4.49**	88.2±4.45**	70.24±5.03**	11.2±0.15**	76.6±2.09**	0.73±0.01**	5.2±0.21**
AQEFMC	100	65.3±1.24 (NS)	258±4.50 (NS)	75.02±10 (NS)	76.29±4.68 (NS)	163.2±15.52 (NS)	162±6.21 (NS)	7.50±0.24 (NS)	102.06±2.29 (NS)	0.91±0.02 (NS)	4.5±0.28 (NS)
AQEFMC	200	62.2±2.7*	225±3.92**	73.65±12.9*	66.33±4.23**	138.±18.9**	110.3±3.4**	9.92±0.19**	83.9±2.30**	0.80±0.01*	5.31±0.31*
AQEFMC	400	58.4±1.57**	180±2.91**	69.85±6.4**	68.23±4.68**	100.5±5.82**	73.5±2.89**	11.5±0.11**	78.6±3.38**	0.75±0.01**	5.4±0.34**

n=6, significant at *P<0.05, **P<0.01 and NS. Statistical analysis by one-way ANOVA followed by Dunnett's *t*-test. EEFMC: Ethanolic extract of fruit of *M. charanti*, AQEFMC: Aqueous extract of fruit of *M. charanti*; NS: Not significant; *M. charantia*: *Momordica charantia*; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alanine phosphate; BUN: Blood urea nitrogen; CHO: Cholesterol; TG: Triglyceride; TP: Total protein; GLU: Glucose; CRE: Creatinine; ALB: Albumine

Table 4: Effect of ethanolic extract of the fruit of *Momordica charantia* and aqueous extract of the fruit of *Momordica charantia* on organ weights in Freund's adjuvant-induced arthritis model in rats (mean±standard deviation)

Groups	Treatment	Kidney (g/100 g)	Liver (g/100 g)	Lungs (g/100 g)	Spleen (g/100 g)
Normal control	Vehicle only (10 ml/kg p.o)	1.46±0.028	5.78±0.05	1.40±0.01	0.50±0.01
Toxicant control	Freund's adjuvant 0.1 ml	1.25±0.02**	6.58±0.08**	1.37±0.02**	0.74±0.04**
Standard	Ibuprofen 50 mg/kg	1.42±0.01**	5.20±0.11**	1.40±0.01**	0.56±0.03**
EEFMC	Low dose 100 mg/kg	1.29±0.01 (NS)	5.63±0.10 (NS)	1.31±0.01 (NS)	0.70±0.01 (NS)
EEFMC	Med dose 200 mg/kg	1.32±0.01 (NS)	5.61±0.09 (NS)	1.30±0.01 (NS)	0.66±0.01 (NS)
EEFMC	High dose 400 mg/kg	1.38±0.05*	5.40±0.04*	1.29±0.09*	0.60±0.01*
AQEFMC	Low dose 100 mg/kg	1.27±0.02 (NS)	5.71±0.06 (NS)	1.37±0.01 (NS)	0.72±0.01 (NS)
AQEFMC	Med dose 200 mg/kg	1.33±0.01 (NS)	5.76±0.06 (NS)	1.35±0.01 (NS)	0.68±0.01 (NS)
AQEFMC	High dose 400 mg/kg	1.37±0.03*	5.46±0.04*	1.32±0.01*	0.62±0.009*

n=6, significant at *P<0.05, **P<0.01 and NS. EEFMC: Ethanolic extract of fruit of *M. charantia*; AQEFMC: Aqueous extract of fruit of *M. charantia*; NS: Not significant; *M. charantia*: *Momordica charantia*

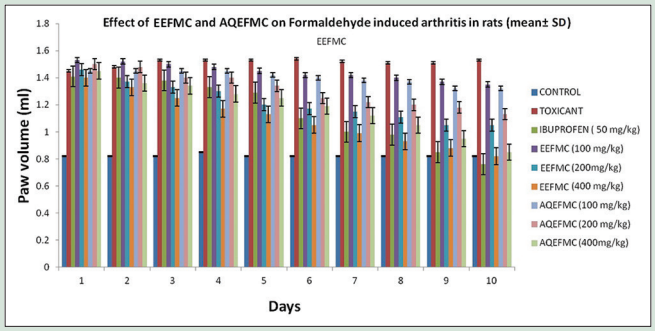

Figure 1: Effect of both the extract on formaldehyde-induced arthritis in Rat

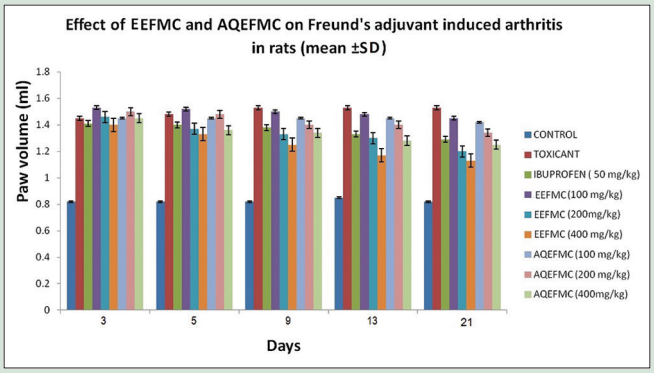

Figure 2: Effect of both the extract on Freund's adjuvant-induced arthritis in rats

