Journal of
Clinical and Experimental Pharmacology

Sarkar et al., Clin Exp Pharmacol 2017, 7:2
DOI: 10.4172/2161-1459.1000231

Research Article OMICS International

# Anti-inflammatory and Anti-arthritis Activity of Flavonoids Fractions Isolated from *Centipeda minima* Leaves Extracts in Rats

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**Received date:** February 13, 2017; **Accepted date:** February 23, 2017; **Published date:** March 02, 2017


## Abstract

*Centipeda minima* has been used since centuries as a traditional medicinal plant in treating a number of disease conditions, the bioactive components responsible for its anti-inflammatory and anti-arthritis activity have not been identified. The present study was planned to isolate flavonoids fractions from *Centipeda minima* leaves extracts, and assessed anti-inflammatory and anti-arthritis activity in rats. The hydroalcoholic and aqueous extracts of *Centipeda minima* leaves were prepared and evaluated for *in vitro* antioxidant activity namely DPPH, total polyphenol content, total flavonol content and reducing power assay. The different fractions were isolated from hydroalcoholic extracts by using column chromatography. The Flavonoids fractions were investigated for anti-inflammatory and anti-arthritis activity in carrageenan rat induced paw oedema, cotton pellet-induced granuloma model and adjuvant induced chronic arthritis in rats. The findings of *in vitro* antioxidant activity confirmed that hydroalcoholic extracts expressed higher antioxidant activity compared to aqueous extract, and hence hydroalcoholic extracts was further selected for the isolation of various fractions. The results of phytochemical study suggest that FCM6, FCM7 and FCM8 exposed the presence of polyphenol and flavonoids. The fraction FCM6, FCM7 and FCM8 (25 mg/kg) exhibits significant anti-inflammatory and anti-arthritis activity. The outcomes suggest that the anti-inflammatory and anti-arthritis activity of isolated fraction was due to presence of flavonoids and polyphenols.

**Keywords:** *Centipeda minima*; Anti-inflammatory activity; Anti-arthritis activity; Flavonoids; Carrageenan paw oedema; Cotton pellet; Adjuvant induced chronic arthritis

## Introduction

Rheumatoid arthritis is identified by synovial inflammation and irreversible joint destruction. The rheumatoid arthritis caused to significant disability. Mostly old age people are affected by this disorder. It is considered that females are more susceptible to the rheumatoid arthritis compared to male. The etiology of this disease is still unknown [1,2]. The various pro-inflammatory molecules including reactive oxygen species, prostaglandins, leukotrines and cytokines released by macrophages are involved in the cause of this disorder [3]. NSAIDS are used to control the rheumatoid arthritis; it leads to inhibit COX and LOX used for the metabolic modulation of arachidonic acid. NSAIDS are mostly associated with various side effects, severe adverse reactions and toxicity [4]. Consequently, research is required to develop novel anti-arthritis drugs, and it produces maximum therapeutic effects with lesser toxicity effects. Herbal remedies can form an alternative source to relieve patients from arthritis as well as to address the drawbacks associated with present treatment methods with allopathic drugs [4,5].

Compound obtained from plants imparts a remarkable role to cure and control various diseases from ancient times. A study conducted by World Health Organization (WHO) has reported that about 80% of world's population relies on traditional medicine [6]. The numerous studies has been conducted and validated the secondary metabolites presents in the plants imparts pharmacological activity to combat numerous disease [7,8]. Plant containing flavonoids or polyphenol is good choice to use as anti-arthritis drugs. The flavonoids smoothly inhibit the production of free radical, and lead to impede COX and LOX stimulation. Consequently, flavonoids control the inflammation and arthritis [8].

*Centipeda minima* (Asteraceae) is found in moist places. The aerial parts of the *Centipeda minima* are used to treat headaches, head colds, conjunctivitis, piles and malaria. Phytochemical studies of its composition have led to the identification of a number of flavonoids, polyphenol and terpenes. The former class contained the major active constituents contributing to the anti-inflammation and anti-arthritis activities of the herb [9]. We planned to isolate the flavonoids and polyphenol components from *Centipeda minima* extracts. Further the anti-inflammatory and anti-arthritis potential of flavonoids fraction isolated from *Centipeda minima* extracts were screened.

## Material and Methods

### Plant material

The leaves of *Centipeda minima* were selected for the proposed study. The plant material was authenticated by Dr. A.P. Singh, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

## Preparation of extract

The powdered leaves of *Centipeda minima* about 1 kg were packed in soxhlet apparatus and extracted with petroleum ether, hydro alcohol (mixture of 70% ethanol and 30% distilled water) and distilled water separately, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator.

