

ACUTE AND SUBACUTE ANTI-INFLAMMATORY ACTIVITIES OF DICHLOROMETHANE  
EXTRACT OF *CASSIA ALATA* (LINN.) LEAVES IN WISTAR RATS

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**Abstract**

**Background:** In Burkina Faso, leaves of *Cassia alata* are used in the treatment of several diseases, including inflammation. This study evaluated the anti-inflammatory effects of the dichloromethane extract of *Cassia alata* leaves (CF-AECal) on different models of inflammation in wistar rats in order to enhance its use in traditional medicine.

**Materials and Methods:** Acute inflammation was induced among rats with 0.1 ml of carrageenan, serotonin, histamine and dextrane 1%. The effects of the CF-AECal 50 mg/kg and 100 mg/kg were compared to the effects of diclofenal 5 mg/kg, prednisone 5 mg/kg, promethazine 1 mg/kg, cyproheptadin 2 mg/kg. Cotton pellet and air pouch - induced granuloma permitted to study CF-AECal effects on the subacute inflammation. The ways of administrations were intra peritoneal for the substances of inductions and oral for the molecules of references and CF-AECal.

**Result:** Carrageenan-induced paw edema has been inhibited to 52.39% by CF-AECal 50 mg/kg and 50.17% by CF-AECal 100 mg/kg. Wet granulomas formation has been inhibited by CF-AECal 50 and 100 mg/kg to 20.94 and 57.82%. The dry granulomas were inhibited at 24.32 and 59.04% by CF-AECal 50 and 100 mg/kg. Air pouch fluid was significantly reduced by CF-AECal in comparison to the control group. There was a massive recruitment of leukocytes in the pocket granuloma of the control group. CF-AECal caused strong inhibition of this infiltration of leukocytes ( $p < 0.001$ ).

**Conclusion:** Dichloromethane extract of *Cassia alata* showed anti-inflammatory effects.

**Key words:** *Cassia alata*, anti-inflammatory effect, animal models, edema, granuloma

**Abbreviations:** CF-AECal: dichloromethane extract of *cassia alata*

**Introduction**

Inflammation is a local response of living tissue of mammals to consecutive various attacks that can be physical, chemical, biological (immune response) or infectious. Several mediators such as histamine, serotonin, bradykinins and prostaglandins are involved in different phases of inflammation. Inflammation starts by a local vasodilation and an increase of capillary permeability causing an inflammatory edema consecutive to the passage of an exudate. Then the leukocytes infiltrate the inflamed tissue and finally there is formation of the granuloma tissue. Inflammatory diseases constitute a health problem in the world. Indeed, the non-steroidal anti-inflammatory drugs prescribed although efficient, generate side effects such as gastric intolerance and renal failure that impede their use to the long short (Gaziano *et al.*, 2006; Corrado *et al.*, 2009). These side effects are related to inhibition of cyclooxygenase constitutive and inducible by the conventional NSAIDS. The NSAIDS with selective action on cyclooxygenase inducible reduce gastro intestinal ulcers but increase the cardiovascular risk (Zeilhofer, 2007). For bearing to these secondary effects, patients use medicinal plants with anti-inflammatory activity. Among them, *Cassia alata* or *Senna alata* (Caesalpinaceae) is used in the tropical countries. Previous studies showed that *Cassia*

*alata* possesses broncho-relaxing and anti-genotoxic activities (Ouedraogo *et al.*, 2013), laxative, anti-inflammatory, anti-mutagenic, analgesic and antimicrobial properties (Khan *et al.*, 2001; Somchit *et al.*, 2003; Villasenor *et al.*, 2002).

Other studies showed that *Cassia alata* inhibits the installation of diabetes (Villasenor *et al.*, 2002). In Burkina Faso, the leaves of *Cassia alata* are used in the treatment of several diseases; including inflammation (Nacoulma/Ouedraogo, 1996). The aim of this study was to evaluate the anti-inflammatory activity of dichloromethane fraction of *Cassia alata*, on acute and subacute animal models.