reduction in paw volume with three different doses selected, i.e., 100, 200, and 400 mg/kg are 4.70%, 12.56%., 4.08%, 25.23%, and 4.6%, 39.5%, respectively. Results are shown in Table 1 and Figure 1.

Effect of complete Freund's adjuvant-induced arthritis in rats

After administration of 0.1 ml of Freund's adjuvant, the minimum and maximum percent increase in paw volume noted on 3rd and 21st day of the experimental study are 46.87% and 88.54%, respectively. In standard drug ibuprofen 10 mg/kg, the percent reduction in paw volume (minimum and maximum) recorded on 3rd and 21st day are 21.2% and 70.99%, respectively. And a time-dependent reduction is observed. In the EEFMC treatment with three different doses, as mentioned previously, the minimum and maximum percent reduction in paw volume recorded with the three doses on 3rd and 21st day are 5.62%, 46.03%; 12.67%, 56.1%, and 16.33%, 66.51%, respectively. Similarly, for AQEFMC treatment, the minimum and maximum percent reduction in paw volume recorded with 3 doses on 3rd and 21st days of experimental study are noted as 4.92% 43.83%, 9.23%, 52.6%, and 14.25%, 63.83%, respectively. Results are shown in Table 2 and Figure 2.

Estimation of biochemical parameters in complete Freund's adjuvant method

The biochemical parameters include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine phosphate (ALP), blood urea nitrogen (BUN), cholesterol (CHO), triglyceride (TG), total protein (TP), glucose (GLU), creatinine (CRE), albumin (ALB), have been investigated for control, standard, and both ethanolic and aqueous extracts. As a result of inflammation induced by adjuvant, the levels of all the biochemical parameters were increased in all arthritis rats as compared to control rats. After extract treatment, the levels of these enzymes were significantly decreased in all groups compared to control rats. Ibuprofen treatment prevented biochemical changes to a greater extent than the aqueous and ethanolic extract of the plant. All the biochemical parameters levels of all the groups were evaluated and compared with each other, and the respective results are shown in Table 3.

Effect of organ weight in complete Freund's adjuvant-induced arthritis in rats

In this study, the effect on different organs, including, kidney, liver, lungs, and spleen have been investigated. The organ weight changes have been observed in control, standard, and both of the aqueous and ethanolic extracts.

In the present study, it is clear from the data obtained that there is a close relationship between the extent of joint inflammation and the degree of weight loss of the organs. The control group when compared to the standard- and test-treated groups, it was found that the weights of the kidney, liver, lungs, and spleen were highest in case of the standard group 1.42 ± 0.01,5.20 ± 0.11,1.40 ± 0.01, and 0.56 ± 0.03 gm. Similarly, the aqueous extracts also increase the organ weight significantly but not more than standard values. The respective results are shown in Table 4.

Collagen-induced arthritis model in mice

Collagen (0.1 ml) + 0.1 ml of Freund's adjuvant-induced arthritis model intoxicant control arthritis index is noted as 8.54 and standard drug ibuprofen has significantly reduced this to 4.32. The selected extracts EEFMC and AQEFMC with three different doses as mentioned earlier

Table 5: Effect of ethanolic extract of fruit of *Momordica charantia* and aqueous extract of fruit of *Momordica charantia* on biochemical parameters in collagen-induced arthritis in mice

