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K . H . Janbaz
Bahauddin Zakariya University

U . Zia
Bahauddin Zakariya University

Hassan Mehmood
Aga Khan University, hassan.mehmood@aku.edu

S . Bashir
Aga Khan University

M . I . Choudhary
University of Karachi

See next page for additional authors

Authors
K . H . Janbaz, U . Zia, Hassan Mehmood, S . Bashir, M . I . Choudhary, and A . H . Gilani

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ANTI-INFLAMMATORY, FREE RADICAL SCAVENGING AND CALCIUM ANTAGONIST ACTIVITIES OF *CORCHORUS FASCICULARIS* LAM. CRUDE EXTRACT

K. H. Janbaz, U. Zia, M. H. Mehmood[**], S. Bashir[**], M. I. Choudhary[*] and A. H. Gilani[**]

Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[*]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan,
[**]Natural Product Research Unit, Department of Biological and Biomedical Sciences, Aga Khan University Medical College, Karachi-74800, Pakistan.
Corresponding author's email: anwar.gilani@aku.edu

ABSTRACT

The plants of genus *Corchorus* have a folkloric repute to possess anti-inflammatory activity. The crude ethanolic extract of *Corchorus fascicularis* Lam. (CFE) was screened for presence of anti-inflammatory, free radical scavenging and calcium channel blocking (CCB) activities to validate its vernacular use. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to assess free radical scavenging activity, anti-inflammatory potential was estimated through inhibitory effect on carrageenin-induced rat paw edema, whereas calcium channel blocking (CCB) effect was explored on isolated rabbit jejunum preparations. The CFE (50 and 100 mg/kg) administration resulted in a significant ($P < 0.05$) decrease in rat paw edema similar to aspirin, while a concentration-dependant (IC_{50}: 95.91 ± 3.07 µg/ml) inhibition of DPPH was achieved in a manner comparable to propylgallate (standard). The CCB-like effect was evidenced as CFE exhibited a concentration-dependant (0.01–1 mg/ml) non-specific relaxant effect in rabbit jejunum, which was supported further by a rightward shift of the Ca^{++} concentration-response curves, similar to verapamil. These results indicated presence of free radical scavenging, anti-inflammatory and CCB activities in *Corchorus fascicularis* and may be attributed to the possible presence of flavonoids and tannins among the plant constituents. The present study thus validates the folkloric use of *Corchorus fascicularis* in inflammatory diseases.

Keywords: *Corchorus fascicularis*, Anti-inflammatory, DPPH scavenging, Calcium antagonist.

INTRODUCTION

Corchorus fascicularis Lam. (Syn: *Corchorus brachycarpus* Guill.; Family: Tiliaceae), has common names of "Hirankhori" and "Lailaboti" and is a sub-erect annual shrub about 50-60 cm in height and wildly grown to dry and hot lands in Pakistan (Ghafoor, 1974). The grinding of plant parts produces mucilage of jelly like consistency, which is prescribed as general tonic by the native physicians due to its claimed cooling, laxative, astringent and restorative properties (Dymock *et al*., 1972). The aerial parts of *C. fascicularis* has also been recommended in native systems of medicine for the management of a number of ailments including pruritis, itching, inflammation, scabies, worm infestation, gonorrhea, syphilis, leprosy, edema, tumours, and sexual incompetence (Kiritikar and Basu, 1987; Khan *et al*., 1997).

The phytochemical studies revealed the presence of corchorin (glycoside), corchortin (phenolic) and fatty acids (i.e., oleic and linoleic acids) among the plant constituents (Anjaria *et al*., 2002), whereas antibacterial potential was explored through biological investigations (Godbole and Pendse, 1960). The present study on crude ethanolic extract of *Corchorus fascicularis* Lam. was undertaken in an attempt to explore its possible anti-inflammatory, antioxidant, and calcium channel blocking activities to validate its medicinal claims in native systems of medicine.