## Materials and Methods

### Preparation of the plant

Leaves of *Cassia alata* were harvested in Ouagadougou at September 2013 and identified by Dr Ouedraogo of University Ouagal Pr Joseph KI-ZERBO, where a voucher specimen n°15965 was deposited. The leaves were dried at room temperature and crushed into powder. One kilogram of powder of *Cassia alata* was macerated at room temperature in alcohol 80% for 48 hours. Preparation was filtered using a whatman paper, concentrated in a rotary evaporator under reduced pressure and lyophilized. The yield was 13.75 %. This extract (10g) was dissolved in distilled water (75 ml). The aqueous extract obtained was introduced into a separating funnel and 75 ml of hexane was added. The mixture was shaken vigorously. The agitation was marked by periods of lower pressure in the bulb. After thirty minutes of decantation, hexane fraction which was distinguished clearly from aqueous fraction was collected. The same procedure was repeated three times with the aqueous fraction that remained. All fractions in the hexane were concentrated in the rotavapor. Dichloromethane (75 ml) was added to the aqueous fraction. The same procedure described above was followed to obtain the final fraction of dichloromethane (CF-AECal).

### Qualitative phytochemical analysis of *Cassia alata*

Preliminary chemical tests have been done with the dichloromethane extract to identify different phyto-constituents (Odebiyi et Sofowora, 1978)

### Drugs and reagents

Carrageenan, serotonin, histamine, dextran were purchased from Sigma Aldrich Chemicals, Bangalore; diclofenal (Gift sample from Glenmark Laboratories Ltd. Mumbai), prednisone, promethazin, cyproheptadin were used for the various tests. All drugs were dissolved in sterile saline (NaCl 0.9%).

### Animals

*Albino swiss* rats of Wistar stump from the pet center of the Faculty of Science, University Yaoundé I; was used. The rats were from 7 to 9 weeks-olds and weighing 120 g to 150 g. The animals received water and food *ad libitum*, at room temperature conditions ( $25 \pm 2^\circ\text{C}$ ) and 12:12 h light/dark cycle. They were divided into groups of five rats each. Twelve hours before the experiment, animals were fasted. The rats were anaesthetized with intraperitoneal injection of diethyl ether. All animal handling and procedures strictly conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication no. 85-25, revised 1996).

### Acute inflammation

#### Carrageenan induced rat paw edema

Four groups of rats were used for experimentation. For each rat, initial volume ( $V_0$ ) of the right hind paw was measured with the plethysmometer 37140 Ugo Basile. The rats of group 1 received distilled water orally (negative control). Animals of groups 2 and 3 received 50 and 100 mg/kg of dichloromethane extract of *Cassia alata* respectively by oral route. Rats of group 4 received diclofenal (5 mg/kg) by oral gavage (positive control). Thirty minutes after this treatment, each rat received subcutaneous injection of carrageenan 1 % (0.1 ml) under plantar fascia on the right hind paw. Paw oedema evolution was determined 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after induction of inflammation (Dongmo *et al.*, 2003; Winter, 1962).

Oedema size was appreciated by the determination of increase of rat paw volume. Percentage of inhibition ( $P_i$ ) was obtained by the following formula:

$$P_i (\%) = \frac{(V_t - V_0) \text{ control} - (V_t - V_0) \text{ test}}{(V_t - V_0) \text{ control}}$$

Where  $P_i$  is the percentage of inhibition of oedema,  $V_0$  and  $V_t$  are the average initial paw volume of rats and the average volume of paw at a time "t" after induction of inflammation, respectively.

