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## Antinociceptive Effects of an Ethanolic Extract of *Capparis erythrocarpos* Isert Roots in the Mice Formalin Test

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**Abstract:** Antinociceptive effect of an ethanolic extract of *Capparis erythrocarpos* Isert roots (10-300 mg kg<sup>-1</sup>; p.o.) was evaluated in the mouse formalin test. Morphine (1-10 mg kg<sup>-1</sup>; i.p.) was used as positive control. The extract dose-dependently reduced pain scores in both phases of formalin-induced nociception with the 100 mg kg<sup>-1</sup> dose significantly reducing formalin-induced pain by 47.54±5.65 and 80.01±3.77% in the first and second phases, respectively. Naloxone (an opioid antagonist) did not block the antinociceptive effect of the extract in both the neurogenic phase and the inflammatory phase; however, theophylline (an adenosine antagonist) completely blocked the effect in the neurogenic phase and significantly inhibited the effect in the inflammatory phase. These findings demonstrate that the ethanolic extract of *C. erythrocarpos* roots has both central and peripheral antinociceptive effect with possible involvement of adenosinergic mechanism.

**Key words:** *Capparis erythrocarpos*, formalin, morphine, theophylline, naloxone, mice

### INTRODUCTION

The search for an efficacious analgesic drug that can be used in the management of pain (from mild to severe) and that can be taken chronically without significant side effects is still an active research goal with emphasis on the use of medicinal plants, however, a large number of medicinally useful plants especially in Africa have not been scientifically explored. The fact that traditional health care and the use of medicinal plants is familiar, affordable and available at the local level is enough evidence to suggest that it will continue to play an important role in national health care delivery of most countries well into the twenty-first century (Breuer *et al.*, 2006; Kaplan *et al.*, 2007; Lee *et al.*, 2008). This increasing demand worldwide for medicines from natural sources has motivated the search for plants with potential pharmacological activity bearing in mind that 25% of modern medicines were made from plants first used traditionally and medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including pain and inflammation (Balunas and Kinghorn, 2005; Khurdayan and Davies, 2005; Amr and Abdulla, 2006; Braddock, 2007).

The plant *Capparis erythrocarpos* Isert (family Capparaceae), commonly called salt bush or capers is reputed traditionally in Ghana and other parts of Africa for the management of various inflammatory and pain

conditions. In Ghana, the local names include *woresenakyiame* (salute me when passing) in Ashanti, *aypo* (Thorns) in Ewe, *okyerabran* (giant catcher) in Fante, *aymatyia* in Ga and *apana* in Twi (Mshana *et al.*, 2000). Powdered roots of *C. erythrocarpos* are used for the treatment of musculoskeletal and joint disorders such as rheumatoid arthritis, rheumatism and swellings of the joints as well as pain associated with these conditions. In addition, the powdered root bark of *C. erythrocarpos* is applied to the head to treat headache and scraped roots juices are instilled in the ear to treat ear pain and otitis; and also for conjunctivitis (Mshana *et al.*, 2000; Irvine, 1961). Although, the medicinal uses and general safety of this plant are well known to the native people, its place is yet to be rationalized in therapeutics, using current methodology. Scientific studies are therefore required to judge its efficacy and some of the medicinal properties popularly claimed, as well as other limitations to widen the scope of this drug. To date, there is little scientific evidence to support the traditional use of *C. erythrocarpos* in the treatment of pain-related diseases and the possible mechanisms involved however previous work has been done on the antinociceptive effect of other species such as *Capparis spinosa* and *Capparis decidua* using the hot plate test (Ageel *et al.*, 1986).

There is no indication in literature of the scientific validation of the plant's use. The objective of this study therefore was to carry out a pharmacological evaluation of

the antinociceptive effect of the ethanolic root extract using the mouse formalin test which is one of the most predictive and valid model of acute clinical pain.

## MATERIALS AND METHODS

**Plant material:** The roots of the plant *C. erythrocarpos* were obtained and authenticated at the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapim, Ghana in April, 2007 and a voucher specimen (No. FP/08/024) was kept in the Faculty of Pharmacy Herbarium.