Groups	Treatment	Biochemical parameters									
		ALT (U/L)	AST (U/L)	ALP (mg/dl)	BUN (U/L)	CHO (mg/dl)	TG (mg/dl)	TP (U/L)	GLU (mg/dl)	CRE (U/L)	ALB (U/L)
Normal control	Vehicle only (10 ml/kg p.o)	44.03±2.53	94.48±4.35	64.72±1.87	60.49±2.67	74.16±4.7	54.05±1.57	9.92±0.37	55.33±1.70	0.28±0.01	4.82±0.04
Toxicant control	0.1 ml collagen + 0.1 ml Freund's adjuvant, intradermally	60.94±2.50**	286.19±12.3**	105.1±2.77**	49.59±2.68**	102.91±2.10**	128.7±4.60**	4.71±0.32**	100.1±1.40**	0.40±0.017**	3.72±0.07**
Standard ibuprofen	Ibuprofen 50 mg/kg	49.25±3.59**	130.23±5.31**	69.1±4.52**	68.25±2.81**	71.68±6.27**	59.23±4.79**	8.30±0.19**	60.95±2.61**	0.30±0.01**	5.60±0.24**
EEFMC	Low dose 100 mg/kg	58.89±4.68 (NS)	278.7±12.0 (NS)	100.91±9.61 (NS)	46.3±2.16 (NS)	99.6±3.58.(NS)	123.6±7.13 (NS)	5.07±0.22 (NS)	100.0±1.2 (NS)	0.38±0.01 (NS)	4.02±0.17 (NS)
EEFMC	Med dose 200 mg/kg	55.22±3.84*	210.29±10.09*	81.41±8.43**	58.06±1.24**	87.95±2.56**	98.3±7.90**	7.10±0.25**	86.16±1.14**	0.35±0.008*	5.03±0.24*
EEFMC	High dose 400 mg/kg	52.62±3.75**	160.3±8.13**	73.44±4.29**	62.6±2.33**	80.28±10.8**	62.6±3.40**	10.48±0.06**	70.66±2.88**	0.32±0.005**	5.35±0.11**
AQEFMC	Low dose 100 mg/kg	59.2±3.15 (NS)	280.9±13.6 (NS)	100.4±3.01 (NS)	47.8±0.62 (NS)	101.0±2.78 (NS)	124.0±3.40 (NS)	5.25±0.27 (NS)	104.9±1.20 (NS)	0.39±0.008 (NS)	4.15±0.13 (NS)
AQEFMC	Med dose 200 mg/kg	57.14±6.55*	218.7±8.69**	84.3±3.41**	62.67±3.44**	89.60±6.36**	103.7±7.0**	7.30±0.35**	83.33±1.77**	0.37±0.006*	5.23±0.21*
AQEFMC	High dose 400 mg/kg	53.61±2.21**	169.9±4.35**	76.7±5.04**	65.86±1.70**	84.94±4.0**	68.7±2.40**	11.3±0.19**	72.32±1.75**	0.36±0.005**	5.48±0.21**

n=6, significant at $^*P<0.05$, $^{**}P<0.01$ and NS. EEFMC: Ethanolic extract of fruit of *M. charantia*; AQEFMC: Aqueous extract of fruit of *M. charantia*; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alanine phosphate; BUN: Blood urea nitrogen; CHO: Cholesterol; TG: Triglyceride; TP: Total protein; GLU: Glucose; CRE: Creatinine; ALB: Albumin; NS: Not significant; *M. charantia*: *Momordica charantia*

too have significantly reduced arthritis index with medium and high doses only, i.e., 6.02, 3.68, and 5.6, 4.03. Low doses of both the extracts failed to reduce the arthritis index to significant extent, i.e., 7.57 and 7.30. The estimated biochemical parameters and organ weight changes as mentioned in complete Freund's Adjuvant method have also investigated in collagen-induced arthritis, the detail results were shown in Tables 5 and 6. The arthritis index values were mentioned in Table 7.

DISCUSSION

RA is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant-induced arthritic model of chronic inflammation is considered as the best available experimental model of RA. Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble RA.[20] The determination of paw swelling is apparently simple, sensitive, and quick procedure for evaluating the degree of inflammation and assessing of therapeutic effects of drugs.[21] In adjuvant-induced arthritis and collagen-induced arthritis models, rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage, and bone destruction and remodeling which have close similarities to human rheumatoid disease. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal. In addition, the complete Freund's adjuvant-administered rats showed soft-tissue swelling around the ankle joints during the development of arthritis, which was considered as edema of the particular tissues. The collagen-induced arthritis is an autoimmune inflammatory disease, and the reactive oxygen species secreted by inflammatory cells induce inflammation and regulate immune response. Arthritis characteristic events are IL-1 induced production of progelatinase-B and PGE_2, synovial fibroblast proliferation. It is a consequent increased production of cytokines and nitric oxide synthase. Overstimulation of reactive oxygen species has been reported as the underlying pathological condition for the development of collagen-induced arthritis.[22]

Assessment of the levels of ALT, AST, ALP, BUN, CHO, TG, TP, GLU, CRE, and ALB provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The activities of these enzymes were increased significantly in arthritic rats and mice. These are good indicators of liver and kidney impairment and are also considered to be features of adjuvant arthritis and collagen-induced arthritis models.[23,24]