## *In vitro* antioxidant activity of extract

**Hydrogen-donating activity:** The methanol solution of DPPH (100 mM, 2.95 ml), 0.05 ml of extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (%AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation [10]:

$$\%AA=100-\left\{\left[\left(Abs_{sample}-Abs_{blank}\right)\times 100\right]/Abs_{DPPH}\right\}$$

The $IC_{50}$ parameter of hydroalcoholic and aqueous extracts were determined using Microsoft Excel 2007.

**Total polyphenol content:** Total polyphenol content was determined using colorimetric method 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin- Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve [11]. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).

**Total flavonol content:** Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve [12]. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

**Reducing power assay:** The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg mL-1 extracts aliquot [12].

## Isolation of compound from hydroalcoholic extracts

The *Centipeda minima* extract was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the following solvent ratios of Hexane: dichloromethane (DCM), 100:0, 80:20, 60:40, 40:60, 20:80, 0:100, then with 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100, DCM:Ethanol (Eth). Finally eluted with 100:0, 90:10, 80:20, 70:30, 60:40, 50;50, 40:60, 30:70, 20:80, 10:90, 0:100, DCM:Methanol (MeOH). The fractions (25 ml) were collected from the column. Elutes collected were monitored by thin layer chromatography (eluent: DCM-MeOH, 9:1 and 3:2) for homogeneity and the similar fraction were pooled together. The eight different fractions were collected and dried [13]. The phytochemical screening was performed according to Harborne [14] and Kokate [15].

## Pharmacological activity

**Selection of animals:** Male Wistar rats (150-200 g), were used, and kept in qurantine for 10 days under standard husbandry conditions (27.3°C, Relative humidity 65 ± 10%) for 12 h in dark and light cycle respectively and were given standard food and water ad. libitum. All experiments were approved by the institutional ethical committee (1321/PO/ReBi/S/10/CPCSEA dated 22/10/2014) and were carried out according to the animal ethics committee guidelines.

**Anti-inflammatory activity:** The Carrageenan-induced oedema and cotton pellet-induced granuloma model were performed to assess the anti-inflammatory activity of isolated compounds from *Centipeda minima* extract.

*Effect of isolated compound of Centipeda minima on carrageenan-induced oedema*: Albino Wistar rats of either sex weighing between (150-200 g) were divided into various groups and six animals in each group. The groups were as follows:

Group I: Treated with distilled water (control group).

Group II: Treated with standard drug Indomethacin at 10 mg/kg body weight.

Group III: Treated with 6 at 25 mg/kg body weight.

Group I: Treated with FCM7 at 25 mg/kg body weight.

Group V: Treated with FCM8 at 25 mg/kg body weight.

Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan suspension in normal saline into the sub plantar region of right hind paw after 60 min of drug administration. The control group was administered only distilled water. The isolated compound and standard drugs administered intraperitoneally 1 h before carrageenan suspension administration. The isolated compounds are active component and produce potent therapeutic activity compared to crude extract. Hence one tenth part of extract dose (250 mg/kg) was selected for the isolated compound to evaluate pharmacological activity.

A mark was made on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The volume of paw oedema volume was measured with the help of plethysmograph by mercury displacement method immediately before and 5 h after the drug administration. The inhibition of oedema in various treated groups was then calculated by using statistical analysis.

*Effect of isolated compound of Centipeda minima on Cotton pellet-induced granuloma model*: Albino Wistar rats of either sex weighing between (150-200 g) were divided into various groups and six animals in each group. The groups were as follows:

Group I: Treated with distilled water (control group).

Group II: Treated with standard drug Indomethacin at 10 mg/kg body weight.

Group III: Treated with FCM6 at 25 mg/kg body weight.

Group IV: Treated with FCM7 at 25 mg/kg body weight.

Group V: Treated with FCM8 at 25 mg/kg body weight.

The animals were grouped as described above to study the anti-inflammatory activity. The groups were fasted and treated with drugs/doses similar to that of carrageenan-induced hind paw edema. Sterile cotton pellets each weighing 30 ± 5 mg were prepared and sterilized in a hot air oven at 123°C for 3 h. Each animal was placed under light ether anaesthesia and subcutaneously implanted with four cotton pellets, one each into both the axillae and the groin region under aseptic conditions. The drugs were administered orally for seven days starting from the day of implantation of the pellets. All the animals had free access to drinking water and food. On the 8$^{th}$ day, all the animals were sacrificed and the implanted cotton pellets were recovered, cleaned of surrounding tissues, and blotted with filter paper. These cleaned pellets were weighed and dried in a hot air oven overnight at 70°C and the dry weights were noted [16].

