Comparative anti-inflammatory properties of Capsaicin and ethyl-aAcetate extract of *Capsicum frutescens* linn [Solanaceae] in rats

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Abstract

Background: The analgesic effect of capsaicin (the active ingredient in *Capsicum frutescens* Linn. *[*Solanaceae*]*) had been reported in several studies. Current research is being directed at producing analgesics, anti-inflammatory agents with better side effect profile.

Objectives: To investigate if either the ethyl acetate extract of *Capsicum frutescens* Linn. *[*Solanaceae*]* (CFE) or capsaicin (Fluka Biotechnika-CPF) (in addition to the known analgesic properties) has any anti-inflammatory effect comparable to non-steroidal anti-inflammatory analgesics (NSAIDS).

Methods: The effects of ethyl acetate extract of *Capsicum frutescens* Linn. *[*Solanaceae*]* (CFE) and capsaicin (Fluka Biotechnika-CPF) was examined on rat hind paw. Inflammation was induced in the rat's hind paw by subplantar injections of fresh egg albumin (0.5 ml/kg). Diclofenac (100 mg/kg) was used as the reference anti-inflammatory agent for comparison, while distilled water was used as the placebo. The leucocytes count, corticosterone and C - reactive protein (CRP) levels were measured as biomarkers of inflammation. Data obtained were pooled and analysed using repeated ANOVA, in a general linear model with the CPSS software.

Results: Sub-plantar injections of fresh egg albumin (0.5 ml/kg) produced profound and time-related oedema in the rat hind paw of the 'control' rats. Diclofenac (DIC, 100 mg/kg, i.p.) and reference capsaicin (CPF, 2.5 mg/kg, i.p.) significantly inhibited paw swelling at ($p<0.05$–0.001) (CI 95%) compared to distilled water-treated 'controls'.

While the corticosterone levels were all very low in 7 rats treated with capsaicin, the leucocytes count was within normal range in 9 rats. However, in 16 specimens randomly assigned for CRP levels, there were very high CRP readings, up to a magnitude of 10 times the normal range.

Conclusion: Capsaicin in both forms (CFE and CPF) produced anti-inflammatory effects that were comparable to diclofenac in the experimental rat model at $p<0.05$. It may be concluded that capsaicin has both analgesic and anti-inflammatory properties.

Keywords: *Capsicum frutescens, 'chili',* ethylacetate extract, capsaicin, anti-inflammation.

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Introduction

Capsicum has been known since the beginning of civilization and has been a part of the human diet since about 7500 BC (MacNeish 1964). The genus Capsicum is a member of the Solanaceae family that includes tomato, potato, tobacco, and petunia. The genus Capsicum consists of approximately 22 wild species (*C. annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens*) and five domesticated species[1].

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As a medicinal plant, Capsicum species has been used as a carminative, digestive irritant, stomachic (beneficial to gastric digestion), stimulant, rubefacient, and tonic. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache. *Capsicum frutescens L.* has been reported to have hypoglycaemic properties[1]. Prolonged contact with the skin may cause dermatitis and blisters, while excessive consumption can cause gastroenteritis and kidney damage. Paprika and cayenne pepper may be cytotoxic to mammalian cells in *vitro*. Consumption of red pepper may aggravate symptoms of duodenal ulcers. High levels of ground hot pepper have induced stomach ulcers and cirrhosis of the liver in laboratory animals. Capsicum peppers may stimulate body temperature, flow of saliva, and gastric juices[1].

This study was undertaken to investigate the anti-inflammatory property of *Capsicum frutescens* ethyl acetate extract (CFE) and capsaicin (CPF) in rat model to providing a pharmacological rationale for the folkloric use to treat arthritis, muscle sprain and other inflammatory conditions in some communities.

Methods

Plant material

Capsicum frutescens Linn. (family:Solanaceae) fruits were purchased from Warwick market in Durban, South Africa. The fruits were identified and authenticated by a botanist, Professor Himansu Baijnath, the Chief Taxonomist/Curator of the then University of Durban-Westville's Department of Botany, as those of *Capsicum frutescens***.** A voucher specimen of the plant (with its ripe fruits) has been deposited in the University's Herbarium (JAT/01). The total weight of the dried red chilli pepper fruits purchased from the local market was 3 kg.