MATERIALS AND METHODS

Plant Material and Preparation of Crude Extract: The dried aerial parts of plant were procured from a local herbal store and were identified by Late Professor Mumtaz Hussain Bukhari (plant taxonomist), Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan and a voucher specimen was deposited in the herbarium (Cf-4). The adulterants were removed through manual picking and plant material was grinded into a coarse powder through an electrical grinding device. By following a previously described method (Gilani *et al.,* 1991), the powder was macerated at room temperature in 70% aqueous-ethanol in amber coloured container for 7 days with twice a day shaking. Screening through a muslin cloth was carried out to get rid of the vegetative debris and the emerging fluid was filtered through Whatman No. 1 filter paper. The filtrate was evaporated on a rotary evaporator (Model 9230; Buchi Labortechnik AG, Switzerland) under reduced

pressure at 35°C into a concentrated brownish paste (CFE) and obtained yield was 21% approximately. The CFE was found soluble in a 10% mixture of DMSO in normal saline.

Animals: The adult (♂ or ♀) Wistar rats (200-310 g) and local strain rabbits (1-1.2 kg) were maintained in plastic cages (47 × 34 × 18 cm^3) with sawdust lining (renewed on alternate days), kept at 23-25°C, 60 ± 4% humidity and 12/12 h light/dark cycles in the Animal House of Aga Khan University, Karachi. The animals were provided with standard diet and tap water *ad libitum*. The diet constituted of mixture of the following ingredients (g): flour (380), fibre (380), molasses (11.5), NaCl (5.8), vitamins mixture (2.5), potassium metabisulphate (1.2), vegetable oil (38), fish meal (170) and powdered milk (150). Experiments were performed in accordance with the rules formulated by Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and adopted by the Ethical Committee for Research on Animals of The Aga Khan University, Karachi.

Chemicals: All the chemicals, solvents, and reagents used in the experiments include: acetylcholine chloride (ACh), atropine sulphate, potassium chloride (KCl), verapamil hydrochloride, λ-carrageenin, aspirin, propylgallate (PG), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and calcium chloride ($CaCl_2$) were purchased from Sigma Chemicals Company, St. Louis, MO, USA.

Preliminary Phytochemical Analysis: Phytochemical screening test were performed on CFE for the detection of saponins, tannins, phenols, coumarins, alkaloids and anthraquinones as possible plant constituents (Tona *et al.*, 1998). The froth formation on vigorous shaking of the aqueous solution of extract was indication for saponin presence, while formation of blue green or dark green coloration on adding aqueous $FeCl_3$ to the extract revealed presence of phenols and tannins. The coumarins presence was detected by emergence of fluorescence on exposure to UV light out of NaOH treated of filter papers already impregnated with boiling vapors of aqueous plant extract. The presence of alkaloid was detected by appearance of yellowish brown coloration on mixing of Dragendorff's reagent to the HCl treated aqueous plant extract. The anthraquinones presence was detected on appearance of pink, violet or red coloration subsequent to exposure to NH_4OH of the mixture containing benzene and aqueous plant extract being acidified with 1% HCl.

Anti-inflammatory Activity: The test material was screened for possible anti-inflammatory activity through slight modification of the earlier reported method (Winter *et al.,* 1962). A total of 20 rats (200-310 g) of either sex were subjected to 12 h fasting prior to experiment and deprived of water during the experiments to achieve uniform hydration status in experimental animals to minimize variations in edema formation. The rats were divided into 4 groups each containing 5 animals. Group 1st animals were designated as vehicle control group and were treated by intra-peritoneal injection (5 ml/ kg) of the vehicle (10 % DMSO in normal saline) and followed after 30 min by edema induction through carrageenin (1%) injection (0.05 ml) in the sub-planter region of the right paw of each rat. Group 2nd animals were regarded as positive control and received treatment similar to group 1st animals except intra-peritoneal injection of vehicle was replaced by aspirin (10 mg/kg). The group 3rd and 4th animals were regarded as test groups and treated similar to the group 1st animals except the extract at respective doses of 50 and 100 mg/kg solublized in 10% DMSO in normal saline was substituted for the vehicle. The paw volume was measured by displacement technique through the Plathysmometer (Model 76014, Ugo, Japan) at 1, 2, 3, and 4 h after the carrageenin injection. The degree of edema inhibition in test animals groups versus control animals group was assessed through calculation of percentage inhibition. Percent inhibition: $(1 - V_T/ V_0) \times 100$; Where, V_0 = volume of paw of vehicle control group; V_T = Volume of paw for test sample (Suleyman *et al.,* 1991).