**Anti-arthritis activity:** The *mycobacterium* induced adjuvant arthritis model was used for exploring the anti-arthritis potential of isolated compounds.

*Effect of isolated compound of Centipeda minima on adjuvant induced chronic arthritis*: One week before the commencement of the experiment, the rats were randomly divided into five groups of six rats per group. On day 0 rats were injected with 0.1 ml of Freund's complete adjuvant (FCA) in to the sub plantar (s.p) region of the left hind paw of all the animals. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil by thoroughly grinding with a pestle and motor to give a final concentrate of 0.6 mg/mL. Administration of test compounds and standard drug was started on the next day and continued for 28 days. The paw was marked with ink at the level of the malleolus laterials and paw volumes were recorded by the plethysmometer immediately after injection and on 7$^{th}$, 14$^{th}$, 21$^{s}$t and 28$^{th}$ day. The experimental rats were randomly divided into five groups of six rats per group and treated as follows:

Group I: Arthritis rats treated with distilled water (control group).

Group II: Arthritis rats treated with standard drug Indomethacin at 10 mg/kg body weight.

Group III: Treated with FCM6 at 25 mg/kg body weight.

Group IV: Treated with FCM7 at 25 mg/kg body weight.

Group V: Treated with FCM8 at 25 mg/kg body weight.

*Hematological screening*: On the 28$^{th}$ day blood samples for hematological assays were obtained through ocular puncture of the rats and collected into EDTA-treated sample bottles. White blood cell (WBC) and Red blood cell (RBC) counts were assessed as stated in the method of Chesbrough and McArthur [17]. Drabkin and Austin method was used to confirming the Hemoglobin (Hb) content [18]. Estimation of erythrocyte sedimentation rate (ESR) was carried out by the method of Westergren [19].

*Data analysis*: Results were analyzed using one way analysis of variance (ANOVA) followed by the Tukey's test by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the $p<0.05$ were considered as statistically significant.

## Results and Discussions

In present study, *Centipeda minima* was selected for isolation of active constituents from crude extract; and check its anti-inflammatory and anti-arthritis activity of active constituents.

### *In vitro* antioxidant activity

The hydroalcoholic and aqueous extract of *Centipeda minima* were subjected to *in vitro* antioxidant studies to determine and compare the antioxidant activities of both extracts. Antioxidant ability of *Centipeda minima* extract was assessed by establishing its efficacy in hydrogen-donating, total polyphenol content, total flavonol content and reducing power assay models.

**Hydrogen-donating activity of Centipeda minima:** DPPH is stable nitrogen centered free radical that can adopt an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals act with appropriate reducing agents, and then depriving colour stoichometrically with the number of electrons depleted which is measured spectrophotometricallty at 517 nm [20]. As shown in Table 1, *Centipeda minima* of hydroalcoholic and aqueous extracts strongly scavenged DPPH radical with the IC50 being 67.01 and 116.49 µg/ml, respectively. The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 93.42. The hydroalcoholic extracts exhibited highest scavenging property compared to aqueous extracts.

| Concentration (µg/ml) | DPPH Scavenging % | | |
|---|---|---|---|
| | Hydroalcoholic Extract | Aqueous Extract | Ascorbic Acid |
| 50 | 40.23 ± 0.36 | 29.32 ± 0.43 | 93.42 ± 0.97 |
| 100 | 61.37 ± 0.72 | 48.63 ± 0.75 | - |
| 150 | 79.82 ± 0.49 | 59.73 ± 0.95 | - |
| 200 | 89.16 ± 1.02 | 72.58 ± 0.68 | - |
| 250 | 97.42 ± 0.58 | 85.16 ± 1.12 | - |
| IC50 | 67.01 | 116.49 | - |

**Table 1:** Free radical scavenging capacity of hydroalcoholic and aqueous extracts of *Centipeda minima*. Values are mean ± SEM of six determinations.

**Total phenolic content of Centipeda minima:** The hydroalcoholic and aqueous extract of *Centipeda minima* was evaluated for investigation of the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled

water for determining absorption data (Table 2). From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y=0.0383x+0.0021 (Figure 1). Results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in Table 2. The total phenolic content in extracts, expressed as gallic acid equivalents. Total phenolic content of hydroalcoholic and aqueous extract of *Centipeda minima* were 91.25 and 82.61 GAE mg/g respectively. Hydroalcoholic extracts exhibited highest amount of total polyphenol content compared to aqueous extracts.

| Extract | Total polyphenol content (GAE mg/g) |
|---|---|
| Hydroalcoholic | 91.25 ± 1.05 |
| Aqueous | 82.61 ± 1.36 |

**Table 2:** Determination of total polyphenol content of *Centipeda minima*. Data expressed as gallic acid equivalent (GAE) mg per g of the extract, Values are mean ± SEM of triplicate determinations.