In the present study, the fruit extracts of *M. charantia* of aqueous and ethanolic extracts showed a significant antiarthritic activity in a dose-dependent manner. In the present study, we showed that aqueous extract of *M. charantia* could significantly inhibit the progression of the RA in treated animals. However, standard drug and aqueous extract significantly suppressed the swelling of the paws in both acute and chronic phase which may be due to the suppression of inflammatory mediator released due to induction of Freund's adjuvant. In collagen-induced arthritis model, the effect of anti-arthritic activity of both the extracts assume that the well-established anti-arthritic properties of *M. charantia* and its ability to block the cyclooxygenase-2 pathway during the progression of inflammation, which justified the uses of the plant extract in the treatment of RA. Although the actual mechanism of suppressing inflammation is not known it can be correlated with the presence of saponins, flavonoids, tannins, and alkaloids and in suppressing the inflammation. Numerous studies have suggested a role of oxidative stress in the pathogenesis of RA.[25] Therefore; it was assumed that the reported and well-established antioxidant properties of *Cheilocostus speciosus* and its ability to block the COX-2 pathway during the progression of inflammation justify the usage of the plant extract in the treatment

Table 6: Effect of ethanolic extract of fruit of *Momordica charantia* and aqueous extract of fruit of *Momordica charantia* on organ weights in collagen-induced arthritis in rats (mean±standard deviation)

Groups	Treatment	Kidney (g/100 g)	Liver (g/100 g)	Lungs (g/100 g)	Spleen (g/100 g)
Normal control	Vehicle only (10 ml/kg p.o)	0.44±0.009	1.21±0.009	0.24±0.006	0.14±0.007
Toxicant control	0.1 ml collagen + Freund's adjuvant, intradermally	0.40±0.007	1.41±0.01	0.18±0.007	0.09±0.007
Standard	Ibuprofen	0.43±0.01**	1.12±0.008**	0.24±0.01**	0.21±0.01**
EEFMC	Low dose 100 mg/kg p.o	0.35±0.008 (NS)	1.31±0.02 (NS)	0.19±0.003 (NS)	0.16±0.005 (NS)
EEFMC	Med dose 200 mg/kg p.o	0.37±0.01 (NS)	1.19±0.01 (NS)	0.20±0.007 (NS)	0.17±0.006 (NS)
EEFMC	High dose 400 mg/kg p.o	0.38±0.01*	1.15±0.006*	0.22±0.007*	0.19±0.006**
AQEFMC	Low dose 100 mg/kg p.o	0.35±0.005 (NS)	1.35±0.01 (NS)	0.18±0.01 (NS)	0.13±0.006 (NS)
AQEFMC	Med dose 200 mg/kg p.o	0.36±0.008 (NS)	1.25±0.007 (NS)	0.21±0.01 (NS)	0.16±0.005 (NS)
AQEFMC	High dose 400 mg/kg p.o	0.37±0.01*	1.21±0.009*	0.22±0.01*	0.17±0.008*

n=6, significant at *P<0.05, **P<0.01 and NS. EEFMC: Ethanolic extract of fruit of *M. charantia*; AQEFMC: Aqueous extract of fruit of *M. charantia*; NS: Not significant; *M. charantia*: *Momordica charantia*

Table 7: Arthritis index of ethanolic extract of fruit of *Momordica charantia* and aqueous extract of fruit of *Momordica charantia* in collagen-induced arthritis in rats (mean±standard deviation)

Group	Treatment	Arthritis index
1	Toxicant control	8.54±0.27**
2	Standard (ibuprofen 50 mg/kg)	4.32±0.20**
3	EEFMC 100 mg/kg	7.57±0.4 (NS)
4	EEFMC 100 mg/kg	6.02±0.52*
5	EEFMC 400 mg/kg	3.68±0.14**
6	AQEFMC 100 mg/kg	7.3±0.39 (NS)
7	AQEFMC 200 mg/kg	5.66±0.22*
8	AQEFMC 400 mg/kg	4.03±0.20**

n=6, significant at *P<0.05, **0.01 and ***0.001. NS: Not significant; EEFMC: Ethanolic extract of fruit of *M. charantia*; AQEFMC: Aqueous extract of fruit of *M. charantia*; *M. charantia*: *Momordica charantia*

of RA. In all the three arthritic models selected for evaluation of antiarthritic activity of aqueous and ethanolic extracts, aqueous extract was recorded relatively better antiarthritic activity than ethanolic extract. The difference in antiarthritic activity can be accounted for the presence and quantity of phytoconstituents.

CONCLUSION

Fruit extracts of *M. charantia* exhibited significant antiarthritic activity in experimental animal's rats/mice. A significant antiarthritic activity was noted with both the extracts but relatively more activity with ethanolic extract which can be accounted for difference in phytoconstituents i.e., sterols as these were presented with ethanolic extract only.

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Conflicts of interest

There are no conflicts of interest.

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