**Preparation of extract:** Chopped roots were sun-dried for four days and powdered using a hammer mill. The ethanolic extract was obtained by cold maceration of the root powder (5 kg) using 70% (v/v) ethanol over a period of 6 days and the filtrate was concentrated under reduced pressure at a temperature of 60°C in a rotary evaporator to obtain a brown syrupy mass. This syrupy mass was then dried to a dark brown semi-solid using a water bath and kept in a dessicator afterwards. Final yield was 450 g; 9% w/w.

**Drugs:** Formalin, naloxone hydrochloride and theophylline were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA and morphine hydrochloride was purchased from Phyto-Riker, Accra, Ghana.

**Animals:** ICR mice (20-30 g) of both sexes were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and housed in the animal facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST). The animals were housed in groups of six in stainless steel cages (34×47×18 cm<sup>3</sup>) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12 h light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85-23, revised 1985). All protocols used were approved by the Departmental Ethics Committee.

**Phytochemical analysis:** The presence of saponins, tannins, alkaloids, triterpenes, flavonoids, glycosides and reducing sugars were tested by simple qualitative methods as described by Trease and Evans (1989) and Sofowora (1993).

**Formalin-induced nociception:** The mouse paw formalin test used was a slight modification of the method of Dubuisson and Dennis (1977) as described by Woode *et al.* (2009). Briefly, mice were randomly divided into groups of five. Each animal was assigned to one of twenty Perspex test chambers (15×15×15 cm<sup>3</sup>) and allowed to acclimatize for 30 min before the start of the experiment. Test drugs were given 30 min for i.p. route and 1 h for oral route before intraplantar injection of 10 µL of 5% formalin solution into the right hind paw of mice. Animals were immediately returned individually into the testing chamber. A mirror was placed at 45° angle beneath the chambers to allow an unobstructed view of the injected hind paws. The behavior of the animal was then captured (60 min) for analysis by a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed in front of the mirror.

**Two sets of experiment were carried out:** In the first set, the effect of the extract and morphine alone were tested to determine whether the extract had analgesic effect; mice were grouped as follows:

- **Group 1:** *Capparis erythrocarpos* extract (10, 30, 100 and 300 mg kg<sup>-1</sup>)
- **Group 2:** Morphine (1, 3 and 10 mg kg<sup>-1</sup>)
- **Group 3:** Control

In the second set, the effect of the extract and morphine in the presence of naloxone and theophylline were tested to determine the possible mechanism of action of the extract; mice were grouped as follows:

- **Group 1:** *Capparis erythrocarpos* extract 100 mg kg<sup>-1</sup>
- **Group 2:** Morphine 3 mg kg<sup>-1</sup>
- **Group 3:** Naloxone 3 mg kg<sup>-1</sup>
- **Group 4:** Theophylline 5 mg kg<sup>-1</sup>
- **Group 5:** Morphine 3 mg kg<sup>-1</sup> + naloxone 3 mg kg<sup>-1</sup>
- **Group 6:** *Capparis erythrocarpos* 100 mg kg<sup>-1</sup> + naloxone 3 mg kg<sup>-1</sup>
- **Group 7:** *Capparis erythrocarpos* 100 mg kg<sup>-1</sup> + theophylline 5 mg kg<sup>-1</sup>
- **Group 8:** Morphine 3 mg kg<sup>-1</sup> + theophylline 5 mg kg<sup>-1</sup>
- **Group 9:** Control

All drugs were freshly prepared. Extract was prepared in 2% tragacanth mucilage. Drug solutions and suspensions were prepared such that not more than 1 mL of extract was given orally and not more than 0.5 mL of the standard drug was injected intra peritoneally.

Pain responses were scored for 60 min, starting immediately after formalin injection. A nociceptive index was determined for each 5 min time block by measuring the duration of biting/licking of the injected paw (Hayashida *et al.*, 2003). Behavioral responses were scored from the videotapes with the aid of the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia available at <http://www.jwatcher.ucla.edu/>). Average nociceptive score for each 5 min time block was calculated by multiplying the frequency and time spent in biting/licking the injected paw. Data were expressed as the Mean±SEM scores between 0-10 and 10-60 min after formalin injection.

**Toxicity test:** Acute and sub acute toxicity tests were done by administration of high doses of the extract (0.3-3 g kg<sup>-1</sup> p.o.) followed by close monitoring for signs of toxicity for 24 h and 14 days, respectively. Hematological and biochemical parameters were also monitored.