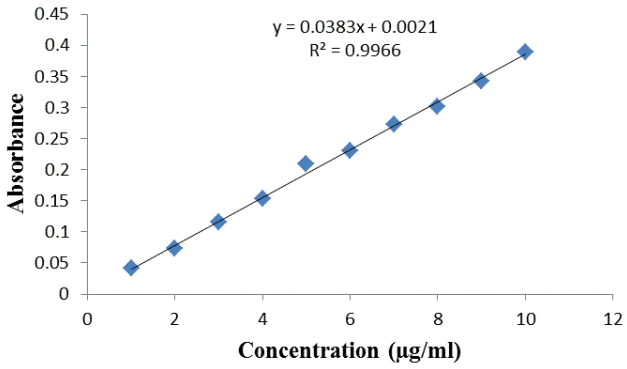


**Figure 1:** Calibration curve of gallic acid in distilled water.

**Total flavonol content of Centipeda minima:** The concentration of flavonoids in hydroalcoholic and aqueous extract of *Centipeda minima* were determined spectrophotometrically using aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalents. Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of quercetin was found to be y=0.0382x+0.0097 (Figure 2). The content of flavonoids identified in the tested extracts is shown in Table 3. The concentrations of flavonoids in hydroalcoholic and aqueous extract of *Centipeda minima* were 69.21 and 47.38 QE mg/g respectively. Hydroalcoholic extracts exhibited highest amount of flavonoids content compared to aqueous extracts.

The flavonoids are good source of medicines and used for the treatment of various diseases. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolic, in general are highly effective in free radicals scavenging, and they are antioxidants [20].

The findings of total polyphenol and flavonol content of hydroalcoholic and aqueous extract of *Centipeda minima* supports the study of DPPH scavenging capacity of extracts.

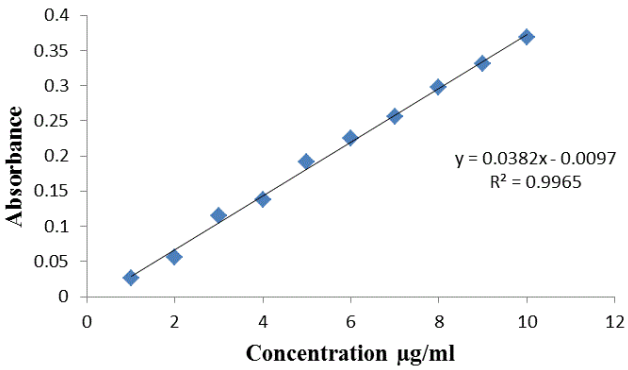


**Figure 2:** Calibration curve of quercetin in distilled water.

| Extract | Total flavonol content (QE mg/g) |
|---|---|
| Hydroalcoholic | 69.21 ± 0.52 |
| Aqueous | 47.38 ± 0.73 |

**Table 3:** Determination of total flavonol content of *Centipeda minima*. Data expressed as gallic acid equivalent (GAE) mg per g of the extract, values are mean ± SEM of triplicate determinations.

**Reducing power assay of Centipeda minima:** The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 4 revealed that the antioxidant activity of hydroalcoholic and aqueous extract of *Centipeda minima*. Reducing power of the hydroalcoholic and aqueous extract of *Centipeda minima* were found to be 52.46% and 45.52% respectively.

| Particulars | Absorbance at 700 nm | Antioxidant activity (%) |
|---|---|---|
| Ascorbic acid | 0.749 ± 0.03 | 100.00 |
| Hydroalcoholic extract | 0.393 ± 0.02 | 52.46 |
| Aqueous extract | 0.341 ± 0.04 | 45.52 |

**Table 4:** Antioxidant activity of *Centipeda minima* extracts. Values are mean ± SEM of triplicate determinations.

The reducing power of ascorbic acid was found to be higher than hydroalcoholic and aqueous extracts. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability [21]. Hydroalcoholic extract of *Centipeda minima* might contain high amount of reductions than aqueous extract. The result indicates that extracts act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions [21]. The findings indicate that antioxidant activity was produced due to the presence of polyphenol compounds.

