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*Full Length Research Paper*

# Analgesic effects of *Capsicum frutescens Linn. (Solanaceae)* fruit aqueous extract in mice

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**The analgesic property of Capsicum frutescens LINN. (SOLANACEAE) extract-CFE (98% Capsaicin by NMR) of South African origin in mice (20-30 g), using 'hot plate' and 'acetic acid' methods was investigated. In the hot plate test, twelve mice in each of 5 groups were administered intraperitoneal (i.p.) CFE or synthetic capsaicin (Fluka Biotechnika-CF) at 0.5-8 mg/kg and at hourly intervals; at 40 C, the mean reaction time (MRT) was estimated. In the 'acetic acid' test method, CFE or CF was administered i.p. at various doses (0.5- 8 mg/Kg) at hourly intervals following single treatment using 0.2 mls of 3 % v/v acetic acid-induced writhing responses, or non treated (n=12). Separate groups of rats (n=12) were pre-treated with 100-mg/kg diclofenac (DIC) or morphine (MPN) 10 mg/kg i.p. to evaluate the peripheral analgesic effects and central analgesia, respectively. Data obtained were pooled and analysed by the student's T-test. The 'hot plate' and 'acetic acid' test methods showed significant ($p<0.0001$) prolongation of the MRT and inhibition of writhing responses, respectively, compared to the control. Similar results with a Pearson correlation of 0.999 were obtained in the CF subgroups. This study shows that capsaicin has superior central analgesia compared to equipotency doses of MPN or DIC and comparable peripheral analgesia to either MPN or DIC.**

**Keywords:** *Capsicum frutescens, 'chili'*, ethylacetate extract, capsaicin, analgesia, 'hot plate' test, 'acetic acid' test, analgesiometer, writhings.

## INTRODUCTION

Chronic pain is a major health hazard resulting in debilitation, sickness role, and adverse drug reactions, including death (Jolayemi, 2002). Pain management is multidisciplinary, with current trend emphasising complimentary health methods (Wallace, 1997). This multi-specialty approach is unaffordable for patients in developing countries (due to poor socio-economic conditions), resulting in poor compliance, self-medication and high complication rate, or death. A review of the evolution of analgesics shows that they are derived primarily from basic foods, natruceaticals, and other edible or chewable substances, and later from conventional medications. For example, eating poppy leaf and cannabinoid tea, or chewing of opioids or addictive substances, have been known to certain racial groups for centuries. It is, therefore, important to use these natural interventions in a more scientific and beneficial way to improve compliance and health; and reduce the unwanted side-effects of conventional analgesics. The development of inhibitors of Substance P of plant origin (reviewed by Bevan *et al*., 1995) provides an opportunity in the treatment of intractable pain.

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*Capsicum species* occur worldwide, and has been used for more than 9000 years by the Chinese, Indians, and Africans for medicinal and non-medicinal purposes (Watt, 1962). One of the main objectives of this study was to determine if *Capsicum frutescens* fruit extract of South African origin, has similar efficacy on peripheral and central components of pain as described for *Capsicum spp* (Linn) (Solanaceae) in other parts of the world, such as India, Mexico, Thailand and South America (Jaiarj *et al*., 1998).

Studies described in the literature suggest that capsaicin could modulate analgesia (Bevan, 1990; Winter, 1997). Capsaicin extracted from *Capsicum spp* act at the vanilloid receptors to inhibit Substance P, and has proved very useful in intractable pain of diabetic neuropathy, as well as herpetic and trigeminal neuralgia (Davis, 2000). Most of these studies have shown the analgesic effect of capsaicin in spinal mechanism. The questions that remain unanswered include elucidation of central and peripheral analgesic mechanisms of capsaicin. The protection of pre-treated rats from thermal pain in the 'hot plate' test method as described by (Ojewole, 2002) and the percentage inhibition of writhings in the pre-treated rats using the 'acetic acid' test method were used in this study.

## Animals

Experiments were performed on mice (20-30 g body weight) of both sexes. The mice were maintained under standard laboratory conditions of light, temperature and relative humidity. The animals were fed with standard diet pellet (Epol-diet 4700, Epol, South Africa) and drinking tap water *ad libitum*. They were kept and maintained in Biomedical Resource Unit, University of KwaZulu-Natal, Durban 4000, South Africa.