Free Radical Scavenging Activity: The free radical scavenging activities of the multiple concentrations of the test material and propylgallate (PG) (10 µl) were determined by method described by Lee *et al*. (1998). The 10 µl aliquots of test materials as well as PG (standard) were allowed to react with 190 µl aliquots of 0.1 mM methanolic solution of DPPH in triplicate, in a light protected 96-well micro-titer plate and shaken vigorously to achieve even mixing prior to incubation at 37ºC for 30 min. The decrease in absorbance at 515 nm was measured in Multiplate Elisa Reader (Spectra Max 340; Molecular Devices, CA, USA), taking PG as a positive control. The percent radical scavenging activities (PRSA) of test samples were calculated in comparison with a DMSO treated control group by using the following formula, i.e., PRSA = 100 – [(Optical density of test material/Optical density of control) x 100]. The IC_{50} value was taken as the concentration (µg/ml) of the crude plant extract capable to inhibit DPPH radical activity by 50%.

Calcium Channel Blocking Activity: The calcium channel blocking (CCB) activity of CFE was investigated on isolated rabbit jejunum preparations as described previously (Gilani *et al*., 2005; 2009). The jejunum was dissected out following incision to rabbit abdomen and placed in Tyrode's solution aerated well with carbogen (5% CO_2 + 95% O_2) to remove mesenteries. About 2 cm long segments were suspended in 10ml tissue baths filled with Tyrode's solution, maintained at 37°C and aerated with carbogen (5% CO_2 + 95% O_2). The contractile

intestinal responses were recorded isotonically by using Bioscience transducers coupled with Oscillograph. The tissues were allowed 30 min incubation for equilibration prior to addition of any test material and were subsequently stabilized through repeated exposure to sub-maximal concentration of acetylcholine (Ach; 0.3µM). The isolated rabbit jejunum preparations exhibited spontaneous rhythmic contractions on which relaxant activity of the test materials can be tested in the absence of any agonist (Gilani *et al*., 2005).The test drugs were added to tissue baths in a cumulative manner to quantify the relaxant effect. The relaxant effects on the part of test materials is measured as the % change in spontaneous contractions of isolated rabbit jejunum preparations as compared to the spontaneous contractions recorded just prior to the addition of test substances.

The CCB activity of the test material was determined by application to high K^+ (80 mM)-induced spastic contractions in isolated rabbit jejunum preparations as described by Farre *et al*. (1991). The concentration-dependent relaxant responses were achieved when test materials were added to the tissue baths in a cumulative fashion. The Ca^{++} antagonist activity of the test materials were confirmed further and the isolated rabbit jejunum preparations were allowed to be stabilized in normal Tyrode's solution, which was subsequently replaced by Ca^{++}-free Tyrode's solution containing EGTA (0.1 mM) for 30 min to remove Ca^{++} from the tissues. This tissue bath solution was replaced further by K^+-rich and Ca^{++}-free Tyrode's solution of following composition (mM): KCl (50), NaCl (91.04), $MgCl_2$ (1.05), $NaHCO_3$ (11.90), NaH_2PO_4 (0.42), glucose (5.55) and EGTA (0.1). The control concentration response curves (CRCs) for Ca^{++} were recorded after an incubation period of 30 min and process was repeated till super-imposable CRCs for Ca^{++} were recorded. The isolated rabbit jejunum preparations were exposed to the particular tissue bath concentrations of the test materials and the CRCs for Ca^{++} were recorded after an incubation period of 60 min. The CRCs for Ca^{++} were recorded in the presence of different tissue bath concentrations of the test materials by repeating experimental procedure.

Statistical Analysis: The *in vivo* anti-inflammatory results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance of the differences between experimental groups was estimated using One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test of all groups versus their respective control group and *P < 0.05 was considered statistically significant. The free radical scavenging activity was reported as mean ± S.E.M. of five parallel measurements in triplicates and the respective IC_{50} values were calculated by the EasyFit software. The logarithmic CRCs of different treatments were plotted for calcium channel blocking activity by using "Graphpad Prism" (computer software).

RESULTS

Preliminary Phytochemical Analysis: The preliminary phytochemical analysis on CFE revealed the presence of flavonoids, tannins, and saponins among the plant constituents.