**Data analysis:** The time-course curves were subjected to two-way (treatment×time) repeated measures Analysis of Variance (ANOVA) with Bonferroni's post hoc test. Nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). To determine the percentage inhibition for each treatment, the following equation was used.

$$\text{Inhibition (\%)} = \left( \frac{\text{AUC}_{\text{control}} - \text{AUC}_{\text{treatment}}}{\text{AUC}_{\text{control}}} \right) \times 100$$

Differences in AUCs were analyzed by ANOVA followed by Newman-Keuls' post hoc test. The (ED<sub>50</sub>) which is the dose for 50% of the maximal effect was determined for each drug by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left( 1 + 10^{(\text{LogED}_{50} - X)} \right)}$$

where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

The fitted midpoints (ED<sub>50</sub>) of the curves were compared statistically using F-test (Motulsky and Christopoulos, 2003; Miller, 2003). GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego,

CA, USA) was used for all statistical analyses and ED<sub>50</sub> determinations. The p<0.05 was considered statistically significant.

## RESULTS

**Phytochemical analysis:** Phytochemical screening revealed the presence of high amount of alkaloids and flavonoids with traces of triterpenes in the crude extract.

**Effect of drugs on nociception:** Formalin induced a clear nociceptive response exhibited as biting or licking of the injected paw. The nociceptive response was biphasic as previously described by Bars *et al.* (2001), Saddi and Abbott (2000) and Wang *et al.* (1999) consisting of an initial intense response to pain beginning immediately after formalin injection and rapidly decaying within 10 min after formalin injection (first phase). This was then followed by a slowly rising but longer-lasting response (second phase) from 10-60 min after formalin injection with maximum effect at approximately 20-30 min after formalin injection (Wang *et al.*, 1999). Pain scores were significantly (p<0.05) lower in the drug-treated groups than in the control (Fig. 1).

The CEE (10-300 mg kg<sup>-1</sup> p.o.) dose-dependently decreased formalin-induced nociceptive behavior in both phases. The highest dose of CEE 300 mg kg<sup>-1</sup> had significant effect in phase 1 (F<sub>3,16</sub> = 15.25, p<0.001) and phase 2 (F<sub>3,16</sub> = 35.34, p<0.001) giving the greatest inhibition in both the neurogenic (phase 1) 52.78±8.12% and inflammatory phase (phase 2) 86.52±3.79% (Fig. 1a, b). Morphine (1-10 mg kg<sup>-1</sup>), an opioid agonist significantly inhibited the formalin-induced nociceptive behavior in both phases in a dose-dependent fashion. The highest dose 10 mg kg<sup>-1</sup> had significant effect in phase 1 (F<sub>3,16</sub> = 9.54, p<0.001) and phase 2 (F<sub>3,16</sub> = 32.05, p<0.001) and produced the greatest inhibition in both the neurogenic (70.28±11.62%) and inflammatory phase (94.80±4.22%) (Fig. 1c, d).

Administration of naloxone and theophylline alone did not produce any significant antinociceptive effect (Fig. 2a, b). The antinociceptive effect of extract in the presence of naloxone and theophylline is shown in Fig. 3a-d. *Capparis erythrocarpos* (100 mg kg<sup>-1</sup>) significantly reduced formalin-induced nociception with a percentage inhibition of 47.54±5.65% (F<sub>3,16</sub> = 15.25 p<0.001) in the first phase and 80.01±3.77% (F<sub>3,16</sub> = 35.34 p<0.001) in the second phase. This antinociceptive effect of the extract was not antagonized by naloxone in both the neurogenic phase (43.44±4.15%) and the inflammatory phase (82.41±0.90%); however, theophylline completely