#### Serotonin and histamine induced edema

Rats received distilled water (*p.o.*), dichloromethane extract of *Cassia alata* (50 or 100 mg/kg, *p.o.*). Prednisone (5 mg/kg, *p.o.*) and promethazine (1 mg/kg) were used as reference drugs for serotonin and histamine

induced paw oedema, respectively. Thirty minutes after the treatment of animals, they received subplantar injection of serotonin 1% (0.1 ml) or histamine 1% (0.1 ml). Oedema was determined 30 or 60 min after serotonin (Lanher *et al.*, 1991; Dimo *et al.*, 2006) or histamine injection (Dimo *et al.*, 2006; Singh *et al.*, 1996), respectively. Percentage of inhibition (Pi) is obtained by the previous formula.

### Dextran-induced paw edema

Rats were treated with distilled water, CF-AECal (50 mg/kg or 100 mg/kg) or cyproheptadin (2 mg/kg) one hour before subplantar injection of dextran solution (1%, 0.1 ml) on the paw rear right (Mandal *et al.*, 2000). Paw edema of each animal was measured before dextran injection (Vo) and 30 min, 1 and 2 hours after induction of inflammation. Percentage of inhibition was calculated as previously described.

### Subacute inflammation

#### Cotton pellet granuloma model

Twenty five rats were anesthetized by intraperitoneal injection of valium (2 ml/kg) and ketamine (10 ml/kg). Two cotton balls weighing 7 mg each were sterilized during 24 hours at  $40 \pm 0.5$  °C. Sterilized cotton pellets were implanted subcutaneously in the inguinal region of each rat, under sterile conditions. The rats of normal group received only distilled water. The other groups of rats were daily treated with distilled water, diclofenal (5 mg/kg), CF-AECal (50 or 100 mg/kg) for seven days. The eighth day of the experiment, the animals were anesthetized and sacrificed by decapitation. Blood was collected in EDTA tubes and used for haematological parameters. Wet granulomas were removed and weighted. They were dried at  $60 \pm 0.5$ °C for 24 hours for determination of dried granulomas (Ismail *et al.*, 1997; Subhadradeci *et al.*, 2010). The percentages of inhibition (Pi) of wet and dry granulomas are obtained by the formula:

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

### Carrageenan-induced inflammation in the air pouch

Twenty five rats were used for the experimentation. They were anesthetized by injection of valium (2 ml/kg) and ketamine (10 ml/kg) intraperitoneally. Pouch was formed by injection of 6 ml of air subcutaneously in the back of the animal. It was immediately filled by injection of carrageenan 2% (4 ml). Animals received a daily treatment with distilled water, diclofenal (5mg/kg), CF-AECal (50 and 100 mg/kg) and by oral route for six days. Normal group received only distilled water. On the 7<sup>th</sup> day of the experiment, rats were anesthetized with ether and then sacrificed by decapitation. Pouch fluid was collected by puncture and quantified. Exudate was diluted in NaCl 0.9% and the number of leukocytes was counted under light microscope (Selye, 1957). The number of cells per mm<sup>3</sup> was calculated by the formula:

$$X = \frac{N \times fd \times 10^6}{n \times V}$$

X = number of leukocytes per liter of blood; N = number of cells counted in the grid; fd = factor of dilution; n = number of grids counted; V = volume of a grid (0.01 mm<sup>3</sup>).

Pouch granuloma was removed and weighted in wet state and dry state after 24 hours in the oven at  $60 \pm 0.5$ °C. Wet and dry granulomas inhibition (Pi) was obtained by the previous formula.

### Statistical analysis

The experimental results were expressed as the mean  $\pm$  SEM for six animals in each group. The biochemical parameters were analyzed statistically using one-way ANOVA followed by Newman-keuls post hoc test with the help of Graph Pad Prism 5.03 software. P value of  $< 0.05$  was considered as statistically significant.