Anti-inflammatory Activity: The anti-inflammatory activity of CFE on carrageenin-induced rat paw edema is shown in Fig. 1. The sub-plantar injection of carrageenin induced paw edema in group 1st rats (control) which was measured in terms of volume (ml) as 0.26 ± 0.01, 0.84 ± 0.09, 0.94 ± 0.06 and 0.79 ± 0.11 at 1, 2, 3 and 4 h respectively. The aspirin treatment to the group 2nd animals (positive control) caused significant ($P < 0.05$) decrease in the paw edema at 1, 2 and 3 h and the respective values (ml) were estimated to be 0.11 ± 0.07, 0.35 ± 0.04 and 0.47 ± 0.06, whereas an insignificant ($P > 0.05$) decrease was recorded at 4 h (0.46 ± 0.07 ml). The group 3rd and 4th animals were treated by CFE and significant ($P < 0.05$) dose-dependent decrease in carrageenin-induced rat paw edema (ml) was noted in comparison with the group 1st animals and estimated values at 1, 2, 3 and 4 h were 0.12 ± 0.03, 0.55 ± 0.05, 0.67 ± 0.07, 0.59 ± 0.6 respectively with 50 mg/kg and 0.17 ± 0.04, 0.22 ± 0.07, 0.47 ± 0.09 and 0.41 ± 0.04 respectively with 100 mg/kg. The maximal (64-73%) inhibitory response was noted at 1-2 h following carrageenin injection in group 2nd, 3rd and 4th animals.

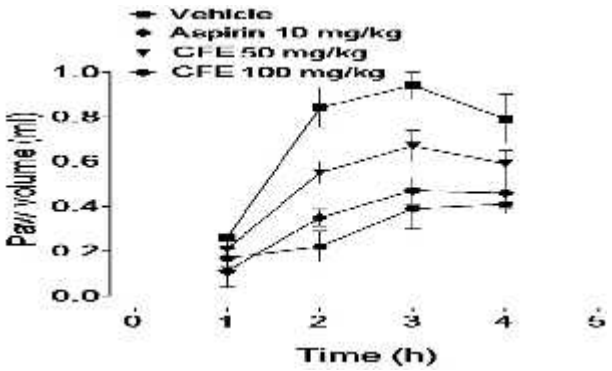

Figure 1. Effect of *Corchorus fascicularis* extract (CFE) and aspirin on the paw volume in carrageenin treated rats at different time intervals. Values shown are mean ± S.E.M. of 5 observations.

Free Radical Scavenging Activity*:* The CFE exerted a significant ($P < 0.001$) concentration-dependent inhibitory influence on DPPH radical with IC_{50} value: 95.91 ± 3.07 µg/ml (Fig. 2). The CFE was found to be less potent than PG, which caused inhibition of the DPPH radicals with IC_{50} value: 50.38 ± 5.24 µg/ml.

Calcium Channel Blocking Activity: The CFE caused relaxation of both spontaneous and high K^+(80 mM)-

induced contractions in isolated rabbit jejunum preparations in a concentration-dependent (0.01-3.0 mg/ml) manner with respective IC_{50} values of 0.32 ± 0.05 and 0.35 ± 0.07 mg/ml (Fig. 3A), in a manner similar to verapamil (Fig. 3B). Similar to verapamil (0.1-1 µM), the addition of CFE to the isolated tissue baths (0.1-1 mg/ml) shifted the CRCs for Ca^{++} towards right, (Fig.4A & 4B).

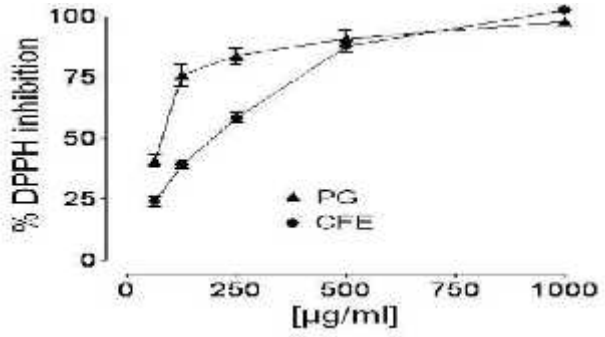

Figure 2. Free radical scavenging activity of crude extract of *Corchorus fascicularis* (CFE) and Propyl gallate (PG) measured as % inhibition of 1,1-diphenyl-2-picryl- hydrazyl (DPPH) radical. The values shown are mean ± S.E.M. of 3 determinations.