From the results of antioxidant activity, it can be concluded that hydroalcoholic extracts of *Centipeda minima* produces higher antioxidant activity compared to aqueous extract and could alleviate the number of oxidative stress induced inflammation and arthritis

[20]. The above study was done only for proper selection of extracts of *Centipeda minima*, to isolate the polyphenol and flavonoids fractions from extract expressing maximum antioxidant activity [22]. Hence the hydroalcoholic extract of *Centipeda minima* was selected for the isolation of polyphenol and flavonoids compounds by column chromatography.

## Isolation of compound from hydroalcoholic extract of *Centipeda minima*

The hydroalcoholic extract of *Centipeda minima* was subjected to column chromatography and fractions were eluted with the gradient polarity of solvent namely hexane, dichloromethane, ethanol and methanol. The eight different fractions were collected and dried. The fraction FCM1 and FCM2 were containing waxy material; the fractions FCM3 and FCM4 were powder but quantity was very little. The yield of fraction FCM5, FCM6, FCM7 and FCM8 were 280 mg, 420 mg, 340 mg and 370 mg. The four fractions were further analysed for phytochemical screening to determine the nature of isolated fractions.

## Preliminary phytochemical analysis of isolated fraction of hydroalcoholic extract of *Centipeda minima*

The phytochemical investigation of FCM5 of *Centipeda minima* leaves revealed the presence of alkaloids. The FCM6 exhibits the presence of alkaloids, saponins, carbohydrates, glycosides, tannins & phenolic compounds and flavonoids. The FCM7 indicates the presence of tannins & phenolic compounds and flavonoids. The FCM8 revealed the presence of alkaloids, glycosides, tannins & phenolic compounds and flavonoids (Table 5).

| Phytoconstituents | FCM5 | FCM6 | FCM7 | FCM8 |
|---|---|---|---|---|
| Alkaloids | + | + | - | + |
| Glycosides | - | + | - | + |
| Carbohydrates | - | + | - | - |
| Tannins & Phenolic compounds | - | + | + | + |
| Flavonoids | - | + | + | + |
| Steroids | - | - | - | - |
| Proteins and Amino acids | - | - | - | - |
| Fixed Oils and Fats | - | - | - | - |

**Table 5:** Preliminary phytochemical analysis of isolated fraction of hydroalcoholic extract of *Centipeda minima*. (+) indicates present and (-) indicates absent.

Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. Especially, some flavonoids were found to inhibit chronic inflammation of several experimental animal models. Thus, it may be valuable to continuously evaluate the anti-inflammatory and anti-arthritis activity of flavonoids, not only for establishing anti-inflammatory and anti-arthritis mechanisms, but also for developing a new class of anti-inflammatory agents [23]. The FCM6, FCM7 and FCM8 containing polyphenol and flavonoids compound and these organic substances impart chief role in anti-inflammatory and anti-arthritis activity. Hence this result supports to evaluate the anti-inflammatory and anti-arthritis activity of the FCM6, FCM7 and FCM8.

## *In vivo* assays

The *in vitro* studies of the FCM6, FCM7 and FCM8 isolated from *Centipeda minima* leaves extracts indicate the presence of flavonoids and polyphenol. Therefore *in vivo* activities were performed with FCM6, FCM7 and FCM8.

### Anti-inflammatory studies

*Carrageenan-induced oedema:* The effect of FCM6, FCM7 and FCM8 isolated from *Centipeda minima* on carrageenan-induced paw oedema is presented in (Table 6). The animals administered only distilled water, the sub plantar injection of carrageenan produced a local oedema that increased progressively from 0.26 ± 0.28 ml after 1 h to reach a maximum within 5 h. Administration of fraction FCM6, FCM7 and FCM8 (25 mg/kg) revealed significant ($P<0.05$) reduction in oedema in the rats compared with the same time of the distilled water treated group. Indomethacin (10 mg/kg) produced a significant ($P<0.05$) decrease in oedema at the 2 h compared with the same time of the distilled water treated group. The decrease order of oedema in the rats for fractions were FCM6>FCM7>FCM8.

| Group | Paw volume after induction | | | | |
|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 5 h |
| Control | 0.26 ± 0.28 | 0.62 ± 1.58 | 0.82 ± 1.27 | 1.02 ± 1.81 | 0.94 ± 0.64 |
| Indomethacin (10 mg/kg) | 0.24 ± 0.53 | 0.28 ± 1.37* | 0.33 ± 1.72* | 0.26 ± 1.64* | 0.18 ± 1.39* |
| FCM6 (25 mg/kg) | 0.27 ± 1.25 | 0.39 ± 1.53 | 0.46 ± 1.42* | 0.42 ± 1.78* | 0.19 ± 1.31* |
| FCM7 (25 mg/kg) | 0.24 ± 1.67 | 0.45 ± 1.53 | 0.58 ± 1.78* | 0.50 ± 1.46* | 0.27 ± 1.28* |
| FCM8 (25 mg/kg) | 0.25 ± 1.83 | 0.48 ± 1.67 | 0.60 ± 1.29* | 0.55 ± 1.38* | 0.30 ± 1.18* |

**Table 6:** Effect of isolated fraction from *Centipeda minima* leaves extract on carrageenan induced paw oedema. Values are expressed as mean ± SEM, n=6 in each group. *$P<0.05$ compared to control group.