## Results

The results of qualitative phytochemical analysis of the dichloromethane extract of *Cassia alata* leaves is shown in Table 1

**Table 1:** Preliminary qualitative phytochemical analysis of *Cassia alata* leaves

Dichloromethane extract of <i>Cassia alata</i> leaves	Results
Alkaloids	+
Phenols	+
Flavonoids	+
Tannins	+
Anthraquinones	+
Saponins	+
Triterpenes	-

(-): No presence, (+): presence

### Acute anti-inflammatory studies Carrageenan induced rat paw edema

The results of the anti-inflammatory effects of CF-AECal on carrageenan-induced edema are summarized in Table 2. CF-AECal 50 and 100 mg/kg and diclofenal exhibited a maximum effect 5 hours after injection of carrageenan, reducing inflammation by 52.39 %, 50.17 % and 75.14 % respectively.

**Table 2:** Effect of CF-AECal on carrageenan induced-paw volume in rats

Treatments	Doses (mg/kg)	Paw volume (ml)						
		0,5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control		0.81 ± 0.02	0.87 ± 0.01	1.68 ± 0.03	1.82 ± 0.07	2.05 ± 0.04	1.71 ± 0.04	1.76 ± 0.05
CF-AECal	50	0.67 ± 0.02** (17.69)	0.79 ± 0.01 (10.04)	1.09 ± 0.04*** (35.47)	1.39 ± 0.06*** (23.49)	1.36 ± 0.07*** (33.85)	0.82 ± 0.06*** (52.39)	0.85 ± 0.05*** (51.53)
CF-AECal	100	0.8 ± 0.04 (1.72)	0.46 ± 0.04 *** (47.26)	0.91 ± 0.03*** (46.26)	1.23 ± 0.08*** (32.27)	1.53 ± 0.06 *** (25.56)	0.85 ± 0.02*** (50.17)	0.9 ± 0.03*** (49.03)
Diclofenal	5	0.62 ± 0.01*** (23.83)	0.47 ± 0.05*** (46.35)	0.83 ± 0.05*** (51.01)	1.15 ± 0.07*** (36.66)	1.33 ± 0.05*** (35.12)	0.43 ± 0.05*** (75.14)	1.33 ± 0.07*** (24.40)

The values are expressed as mean ± SEM, (n = 5). The values in parentheses represent the percentages of inhibition. \*\* p < 0.01, \*\*\* p < 0.001 compared to control animals. CF-AECal = dichloromethane fraction of *Cassia alata* aqueous extract.

### Dextran-induced paw edema

Dextran-induced paw oedema was reduced all over the experiment by CF-AECal, which exhibited a maximum effect of 53.27% at the dose of 100 mg/kg one hour after induction of inflammation. (Figure1).

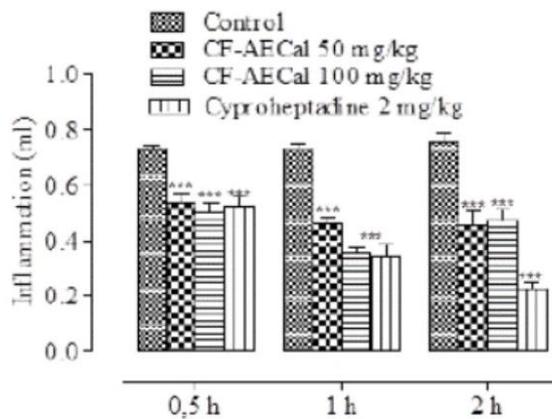


Figure 1: Effect of CF-AECal on dextran-induced rat paw oedema. Every rod represents mean  $\pm$  ESM, n=5. \*\*\*p < 0.001 compared to control animals. CF-AECal = dichloromethan fraction of *Cassia alata* aqueous extract.

### Serotonin and histamine induced oedema

Serotonin and histamine-induced paw oedema were reduced by the extract of *Cassia alata*. Thirty minutes after Serotonin administration, paw oedema was inhibited by 22.22%, 51.06% and 68.79% by CF-AECal (50 and 100 mg/kg) and cortancyl, respectively. When administered at 100 mg/kg, the anti-inflammatory effect of the plant extract (29.45%) against histamine-induced paw oedema was comparable to the effect of promethazine (31.29%) (Table 3).