Preparation of extracts

Dry ripe fruits of *Capsicum frutescens* were separated from the stalk, cleaned and pulverized, using a mechanical grinder. Two-and-a half- kilogram of the powder was put in a big conical flask and exhaustively extracted sequentially in hexane, dichloromethane and ethyl acetate. Preliminary pilot experiments showed that the ethyl acetate extract contains active compounds (capsaicinoids). The ethyl acetate extract was concentrated in a rotary evaporator under reduced pressure to yield 298 gm (10% yields) of the crude extract. The ethyl acetate extract was further purified. NMR analysis of the yield showed 98% capsaicin.

Study animals

Adult rats of both sexes weighing 250-300 g were randomly selected into 5 groups (A-E) of 10 rats per group; distilled water-treated (A), diclofenac-treated (B), CFE- treated (C), CPF-treated (D), and non-treated (E) groups respectively. The animals were kept and maintained under standard laboratory temperature, humidity and light conditions. They were allowed free access to food (standard pellet diet) and tap water ad libitum. Baseline measurements of the circumferences of both the right and left hind limbs were obtained. Each rat in the control Group A received 2 ml/kg intraperitoneal, (i.p.) of distilled water, while each rat in Group B received 100 mg/kg diclofenac (i.p.), respectively. Groups C and D rats received 2.5 mg/kg CFE and CPF, respectively. Each of the 50 rats was then subjected to inflammation by injecting fresh egg albumin (0.5 ml/kg) into the sub-plantar of the right paw according to standard protocol. Thereafter, the circumferences of the paws were repeatedly measured at time intervals of 30 min, 1, 2, 4, 8, 16, and 32 hours, respectively. Leucocyte count and blood levels of C-reactive protein (CRP) and cortisol were measured (and analysed by the only private, University-approved laboratory for animal studies), following prolonged exposure to both forms of capsaicin and the phlogistic agent by intracardiac puncture under deep halothane anaesthesia.

Evaluation of anti-inflammatory effects

Increases in the linear-circumference of the rat hind paw induced by subplantar injection of fresh egg albumin (a phlogistic agent), have been used by several researchers (Ekpendu, et. al., 1994; Moko, et. al., 2000; and Hess, et. al., 1972). In this study, acute inflammation was induced in each of the test rats by injecting 0.5 ml/kg of fresh egg albumin on the subplantar surface of the right hind paw. Linear paw oedema was assessed for 32 hours at 30 min, 1, 2, 4, 8, 16, and 32 hours respectively. The measurements (in centimetres) for the right hind paw oedema were compared to those of the contralateral, non-injected left hind paw circumference[5, 8, 9]. The percent inflammation (oedema) was calculated by using C_0/C_T x 100

While percent inhibition of oedema was calculated from the formula: C_{0-} C_0/C_T x 100

(Where C_0 is the initial paw circumference; and C_T is the average inflammation of the extract- or diclofenac-treated rats at specific time; t)

Statistical analysis

Results are expresses as means (±SEM). In each case, repeated ANOVA was used to determine the level of significance of the difference between the test and the control group data. Values of p d" 0.05 were taken to imply statistical significance.

Results

Sub-plantar injections of fresh egg albumin (0.5 ml/kg) produced profound and time-related oedema in the rat hind paw of the 'control' rats. Plantar swelling and/or oedema (which became evident approximately 20-30 minutes following fresh egg albumin administration) reached its peak approximately 90 minutes after the sub-plantar injection of fresh egg albumin. Diclofenac (DIC,

100 mg/kg, i.p.) and reference capsaicin (CPF, 2.5 mg/kg, i.p.) significantly inhibited paw swelling at ($p < 0.05–0.001$) (CI 95%) compared to distilled water-treated 'controls'.

In comparing the inhibitory effects of CFE, CPF, and DIC; repeated ANOVA between groups showed a statistically significant difference $p < 0.05$, between CPF, DIC groups and non-treated and control groups. Inhibition of inflammation spiked earliest in the CFE group. However, percent reduction of oedema lasted longer in both CPF and diclofenac groups over 2-8 hours, while CPF group lasted for a further 8 hours compared to other groups as shown in table 1.