The present study creates the anti-inflammatory activity of the isolated compound of *Centipeda minima* leaves in a number of experimental rat models, which represent different phases of inflammation. The isolated compound namely FCM6, FCM7 and FCM8 exhibited anti-inflammatory effect on carrageenan-induced paw oedema. The FCM6 isolated from *Centipeda minima* produces maximum anti-inflammatory effect compared to other isolated compounds. Carrageenan-induced oedema is a model of acute inflammation used in the study of NSAIDS drugs. The model is suitable for evaluating the antioedematous effect of natural products and is believed to be biphasic. The first phase which occurs within an hour is believed to involve the release of serotonin and histamine while the second phase which occurs after 1 h has been attributed to prostaglandin and the continuity between the two phases is provided by kinin [23]. That the isolated compound produced marked anti-

inflammatory effect 2 h post-carrageenan injection suggests that its anti-inflammatory activity may involve the inhibition of prostaglandin synthesis and cyclooxygenase products since the carrageenan inflammatory model basically reflects the action of prostaglandins. The isolated compound prevented formation of exudate and leucocytes mobilization induced by intraperitoneal injection of carrageenan. The carrageenan-induced leucocytes migration assay has been adjudged as an excellent acute and sub-acute model for the measurement of fluid extravasation, leucocytes migration and other biochemical parameters which accompany inflammatory stimuli. Production of exudate in this model is related to local release of histamine, kinins and synthesis of prostaglandins. Migration of leucocytes would not be directly related to cyclooxygenase products, but the process is inhibited by non-steroidal anti-inflammatory compounds indicating that many mechanisms may be implicated in its control. The inhibitory effect of the isolated compound on the intra-peritoneal formation of exudate and leucocytes mobilization is probably due to the inhibition of prostaglandins. This possibility is reinforced by the fact that the isolated compound remarkably inhibited paw oedematous process which is believed to be mediated by prostaglandins [24]. Moreover, it is reported that the flavonoids display anti-inflammatory activity by the inhibition of prostaglandin synthesis.

*Cotton pellet-induced granuloma model*: The effect of FCM6, FCM7 and FCM8 isolated from *Centipeda minima* was studied at the doses of 25 mg/kg per body weight. The results revealed that the isolated fraction of *Centipeda minima* shows dose dependent inhibition of weight of both wet and dry cotton pellets. The mean number of decrease in weight of both wet and dry cotton pellets for rats, which received 25 mg/kg body weight of the isolated compound was significant ($p<0.05$) lower than those in the control rats (Table 7). The FCM6, FCM7 and FCM8 demonstrated 32.06%, 29.13% and 22.19% inhibition, respectively in weight of wet cotton pellets. The FCM6, FCM7 and FCM8 demonstrated 65.15%, 57.24% and 51.94% inhibition, respectively in weight of dry cotton pellets. The FCM6 was found to be most effective compared to other isolated compound. The standard drug exhibited 35.00% and 65.15% inhibition in weight of wet cotton pellets and dry cotton pellets respectively.

| Group | Wet weight (mg) | %Inhibitions | Dry weight (mg) | %Inhibitions |
|---|---|---|---|---|
| Control | 302.21 ± 1.35 | -- | 82.14 ± 2.18 | -- |
| Indomethacin (10 mg/kg) | 196.43 ± 1.58* | 35.00 | 28.62 ± 1.63* | 65.15 |
| FCM6 (25 mg/kg) | 205.32 ± 2.05* | 32.06 | 35.12 ± 2.08* | 57.24 |
| FCM7 (25 mg/kg) | 213.61 ± 1.74* | 29.31 | 39.47 ± 1.47* | 51.94 |
| FCM8 (25 mg/kg) | 235.14 ± 2.31* | 22.19 | 51.37 ± 1.72* | 37.46 |

**Table 7:** Effect of isolated fraction from *Centipeda minima* leaves extract on cotton pellet-induced granuloma model in rats. Values are expressed as mean ± SEM, n=6 in each group. *P<0.05 compared to control group.