**Table 3:** Effects of CF-AECal, on acute inflammation induced by serotonin and histamine in Wistar rats

Treatments	Doses (mg/kg)	Paw volume (mL)	
		Serotonin	Histamine
Control		0.846 $\pm$ 0.017	3.26 $\pm$ 0.163
CF-AECal	50	0.658 $\pm$ 0.022*** (22.22)	2.44 $\pm$ 0.067*** (25.15)
CF-AECal	100	0.414 $\pm$ 0.027*** (51.06)	2.3 $\pm$ 0.118*** (29.45)
Cortancyl	5	0.264 $\pm$ 0.036*** (68.79)	-
Promethazine	1	-	2.24 $\pm$ 0.050*** (31.29)

The values are expressed as mean  $\pm$  SEM, n = 5. The values in parentheses represent the percentages of inhibition. \*\*\* p < 0.001 compared to control animals. CF-AECal = dichloromethane fraction of *Cassia alata* aqueous extract.

### Subacute anti-inflammation studies

#### Cotton pellet-induced granuloma formation

As shown in Table 4, CF-AECal inhibited the granuloma formation, with a maximum reduction of wet weight (27.61%) and the dry weight (23.65%) of cotton observed at 100 mg/kg, compared to the control group.

**Table 4:** Effect of CF-AECal on cotton pellet- induced granuloma formation in rats

Treatments	Doses (mg/kg)	Wet weight of cotton (mg)	% INH	Dry weight of cotton (mg)	% INH
Control		221.45 $\pm$ 7.36		42.7 $\pm$ 1.90	
CF-AECal	50	192.6 $\pm$ 6.43 *	13.03	35.8 $\pm$ 1.31 **	16.15
CF-AECal	100	160.3 $\pm$ 8.24 ***	27.61	32.6 $\pm$ 1.2 ***	23.65
Diclofenal	5	162.5 $\pm$ 8.88 ***	26.62	29.6 $\pm$ 0.4 ***	30.68

The values are expressed as mean  $\pm$  SEM, (n = 5). \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 compared to control animals. CF-AECal = dichloromethan fraction of *Cassia alata* aqueous extract.

## Carrageenan induced inflammation in the air pouch

CF-AECal 50 mg/kg inhibits wet granulomas at 20.94 % and dry granulomas at 24.32 %. CF-AECal 100 mg/kg inhibits wet granulomas at 57.82 % and dry granulomas at 59.04 %. Pouch fluid volume and leukocytes infiltration are significantly reduced by CF-AECal in comparison to the control group ( $p < 0.001$ ) (Figure 2).