In the current study, diclofenac, CPF and CFE prevented inflammation induced by egg albumin on the hind paw of the rats (table 1). However, there are notable differences between the degree of anti-inflammation and prevention of paw edema by these agents (tables 1-3).

Table 1: Comparative anti-inflammatory effects of capsaicin in rats treated with Phlogistic agent compared with parallel groups treated with Diclofenac and saline at the same successive time intervals

GROUPS	Distilled Water	Albumin *	Alb. + CFE**	Alb.+DIC***	Alb. + CPF***	Key
%C0/Ct1	94 ± 1	96 ± 1	88 ± 1	86 ± 1	93 ± 1	Ct1=paw circumference at 30 min
%C0/Ct2	97 ± 1	96 ± 1	85 ± 1	83 ± 1	85 ± 1	Ct2=paw circumference at 1 hour
%C0/Ct3	100 ± 1	103 ± 1	83 ± 1	80 ± 1	81 ± 1	Ct3= paw circumference at 2 hours
%C0/Ct4	100 ± 2	103 ± 1	97 ± 2	95 ± 1	85 ± 1	Ct4= paw circumference at 4 hours
%C0/Ct5	102 ± 2	103 ± 2	98 ± 2	96 ± 1	95 ± 1	Ct5= paw circumference at 8 hours
p-value (between control & group) Using repeated					***	C0= initial paw circumference (cm)
ANOVA		*P<0.02	** P<0.001	*** P<0.0001	P<0.0001	

Table 2: Comparative anti-inflammatory effects of capsaicin in rats treated with Diclofenac and saline

Groups	Distilled Water	Albumin*	Alb. + CFE**	Alb.+DIC***	Alb. + CPF***	Key
%CO-Ct/C01	-4.1	-0.86	-12.43	-11	-16	C0-Ct/C01= percentage Inhibition of oedema at 30 min
%CO-Ct/C02	-1.98	-0.04	-0.97	-0.6	-21	C0-Ct/C02= percentage Inhibition of oedema at 1 hour
%CO-Ct/C03	2.8	0	0.42	-24	-28	C0-Ct/C03= percentage Inhibition of oedema at 2 hours
%CO-Ct/C04	3.6	1.43	0.23	-11	-8	C0Ct/C04= percentage Inhibition of oedema at 4 hours
%CO-Ct/C05	3.7	4.31	2.5	-5	-4	C0-Ct/C05= percentage Inhibition of oedema at 8 hours
p-value (between control & group). Using repeated			**		***	C0= initial paw circumference (cm)
ANOVA		*P<0.02	P<0.001	*** P<0.0001	P<0.0001.	

Table 3: Inhibitory effects (in percentage) of DICLOFENAC (DIC), ethyl acetate extract of *Capsicum frutescens* (CFE) and capsaicin (CPF) on fresh egg albumin-induced rat paw inflammation (Oedema) Measurement of circumference was in cm ± SEM

Treatment	Dose (mg/kg)	0.5 Hr.	1.0 Hr.	2 Hr.	4 Hr.	8 Hr.	16 Hr.	32 Hr.	P-value between (control & group).
Control (Distilled H_20)	(2ml/kg)	7.12 ± 0.14	7.7 ± 0.22	8.4 ± 1.48	9.1 ± 1.3	9 ± 1.5	8.2 ± 1.5	7.3 ± 1.3	Using repeated ANOVA.
CFE **	2.5	7.9 ± 0.28 (11%)	7.2 ± 0.27 (64 %)	8.1 ± 0.31 (3.5%)	8.2 ± 0.31 (10%)	7.9 ± 0.31 (12%)	7.2 ± 0.31 (12%)	7.2 ± 0.31 (14%)	**P<0.001
CPF ***	2.5	6.2 ± 0.13 (13%)	7.4 ± 0.15 (3.8%)	5.0 ± 0.16 (40%)	6.5 ± 0.17 (29%)	6.3 ± 0.38 (30%)	6.2 ± 0.42 (24%)	6.1 ± 0.35 (16%)	*** P<0.0001.
Diclofenac ***	100	6.5 ± 0.21 (8.7%)	7.8 ± 0.25 (1.2%)	4.9 ± 0.22 (42%)	5.7 ± 0.21 (37%)	6.5 ± 0.21 (28%)	7.4 ± 0.19 (10%)	7.2 ± 0.19 (1.3%)	*** P<0.0001.
Non-treated *	-	10 ± 0.24	6.5 ± 0.23	11 ± 0.22	6.5 ± 0.17	5.5 ± 0.27	11 ± 0.28	0.3 ± 0.01	*P<0.02.