Generally the cotton-pellet granuloma is employed to assess the transudative and proliferative components of the chronic inflammation. The transudate correlates with the moist weight of the pellets while dry weight of the pellet correlates with the amount of granulomatous tissues. The proliferation of macrophages, neutrophils and fibroblast are responsible for granuloma formation, and leads to chronic inflammation. Indomethacin diminishes the granuloma dimension by deterring the granulocyte infiltration, impeding the production of collagen fibers and quashing muco-polysaccharides [25]. The isolated fractions of *Centipeda minima* showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. Consequently, the pattern of anti-inflammatory activity displayed by the isolated fractions was similar to that of indomethacin. The flavonoids impart anti-inflammatory activity, and phytochemical study of isolated fractions justifies the above statement. The outcome of the study endorses the anti-inflammatory activity of isolated fraction was due to presence of flavonoids.

### Anti-arthritis activity

*Adjuvant Induced Chronic Arthritis*: The anti-arthritis models proposed that mycobacterial infections can trigger autoimmune arthritis, predominantly through T-cell mediated responses. Arthritis was induced in rats by injecting dead mycobacteria in liquid paraffin. There was a significant increase in rat paw volume in FCA injected control rats when compared to the standard and fraction treated rats. The rats treated with isolated fractions at the dose of 25 mg/kg exhibited significant reduction in rat paw oedema volume compared to control group. Table 8 demonstrated the effect of FCM6, FCM7 and FCM8 isolated from *Centipeda minima* on Freund′s adjuvant model induced arthritis. After 28 days, it was found that FCM6, FCM7 and FCM8 significantly displays dose dependent inhibition in paw thickness i.e. the chronic inflammation induced by adjuvant shows decrease in paw thickness. The decreased in paw thickness after administration of FCM6, FCM7 and FCM8 were 0.25 ± 0.24, 0.31 ± 0.62 and 0.39 ± 0.74 ml, respectively. Standard drug indomethacin significantly decreased the paw thickness i.e., 0.23 ± 0.91ml after induction of Freund′s adjuvant. The FCM6 was found to be most effective compared to other isolated compound.

*Hematological parameters*: The administration of FCM6, FCM7 and FCM8 isolated from *Centipeda minima* on Freund′s adjuvant induced arthritis animals enhanced the levels of RBC and Hb compared to control animals (Table 9). The WBC count and ESR were significantly reduced after administration FCM6, FCM7 and FCM8 compared to control animals.

Rheumatoid arthritis is a widespread autoimmune disorder; the FCA incited arthritis model is pondered as one of the prominent animal models of rheumatoid arthritis. The systemic inflammation for arthritis was induced in experimental animals by inoculating with FCA (which was prepared by suspending heat-killed *Mycobacterium butyricum* in liquid paraffin at a dose of 10 mg/ml *Mycobacterium butyricum* in paraffin oil).

The investigation of paw oedema is according to the grapevine simple, inclined and rapid procedure to evaluate the degree of inflammation, and assess the therapeutic effects of drugs [25]. The adjuvant-induced arthritis rats developed a chronic swelling in several joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodelling which have close similarity to

human rheumatoid arthritis [24]. These inflammatory changes eventually result in the complete destruction of joint stability and mobility in the arthritis rats. Also, soft tissue swelling around the ankle joints was appeared during the progress of arthritis in FCA injected rats, which was considered as oedema of the exacting tissues [26].

| Group | Paw volume after induction | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
| Control | 0.21 ± 0.32 | 0.73 ± 0.47 | 0.98 ± 0.96 | 1.12 ± 0.25 | 0.98 ± 0.42 |
| Indomethacin (10 mg/kg) | 0.27 ± 0.54 | 0.53 ± 0.83* | 0.45 ± 0.63* | 0.32 ± 0.59* | 0.23 ± 0.91* |
| FCM6 (25 mg/kg) | 0.22 ± 0.38 | 0.55 ± 0.72 | 0.42 ± 0.83* | 0.37 ± 0.39* | 0.25 ± 0.24* |
| FCM7 (25 mg/kg) | 0.26 ± 0.92 | 0.60 ± 0.21 | 0.49 ± 0.15* | 0.41 ± 0.57* | 0.31 ± 0.62* |
| FCM8 (25 mg/kg) | 0.23 ± 0.41 | 0.66 ± 0.35 | 0.58 ± 0.48* | 0.50 ± 0.82* | 0.39 ± 0.74* |

**Table 8:** Effect of isolated fraction from *Centipeda minima* leaves extract on FCA induced paw volume. Values are expressed as mean ± SEM, n=6 in each group. *P<0.05 compared to control group.