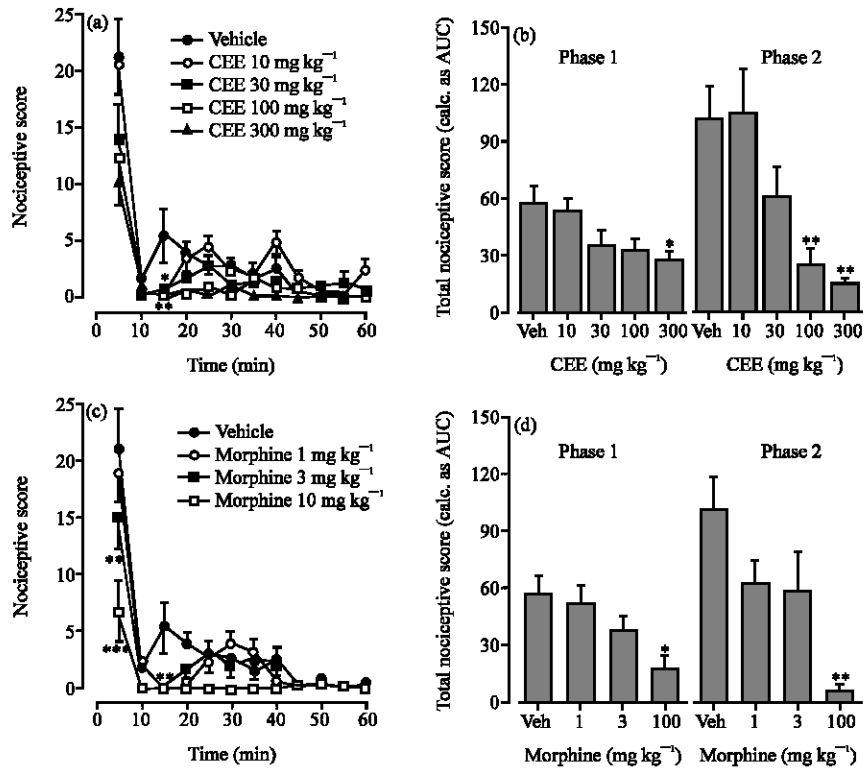


Fig. 1: Effect of CEE (10-300 mg kg<sup>-1</sup> p.o.) and morphine (1-10 mg kg<sup>-1</sup> i.p.) (a, c) on the time course of formalin-induced pain in mice. Nociceptive/pain scores are shown in 5 min blocks up to 60 min post formalin injection; the AUC (total response) for phase 1 and phase 2 (b, d). Each column in b and d represent the Mean±SEM. n = 5 \*\*p<0.001; \*p<0.05

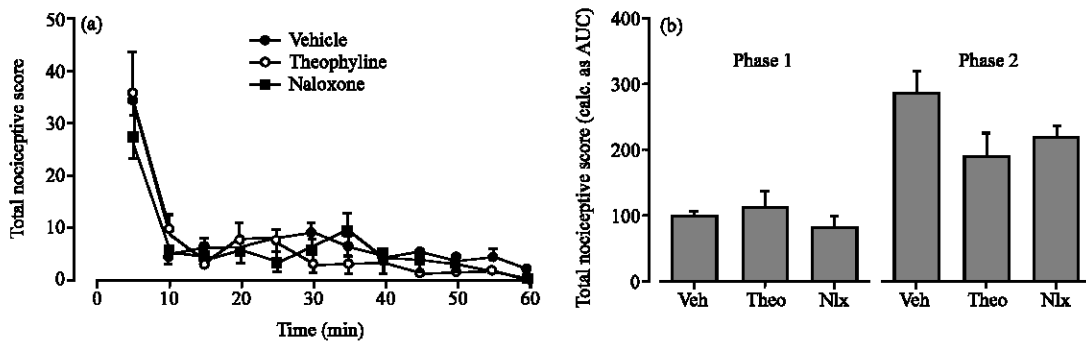


Fig. 2: Effect of intraperitoneal injection of naloxone and theophylline on the time course (a) and the AUC (total response) (b) of formalin-induced pain in mice

inhibited the first phase and significantly inhibited the second phase with a percentage inhibition of 57.08±1.88%. In the formalin test, naloxone inhibited the antinociceptive effect of morphine in both the neurogenic and inflammatory phases with percentage inhibitions of 9.14±9.14 and 22.00±4.05%, respectively. Theophylline on the other hand, could not inhibit the antinociceptive effect of morphine in both phase 1 and phase 2.

From the ED<sub>50</sub> values calculated from the dose response curves in Fig. 4a and b, the extract was found to be approximately 24.6 and 21.98 times less potent than morphine in the first and second phases of the formalin test, respectively.