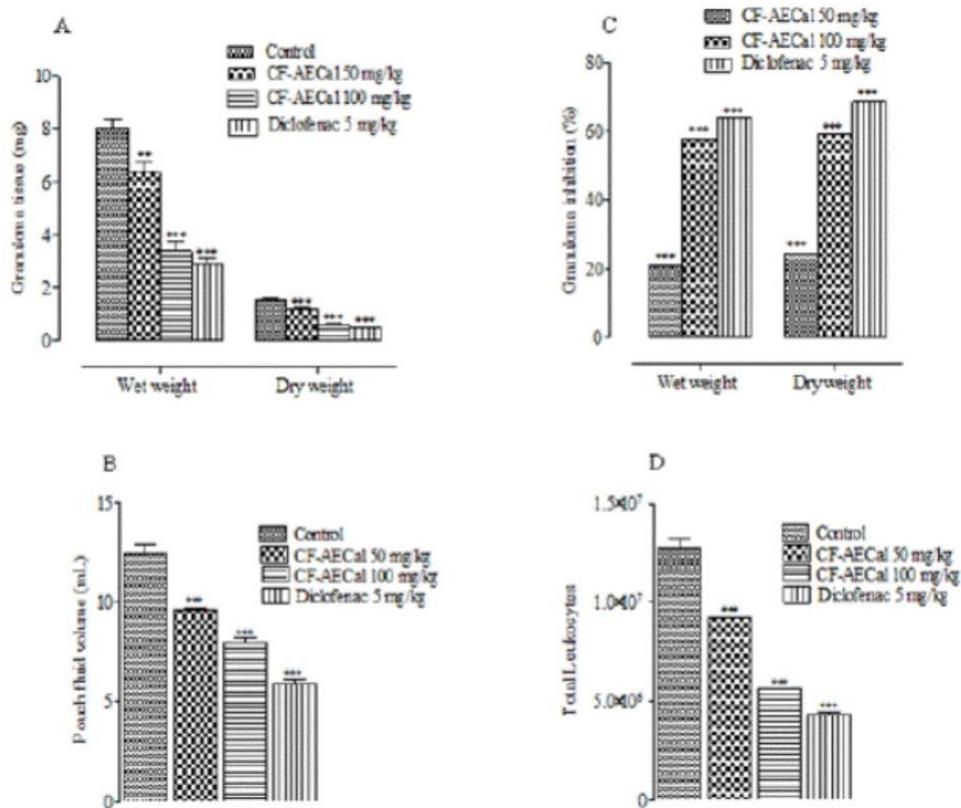


Figure 2: Effects of CF-AECal on capillary permeability and leukocytes recruitment of air pouch inflammation. A: effect of CF-AECal on the wet and dry weight of granuloma tissues; B: effect of CF-AECal on pouch fluid volume; C: inhibitory effect of CF-AECal on pouch induced granuloma tissue; D: total number of leukocytes in the pouch fluid. The values are expressed as mean  $\pm$  SEM on average, ( $n = 5$ ) \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control animals.

Carrageenan-induced granuloma was responsible for an increase of white blood cells count. The treatment of rats with CF-AECal (50 or 100 mg/kg) significantly decreased the number of lymphocytes and monocytes compared to negative control (Table 5).

**Table 5:** Effect of CF-AECal on various haematological parameters in air pouch inflammation

Haematological parameters	Treatments and doses (mg/kg)				
	Neutral control	Negative Control	CF-AECal (50 mg/kg)	CF-AECal (100 mg/kg)	Diclofenal (5 mg/kg)
WBC x 10 <sup>3</sup> / $\mu$ L	10.13 $\pm$ 2.71 ###	22.80 $\pm$ 2.1 ***	19.5 $\pm$ 2.40 #	18.93 $\pm$ 4.06 #	14.03 $\pm$ 0.66 ##
Lymphocyt x 10 <sup>3</sup> / $\mu$ L	6.52 $\pm$ 1.71 ##	13.57 $\pm$ 2.24 **	11.73 $\pm$ 1.44 #	10.27 $\pm$ 2.71 #	10.89 $\pm$ 0.50 #
Granulocyt x 10 <sup>3</sup> / $\mu$ L	1.39 $\pm$ 0.47 ###	4.25 $\pm$ 0.29 ***	4.00 $\pm$ 0.65 **	4.97 $\pm$ 0.76 *** #	0.95 $\pm$ 0.07 ###
Monocyt x 10 <sup>3</sup> / $\mu$ L	2.23 $\pm$ 0.67 ###	4.99 $\pm$ 0.34 ***	3.78 $\pm$ 0.31 ##	3.67 $\pm$ 0.71 ##	2.20 $\pm$ 0.12 ###
RBC x 10 <sup>6</sup> / $\mu$ L	6.66 $\pm$ 0.01 ###	5.04 $\pm$ 0.26 ***	5.16 $\pm$ 0.07 ***	5.36 $\pm$ 0.02 ***	5.63 $\pm$ 0.38 *

The values are expressed as mean  $\pm$  SEM, (n= 5) \*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001 compared to negative control animals. #p< 0.05, ##p< 0.01, ###p< 0.001 compared to neutral control animals. CF-AECal = dichloromethan fraction of *Cassia alata* aqueous extract.