## Drugs

Investigative drugs included capsaicin (Fluka-Biotechnika), *Capsicum frutescens*-derived extract, and positive controls (morphine) and diclofenac.

## Assessment of analgesic effects of capsaicin using the 'hot plate' test method

The 'hot-plate' (thermal) test method employed in this study was modified from those described in detail earlier by Eddy and Leimback, (1953) cited by Ojewole, (2006); Lanhers *et al.,* (1992); and Williamson *et al*., (1996) cited by Ojewole, (2006). Mice weighing 20–30 g were divided into two groups (A and B) of control and treated animals. Group A had three subgroups of 12 mice each. The first subgroup consisted of untreated mice, the mice in second and third subgroups were treated with morphine (MPN, 10 mg/kg i.p.) and diclofenac, (DIC 100 mg/kg i.p.), respectively. Group B consisted of 2 main subgroups treated with *Capsicum* fruit aqueous extract (CFE) and CPF as the reference drug. Each subgroup was subdivided into 5 subunits based on the dose regimen of 0.5-8 mg/kg i.p. The animals had similar demographics in terms of sex, age and weight. All the animals were fed with standard diet pellet and drinking tap water *ad libitum*. A 600-ml glass beaker was placed on a 'Heidolph MR 2002' hot-plate (with adjustable temperature). The temperature of the hot-plate was then regulated to 40 1°C. Each mouse was placed in the glass beaker (on the hot-plate) in order to obtain the animal's response to electrical heat-induced nociceptive pain (licking of the forepaws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain. The time taken for each mouse to jump out of the beaker (i. e. reaction time) was noted and recorded. Capsaicin extract and CPF were tested at doses of 0.50, 1.0, 2.0, 4, and 8.0 mg/kg i. p., respectively. Treated 'control' mice received MPN (10 mg/kg i. p.), or DIC (100 mg/kg route) only. Due to the effects of gastric juice, capsaicin was administered intraperitoneally, a route that ensures adequate systemic bioavailability, early onset and satisfactory pharmacological activity of the tested agents.

The mean reaction time for each 'test' mouse was determined and documented using a standard stop watch calibrated in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 1-hour intervals. The mean of these three determinations constituted the 'initial reaction time'– that is, reaction time before the treatment of the mouse. The mean reaction times for all the mice used were pooled to obtain the final, 'control' mean reaction time ($t_1$). Each of the mice in the study was thereafter treated with either CFE or CPF. Thirty minutes after treatment with CFE or CPF, the reaction time was again evaluated ($t_2$). This value was pooled for the mice used in each treatment group, and the final 'test' mean value for each treatment group was calculated. This final 'test' mean value represented 'after-treatment reaction time' for each group of treated mice. This 'test' mean value was subsequently used to determine the percentage thermal pain stimulus relief or protection, by applying the formula:

% protection against thermal pain stimulus = Test Mean – Control Mean X 100/Control Mean = $t_1 - t_2 \times 100 / t_2$

(Where $t_1$ = the mean 'control' reaction time, and $t_2$ = mean 'test' reaction time, respectively).