| Group | RBC (millions/cmm) | WBC (thousands/cmm) | Hb (g /dL) | ESR (mm/h) |
|---|---|---|---|---|
| Control | 3.25 ± 1.24 | 23.17 ± 0.84 | 8.31 ± 1.53 | 35.26 ± 1.32 |
| Indomethacin (10 mg/kg) | 8.12 ± 0.48* | 9.63 ± 1.41* | 13.74 ± 0.58* | 2.31 ± 0.67* |
| FCM6 (25 mg/kg) | 8.36 ± 1.35* | 10.27 ± 0.58* | 13.06 ± 0.76* | 3.47 ± 1.29* |
| FCM7 (25 mg/kg) | 7.31 ± 0.46* | 11.14 ± 0.85* | 12.63 ± 1.41* | 4.14 ± 1.15* |
| FCM8 (25 mg/kg) | 7.16 ± 1.24* | 12.51 ± 0.72* | 12.32 ± 0.29* | 5.42 ± 0.59* |

**Table 9:** Effect of isolated fraction from *Centipeda minima* leaves extract on hematological parameters. Values are expressed as mean ± SEM, n=6 in each group. *P<0.05 compared to control group.

In the present study, experimental arthritis was reliably established with repeated and daily subcutaneous plantar injection of 0.1 ml of FCA over a period of 28 days which was characterized by plantar oedema formation which maximized on day 7 of the treatment and subsequently increase for the remaining part of the study. The inflammation induced by FCA is primarily due to oedema formation and cellular influx.

The progression of arthritis was confirmed in our study by scoring total arthritis lesions. The inflammation associated with AIA is mainly dependent on prostaglandin E2 generated by cyclooxygenases. Besides, the role of cytokines like TNF-α and IL-1 has also been implicated in this model [24]. The outcomes indicate that the FCM6, FCM7 and FCM8 treated arthritis animals showed decreased inflammation of joints. Therefore, the anti-arthritis action of isolated fractions may be mediated by prostaglandin and/or cytokine inhibition. These results are also in line with reports that anti-inflammatory action of *Centipeda minima* leaves has been attributed to polyphenolic component. The inhibition of lipid peroxidation, capillary permeability and fragility, and enzymes such as phospholipase A2, cyclooxygenase, and lipoxygenase may be due to tannins and polyphenols components [27].

In present study, arthritis control rats showed a reduced RBC count, reduced Hb levels, and an increased erythrocyte sedimentation rate (ESR). All these symptoms indicate an anemic condition. The FCM6, FCM7 and FCM8 treated groups showed a significant recovery from the induced anemia. The significant increase in leucocyte count in adjuvant induced arthritis rats may be due to the stimulation of immune system against the invading antigens and significant decrease in FCM6, FCM7 and FCM8 treated groups showed its immunomodulation effect. This clearly indicates the anti-arthritis activity of *Centipeda minima* isolated fractions. This study confirmed that flavonoid fraction obtained from *Centipeda minima* leaves extract are responsible for its anti-arthritis activity and the effects observed are attributable due to the presence of flavonoids in the plant.

It has been validated by various researchers that medicinal plants demonstrating relations between anti-inflammatory/anti-arthritis and phenol or flavonoid content. Several researches suggest that the combination of secondary metabolites with flavonoids compounds produces synergistic pharmacological activity [28]. Although no record of chemical constituents isolated and characterized from *Centipeda minima* was found, and the methods used for the identification of phytochemical constituents are preliminary in nature. The anti-inflammatory and anti-arthritis effects recorded for isolated fractions of *Centipeda minima* in this study, caused by the total polyphenol and flavonoids constituents present in the plant. The phytochemical study of isolated compound justifies the above statement. The results obtained in this study established the anti-inflammatory and anti-arthritis actions for the isolated fractions. However, the mechanism of these actions is uncertain, and the flavonoids and polyphenol imparts chief role for the anti-inflammatory and anti-arthritis activity.

## Conclusion

The flavonoids component isolated from the hydroalcoholic extracts of *Centipeda minima* exhibited significant anti-inflammatory and anti-arthritis activity. The *in vitro* antioxidant activity and phytochemical findings justified that the anti-inflammatory and anti-arthritis activity of the bioactive components might be due to presence of flavonoids compound. Moreover, it required to conduct further study to conclude the possible mechanism responsible for anti-inflammatory and anti-arthritis activity of isolated flavonoids fractions.

## Acknowledgements

We thank to the Institute of Pharmacy, HCPG College, Varanasi, Uttar Pradesh, India and Columbia Institute of Pharmacy, Tekari, Raipur, Chhattisgarh, India for providing research facilities to carry out research work.

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