## Discussion

Inflammation constitutes the response of body to injury and is characterized by a series of events that mainly occur in three distinct phases. The first phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space; the second phase involves the infiltration of leukocytes from the blood into the tissue and the third phase is characterized by granuloma formation and tissue repair (Badgular *et al.*, 2009). One of the causes of inflammatory reactions in our study is the tissue injury that induces synthesis of histamine, prostaglandins, leukotrienes (Ammon *et al.*, 1993), PAF (platelet activating factor), cytokines, NO (nitric oxide) and TNF  $\alpha$  (tumor necrosis factor) (Clarke *et al.*, 1996).

Carrageenan induced rat paw oedema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation (Morebise *et al.*, 2002). Development of edema in the paw of the rats is due to the release of histamine, serotonin and prostaglandin like substances (Umesh *et al.*, 2011). Our study show that CF-AECal inhibited the carrageenan induced rat paw oedema formation during both early and later phases. This result suggests that the inhibitory effect of the extract on oedema formation is probably due to the inhibition of the synthesis or release of the inflammatory mediators, especially histamine, serotonin and cyclooxygenase products. To ascertain the activity of the plant extract on inflammatory mediators, its effect was evaluated on dextran, histamine and serotonin induced paw oedema.

Dextran is a polysaccharide of high molecular weight that induces anaphylactic reaction, characterized by extravasation and oedema formation, as a consequence of liberation of histamine and serotonin from mast cells (Van Wauwe *et al.*, 1989; Prakash *et al.*, 2009).

Histamine, one of the important inflammatory mediators, is a potent vasodilator which increases vascular permeability (Linardi *et al.*, 2000; Cuman *et al.*, 2001). CF-AECal significantly suppressed dextran, histamine and serotonin-induced oedema. The results correlated with those observed during the first phase of carrageenan-induced paw oedema.

Subchronic and chronic models have been employed to assess the transudative and proliferative components of chronic inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granulomas whereas the dry weight correlates well with the amount of granulomatous tissue formed. The results show inhibitions of wet and dry granulomas by CF-AECal. The pouch fluid volume was also significantly reduced by CF-AECal. This reduction in transudate and granuloma formation may correlate with the ability of the plant extract to inhibit collagen and mucopolysaccharides synthesis and reduce the number of fibroblasts involved in the formation of granuloma tissue (Mallat *et al.*, 1995; Chandrasekaran *et al.*, 2013). The migration of leukocytes at the site of inflammation is an important parameter in the inflammatory response. The migration of leukocyte occurs as a result of different processes including adhesion and cell mobility (Meade *et al.*, 1986). CF-AECal inhibited leukocyte accumulation in inflammatory pouch fluid.

Anemia is commonly noted in patients with subchronic inflammation. The two most common explanations are gastrointestinal blood loss due to anti-inflammatory drugs and bone marrow changes in patients with inflammatory arthritis, which prevents the release of iron for incorporation into red blood cells (Mowat, 1971; Allar *et al.*, 1977). In our study, control rats showed a reduced RBC count. Animals treated with CF-AECal showed a significant recovery from the induced anemia. An indicator of infectious and inflammatory diseases, the WBC count increased in control rats (Maria *et al.*, 1983). The migration of leukocytes to the inflamed area was significantly suppressed by CF-AECal.

## Conclusions

The findings of the present study have demonstrated that CF-AECal has potent anti-inflammatory activity and justify its use in traditional medicine to treat inflammatory. The results also show evidence that the beneficial effects of this plant may be due to its free radical scavenging activities.

**Statement on conflict of interest:** We declare that this work does not present any conflict of interests.

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