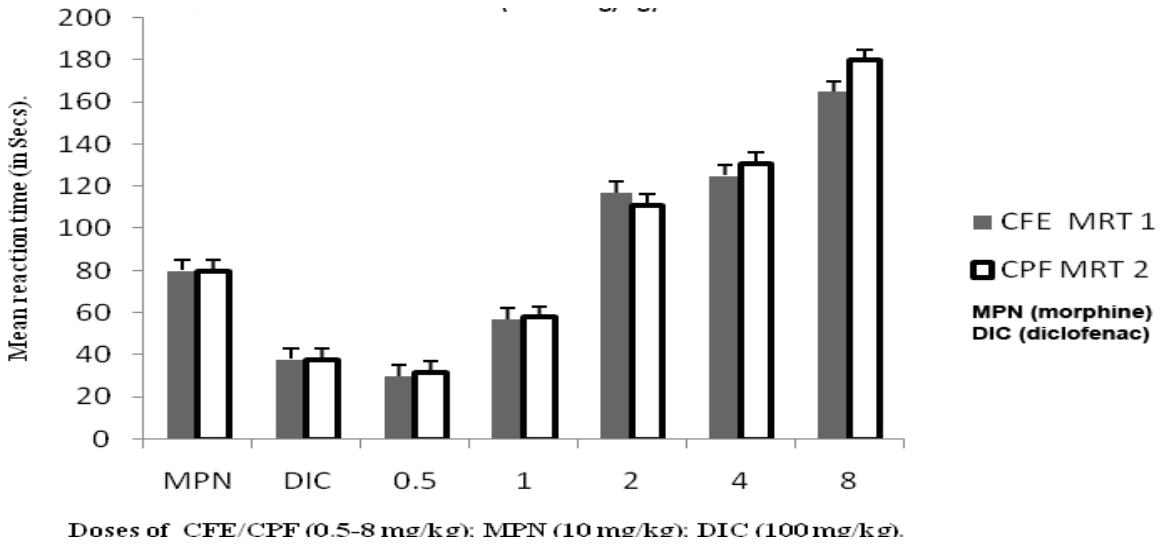


**Figure 1.** Mean reaction time (secs) to thermal pain following treatments of rats with CFE or (0.5-8 mg/kg) n= 12 P<0.01

### Assessment of cfe and cpf analgesia using 'acetic acid' test method

The 'acetic acid' test method used in this study was adopted from those described earlier by Koster*et al,* (1959); Williamson *et al*, (1996);Zakaria*et al,* (2001); and Silva *et al,* (2003). The mice used were divided into groups of 'test' and 'control' animals. The treated group had subunits that received CFE and CPF, respectively, subdivided into 5 treatment groups of 10 mice each for graded doses of CFE or CPF at 0.5, 1, 2, 4 and 8.0 mg/kg (i.p). Group A control mice included an untreated group of 12 mice and two positive 'control' groups of 10 mice each, that were pre-treated with DIC (100 mg/kg i.p.) and MPN (10 mg/kg i.p.). Each of the untreated 'control', and 'test' animals was treated with intraperitoneally-administered 0.2 ml of 3% *v*/v acetic acid solution (Koster*et al,* 1959). Twenty minutes after pre-treatment with DIC, a dose of the capsaicin, or the extract, 0.2 ml of a 3% w/v of acetic acid solution was injected intraperitoneally (i. p.) to each of the 'treated' mice (Koster*et al,* 1959).

The number of writhings (i. e., abdominal contractions and stretches) that occurred within the first 20 minutes following acetic acid administration were counted and recorded. The recorded numbers of acetic acid-induced writhings (abdominal contractions and stretches) that occurred in the CFE-, CPF-, and DIC- pretreated mice were compared with those in the 'untreated Group A 'control' mice. This 'test' mean value was subsequently used to determine percentage inhibition to writhing response using the formula:

% inhibition of writhings response = Control Mean – Test Mean X 100/Control Mean = $w_1 - w_2 \times 100 / w_1$
(Where $w_1$ = mean 'control' and $w_2$ = mean 'test' writhing movements, respectively).

### DATA ANALYSIS

Data obtained were pooled and presented as means (±SEM). Data from 'control' mice were used as baseline values. The mean reaction times to the pain stimulus were recorded and subsequently analysed using a 2-way ANOVA. Wilcoxon and Kruskal-Wallis tests were used to assess any differences. Statistical significance was obtained by using a double tailed CI of 95%, and a P value of less than 0.05. Pearson correlation coefficient was used to assess the activity of the extract compared to that of the synthetic capsaicin as shown in Figures 1-3.

## RESULTS

### Analgesic activity

*Capsicum frutescens*-derived capsaicin extract (0.5–8.0 mg/kg i. p.) produced dose-dependent and significant (P□0.05–0.001) analgesic effects against thermally- and chemically-induced nociceptive pain (see Figures 1-3). Pretreatment of the mice with either CFE or CPF (0.5–5.0 mg/kg i. p.) caused significant (P□0.01–0.0002) delays in the mean reaction times of the animals to thermally-induced pain. (Figure 1). CFE and CPF (0.5-8.0 mg/kg i.p.) also caused dose-dependent and significant (P□0.05–0.001) reductions in the acetic acid-induced writhes of the mice (Figure 2).