No remarkable signs of toxicity were observed either immediately or during the post-treatment period even at the highest dose of 3.0 g kg<sup>-1</sup>. There were also no

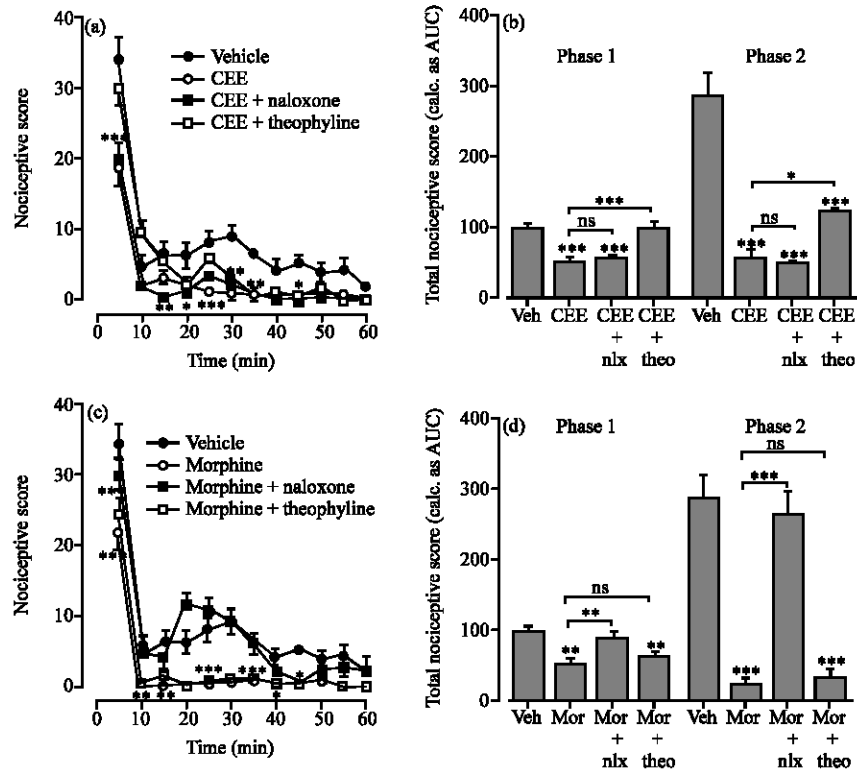


Fig. 3: Effect of intra peritoneal injection of naloxone and theophylline on the antinociceptive effect of CEE ( $100 \text{ mg kg}^{-1}$  p.o.) and morphine ( $3 \text{ mg kg}^{-1}$  i.p.) (a, c) on the time course of formalin-induced pain in mice and the AUC (total response) for phase 1 and phase 2 (b, d). Each column represents the Mean $\pm$ SEM. (n = 5) \*\*\*p<0.0001; \*\*p<0.001; \*p<0.05; ns: Not significant

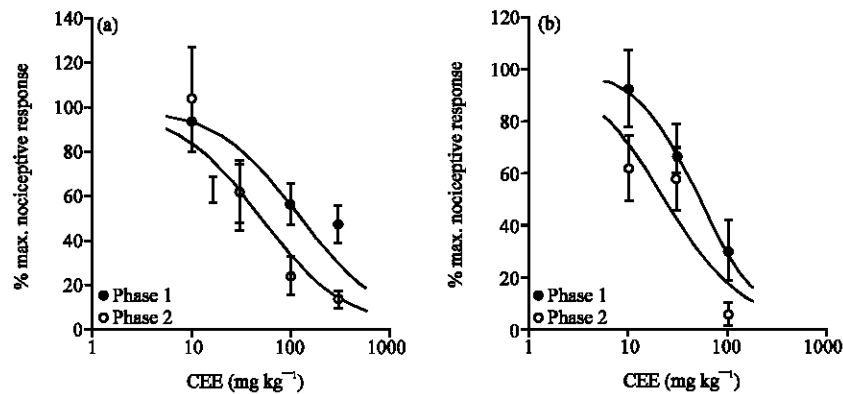


Fig. 4: Dose response curves of CEE (a) and morphine (b) on the total nociceptive score for the first phase and the second phase of the formalin test in mice. Each point represents Mean $\pm$ SEM (n = 5)

changes noted in behavior, activity, posture, or external appearance that were considered to be test drug related and all the animals survived throughout the 24 h and 14 day study period, respectively. There were generally no statistically significant changes in the hematological and biochemical parameters taken.