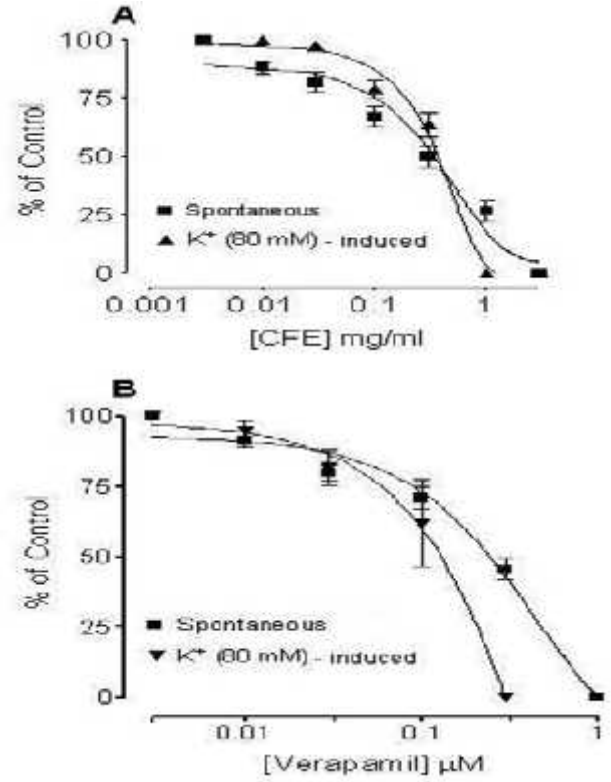

Figure 3. Tracings showing the inhibitory effect of (A) the crude extract of *Corchorus fascicularis* (CFE) and (B) verapamil on spontaneous and K^+ (80 mM) - induced contractions in isolated rabbit jejunum preparations. Values shown are mean±S.E.M. of 5-6 determinations.

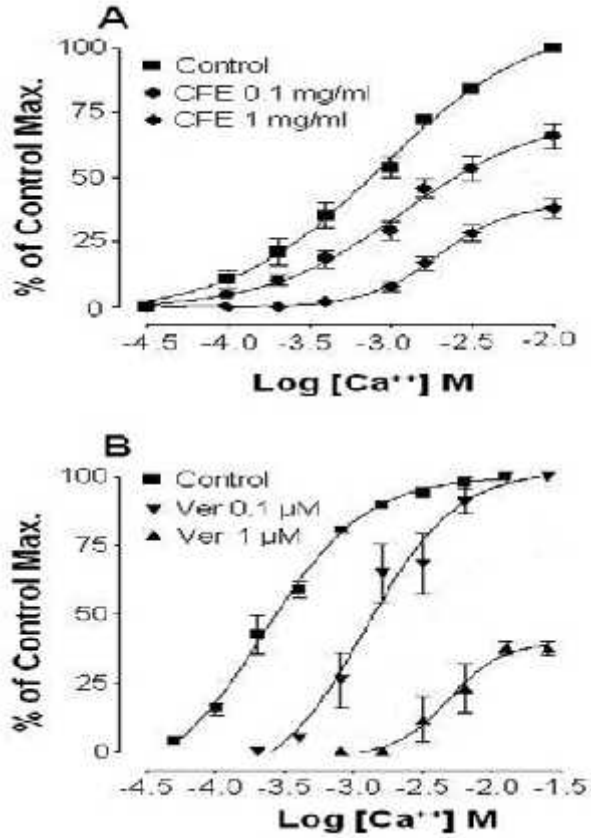

Figure 4. The concentration-response curves of Ca^{++} in the absence (control) and presence of different concentrations of (A) crude extract of *C. fascicularis* (CFE) and (B) verapamil (Ver) in isolated rabbit jejunum preparations. Values shown are mean ± S.E.M. of 5-6 determinations.