### Activity level in mice

A general observation of the effects of capsaicin on the activities, sleep, pain tolerance and mechano-thermal sensation of mice, were noted. These effects were dose

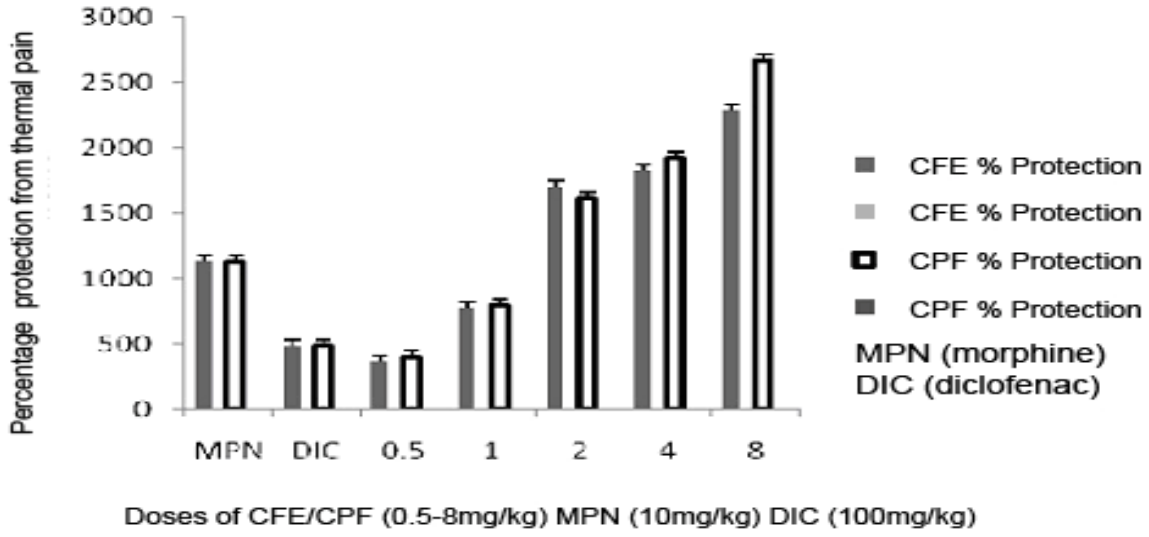


**Figure 2.** Percentage protection from thermal pain following treatment of rats with CFE or CPF (0.5-8mg/kg) n=12 P<0.01

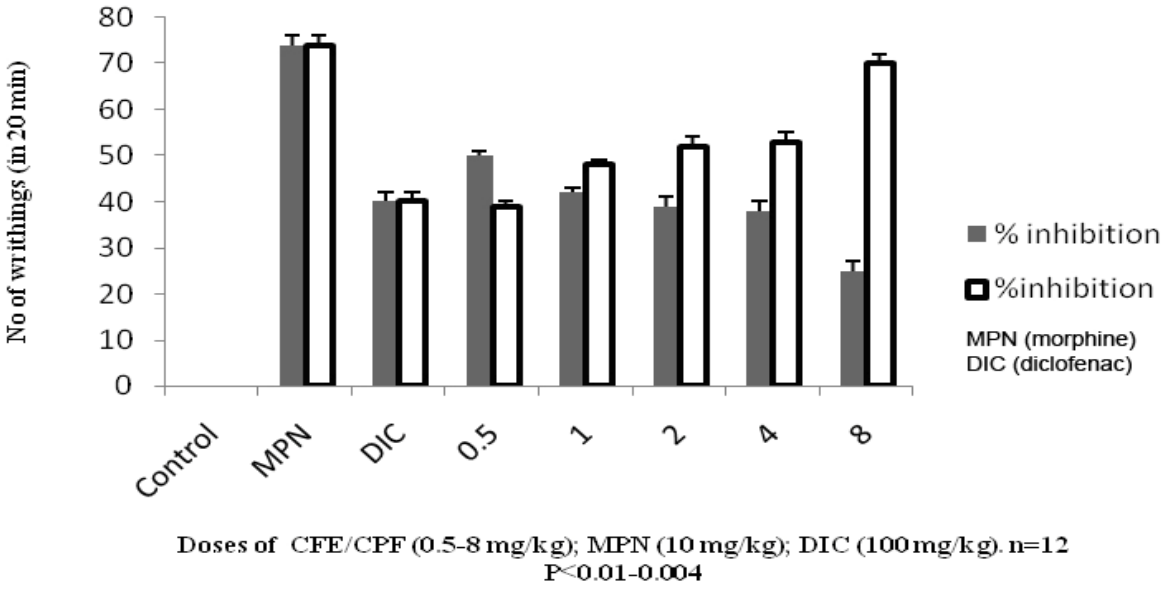


**Figure 3.** Doses of CFE/CPF and writhings (in 20 mins)

dependent, and wear off with time. The animals' quality of sleep was also better. There were initial excitation following injections of capsaicin, but this was followed by playful acts within each cohort, followed by calmness. These occur for both groups on reference and extracted capsaicin.