Capsaicin and Chemical markers of inflammation

Corticosterone levels were all very low (28-31 μmol/L) in 7 rats treated with capsaicin. Leucocytes count was within normal range (2.8-11.8 x 10^6/L) in 9 rats. In 16 specimens randomly assigned for CRP levels, there were very high CRP readings, up to a magnitude of 10 times the normal (3-33 μmol/L).

Discussion

In the present study, the effects of capsaicin on inflammation were compared to placebo (with distilled water), and positive controls (with diclofenac) respectively. The association of vanilloid receptors with inflammation and pain has been reviewed[4].

With increase in the levels of Substance P in inflammatory and neurogenic joint diseases (arthritis), topical or intra-articular injections of capsaicin have shown a significant improvement, as well as reduction in the level of inflammatory mediators[4, 5,6] .

Furthermore, post-operative use of capsaicin to prevent upper airway obstruction (often caused by airway swelling) following Ear, Nose and Throat surgery was also reviewed[2,3]. Although these studies have alluded to the usefulness of capsaicin in the above clinical scenarios, these studies were mainly observational, with no attention to statistical significance resulting from the studies.

Firstly the doses that were used for the experimental drugs, DIC, CPF and CFE, respectively were not equipotent. The doses of 100-mg/kg diclofenac, 2.5 mg/kg of CPF and CFE were based on previous studies showing therapeutic doses of capsaicin for analgesic experiments to be 2.5 to 10 mg/kg[4].

The results should be interpreted in the light of the use of non-equipotent doses of CPF and CFE (2.5 mg/kg) to a 100 mg/kg of diclofenac. Overall, there was a comparable degree of anti-inflammation or inhibition of paw oedema at a dose ratio of 1:40 between CPF and CFE groups and results from diclofenac group (table 2).

Besides, CFE showed a more pronounced percentage inhibition in the first hour compared to diclofenac and CPE. This might be related to fresher preparations compared to diclofenac and CPF groups, both of which have been synthesized and packaged by different suppliers. This could also be a reason for the apparent delayed activity and peaking of CPF and diclofenac in the second hour before the decline in their anti-inflammatory activity (table 1 and 2).

Statistical significance was found in animals that received diclofenac, CPF and CFE, compared to animals in albumin alone and distilled water treated groups, respectively. Diclofenac, CFE and synthetic capsaicin (CPF) groups showed significant inhibition of inflammation (tables 1-3)

Induced inflammation in the treated animals was associated with a corresponding rise in the CRP levels alluded to the inflammation producing properties of egg albumin. It was the objective of the study to determine if a pre-treatment with CPF and CFE could produce a comparable reduction of inflammation (and inhibition of expected oedema induced by egg albumin) to anti-inflammation produced by a standard, non-steroidal anti-inflammatory agent; which was, diclofenac in this study.

The only plausible explanation for the low cortisol levels in all the randomly-sampled animals is that CFE and CPF inhibited the unnecessary stress responses, while not inhibiting the beneficial pro-inflammatory activities to defend the body from infection. This hypothesis tends to suggest that capsaicin could play a useful role in stress reduction and inflammation. However, further work is needed to prove this.

The experimental evidence obtained in this laboratory animal study indicates that *Capsicum frutescens* ethyl acetate extract (CFE) and capsaicin (CPF) have comparable anti-inflammatory effects to diclofenac. This lends credence to the use of *Capsicum spp* in folklore medicine in fevers and other inflammatory conditions. However, the mechanism of its action, in inflammatory conditions needs further investigations.

Conclusion

This study shows that capsaicin possesses a dose-dependent anti-inflammatory effect, which is comparable to inhibition of inflammation produced by diclofenac. More work has to be done to elucidate the delay in onset of anti-inflammatory activity of CPF and early onset of action of CFE in the groups of rats used. Further studies are needed to isolate other possible mediators that capsaicin could inhibit in the inflammatory pathway to justify a more specific role. Capsaicin may present an alternative analgesic, anti-inflammatory agent when current agents are contra-indicated or not suitable.

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