DISCUSSION

The animal treatment with CFE resulted in inhibition of carrageenin-induced inflammatory response similar to aspirin. The anti-inflammatory activity of test materials has been screened on carrageenin-induced rat paw edema, which is one of the manifestations of prostaglandins involvement in the inflammation process (Mujumdar and Misar, 2004). The rat paw edema formation is reported to be a reflection of synergistic interaction among the multiple inflammatory mediators resulting in increased vascular permeability and blood flow (Ialenti *et al.*, 1995). The biphasic sequence of events has been reported to be observed subsequent to carrageenin injection: 1st phase (1-2 h), consisting of synthesis, release and activity of serotonin, histamine, bradykinin and prostaglandins whereas 2nd phase (3-4 h) comprised of neutrophils accumulation and persistent prostaglandin synthesis (Salvemini *et al.,* 1996). The CFE-induced anti-inflammatory activity was observed to be maximal after 2 h of carrageenin injection, indicating

that anti-inflammatory activity is likely to be mediated through inhibition of either synthesis or release of some mediators of inflammation, i.e. serotonin, bradykinin, histamine or prostaglandins.

Moreover, the inflammatory process is reported to be boosted up by the reactive free radical species (Halliwell *et al.,* 1988) and impairment in body antioxidant capabilities caused aggravation of inflammatory diseases (Chapple *et al.,* 2002; Cimen *et al*., 2003), hence, the observed free radical scavenging activity of CFE may also have contributed toward the observed anti-inflammatory effects.

Noxious stimuli-induced inflammation is reported to be mediated through raised intracellular Ca^{++} concentration (Sanchez *et al.,* 1988) and toxicity to chemicals and metals is presumed to be an outcome of disturbed intra-cellular Ca^{++} homeostasis (Nicotera *et al.,* 1992). The presence of inflammatory mediators is essential for the demonstration of inflammatory response (Murray *et al.,* 1988), whereas Ca^{++} influx play pivotal role for their release during inflammation (Sanchez *et al.,* 1988). Verapamil and other known calcium channel blockers are reported to inhibit carrageenin-induced edema (Sanchez *et al*., 1988; Al-Tuwaijri and Mustafa, 1992).

The crude ethanolic extract of *Chorocus fascicularis* was investigated for possible Ca^{++} channel blocking activity on isolated rabbit jejunum preparations. The activation of contractile element in smooth muscle preparations is known to be dependent upon the increased free cytoplasmic Ca^{++} concentration (Karaki *et al.,* 1997). The increase in free cytoplasmic Ca^{++} concentration can either be achieved via influx through the voltage-dependant Ca^{++} channels (VDCs) or alternatively released from Ca^{++} stores located in smooth sarcoplasmic reticulum (Godfraind *et al.,* 1986). The appearance of spontaneous movements in intestine is based on periodic depolarization and re-polarization and Ca^{++} is rapidly influxes through the VDCs to generate action potential in maximally depolarized condition (Brading, 1981). The observed relaxant effect of CFE on the isolated rabbit jejunum preparations is likely to be mediated either through decreased Ca^{++} influx through VDCs or through decrease in release of Ca^{++} from sarcoplasmic stores.

The exposure to high K^+ (80 mM) causes opening of the VDCs and rapid influx of extra-cellular Ca^{++} results in contraction of isolated smooth muscle preparations (Bolton, 1979). The CFE exerted a relaxant effect on high K^+- induced contraction in isolated rabbit jejunum preparations and also displaced the CRCs for Ca^{++} towards right in a manner similar to a standard calcium antagonist (verapamil) (Fleckenstein, 1977), hence, strongly suggested the presence of calcium antagonist among the plant constituents.

The CCBs have been reported to exhibit free radical scavenging activity (Rangan and Bulkley, 1993; Rojstaczer and Triggle, 1994; Mak *et al.,* 1995), hence free radical scavenging activity on the part of CFE may be attributed to the possible presence of CCB constituents, although possibility of other mechanisms cannot be ignored.

The anti-inflammatory (Read, 1995), spasmolytic (Di-Carlo *et al*., 1993) and calcium channel blocking (Revuelta *et al*., 1997) activities of flavonoids has already been reported and flavonoids were detected to be present in CFE . The tannins are known to exhibit high DPPH radical scavenging activity (Amarowicz *et al*., 2000) and the observed presence of tannins among the plant constituents might be a plausible explanation for the observed antioxidant effect.

Conclusion: Results of this study clearly show that *C. fascicularis* crude extract possesses anti-inflammatory, free radical scavenging, and calcium channel blocking activities. These findings provide the scientific basis for the medicinal use of *C. fascicularis* in inflammatory disorders in folk systems of medicines.

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