## DISCUSSION

Vanilloid 1 receptor has been described as the molecular integrator of several nociceptive stimuli (Ferreira *et al*., 2004). Most studies have alluded to the effectiveness of capsaicin-induced central analgesia. The present study

aimed to explore the potential benefits of capsaicin in both central and peripheral analgesia.

The use of 'acetic acid' test method to investigate peripheral analgesic mechanism was validated at different times by Koster*et al.,* (1959); Williamson *et al*, (1996); Zakaria*et al.,* (2001); and Silva *et al.,* (2003). Similarly, the evaluation of central analgesic mechanism using the 'hot plate' test method was validated by Eddy and Leimback, (1953); Lanhers *et al.,* (1992); and Williamson *et al*., (1996). The reduction in the writhings of the abdominal wall and the prolongation of the mean reaction time to thermal pain can be regarded as indicative of positive peripheral and central analgesic mechanism, respectively.

Centrally-acting neuromediators can be classified into excitatory and inhibitory neuromediators. Glutamate and aspartate are examples of excitatory amino acids acting as neurotransmitters centrally. Substance P (SP), calcitonin-gene-related peptide (CGRP) and growth factors (e.g., brain derived neurotrophic factors) are other examples (Cervero, 1983; Chiang, *et al*., 1997, and 1999). Inhibitory neuromediators include endogenous opioids, such as enkephalin and □-endorphins. Others are gamma aminobutyric acid (GABA), glycine and □-adrenergic agonists. The effects of capsaicin at the GABAergic terminals, central MAO receptors, NMDA, acetylcholine and noradrenergic receptor sites were earlier elucidated (Davis, 1995; Davis, 2000; Woolf, 2000; 2001). The finding suggests that capsaicin activity at vanilloid receptors results in analgesia and cerebral depression at low doses, and that it produces paradoxical effects at other dissimilar receptors at high doses (Davis and Marinelli, 1993; Ralevic, 2001).

The aberration of inflammatory and neuropathic enhancement of pain perception as seen in allodynia (painful touch) and hyperalgesia are due to increased release of SP from the *substantiagelatinosa*. This phenomenon is called peripheral sensitization, and three principal neurotransmitters (NMDA, GABA, and Substance P) have been implicated in previous studies. Conversely, any agent acting on these receptors and neuromediators have the ability to modulate pain (Wallace, 1997).

Capsaicin showed a potent analgesic potential in the animals used. The explanation for the statistically often less significant effects at lower doses might have to do with the release of Substance P, due to de-vesiculation mechanism of receptor modification, receptor up-regulation and internalization (Yakish, 1999).

One of the findings of this study is that capsaicin has statistically significant ($P<0.002$) analgesic effects, especially for chronic therapy on centrally-mediated pain mechanisms. Peripherally-induced analgesia is statistically significant ($P< .001$) as compared to the 'control' at $P<0.5$. This finding probably suggests that capsaicin inhibits the inflammatory limb of pain mechanisms, or do so by receptor modification, and/or by the 'wind-up' mechanisms shown in previous studies (Winter, 1995).

The present study also shows that capsaicin has dose-dependent and statistically significant analgesic effects on mechano-thermal and chemically-induced pain. These results corroborate earlier studies that capsaicin is efficacious in neuropathic pain from diabetes, herpes, phantom and stump pain, chronic pain from osteoarthritis, and trigeminal neuralgia (Deal, 1991; Dray, 1990; 1992; Dini, 1993; Winter, 1995).

Furthermore, the stimulant effect of capsaicin results in convulsion and death at high doses (Nagy, 1980; 1983; Gade, 1985). Further work will be required to elucidate the mechanisms of this paradoxical effect.

## CONCLUSION

Both capsaicin and *Capsicum frutescens* Linn (Solanaceae) fruitaqueous extract (CFE) have dose-dependent, statistically significant peripheral and central analgesic properties. More studies, however, need to be done towards evolving structural changes in their chemistry so as to develop capsaicin analogues adaptable for human uses in intractable pain from sports injuries, cancer management, and other forms of acute or chronic pain.

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