## DISCUSSION

The results of this study show that the ethanolic extract of *Capparis erythrocarpos* roots has a potent antinociceptive effect against the chemical stimuli provoked by the intraplantar injection of formalin. The

formalin test is one of the most frequently used models to explain pain and analgesic mechanisms. Results from the formalin test are usually better than those using mechanical or thermal stimulus (Tjolsen *et al.*, 1992).

Intraplantar injection of formalin evoked a characteristic biphasic licking response. There was an early phase (0-10 min post formalin injection) due to the direct stimulation of nociceptors by formalin that corresponds to acute neurogenic pain with release of substance P and is sensitive to central analgesics (Bars *et al.*, 2001; Szolcsanyi *et al.*, 2004). The late phase involves inflammatory components with the release of different pain mediating substances such as serotonin, histamine, bradykinin and prostaglandins and is sensitive to NSAIDs (Yashpal and Coderre, 1998; Malmberg and Yaksh, 1992), corticosteroids as well as analgesics with central effects (Vasconcelos *et al.*, 2003).

Response to pain (defined by pain scores) was significantly lower in the drug treated groups than the control treated with only formalin. *Capparis erythrocarpos* extract exhibited analgesic activity in the rat formalin test by significantly lowering the response of the animals to pain in both phases; it however, inhibited the second phase of the formalin response more than the first phase suggesting that *C. erythrocarpos* could be useful in inflammatory pain. Since the extract had effect on inflammatory pain, it is possible that it has inhibitory effect on inflammatory mediators notably prostaglandins as well as receptor blockade. Furthermore, the potent analgesic activity of the extract in both phases of the formalin test presupposes a possible central analgesic effect in addition to peripheral analgesic effect.

Morphine, an opioid agonist was antinociceptive and inhibited both phases of the formalin response clearly depicting that it is a centrally acting analgesic drug (Bohn *et al.*, 2002; Catheline *et al.*, 1996). The role of opioid receptors located in the Central Nervous System (CNS) and peripheral nervous system in inflammatory pain is well established (Whiteside *et al.*, 2005).

The analgesic effect was further investigated to elucidate the possible mode or mechanism of action(s) of the extract by using the non-selective opioid receptor antagonist naloxone (Herrero and Headley, 1996) and the non-selective adenosine receptor antagonist theophylline (Sawynok *et al.*, 1991). Compounds that mimic or modulate the antinociceptive action of adenosine presents a potential approach to the treatment of pain (Kowaluk *et al.*, 2000; Jarvis *et al.*, 2002).

As expected naloxone reversed the effect of morphine however, naloxone did not reverse the antinociceptive effect of the extract in both phases, suggesting that opioid receptor activation was not involved in the

antinociceptive effect of the extract. Antinociceptive effect of *C. erythrocarpos* was completely reversed in the first phase and significantly reversed in the second phase by theophylline, which suggest the involvement of adenosine in the antinociceptive effect of *C. erythrocarpos* and more specifically the receptor subtype A<sub>1</sub> which are involved in antinociception could be implicated (Poon and Sawynok, 1999; Whiteside *et al.*, 2005). Adenosine interacts with at least four P1 receptor subtypes coupled with G protein, namely A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub> and A<sub>3</sub> (Fredholm *et al.*, 2001). These receptors can affect nociceptive, inflammatory and neuropathic pain states at peripheral, spinal and supraspinal sites (Sawynok, 1998; Dickenson *et al.*, 2000; Sawynok and Liu, 2003). Current evidence shows that A<sub>1</sub> receptor activation produces antinociception while activation of A<sub>2a</sub> and A<sub>3</sub> mediates pronociceptive actions in rodents (Sawynok, 1998; Sawynok *et al.*, 1998). However, detailed research involving the use of specific agonists and antagonists of A<sub>1</sub> receptors are necessary to confirm this assertion. In addition its mechanism of action may involve other pathways which need to be investigated.

The presence of flavonoids, alkaloids and triterpenes in the ethanolic root extract of *Capparis erythrocarpos* may be responsible for some of its pharmacological properties. As has been reported by several researchers, the presence of many biologically active phytochemicals such as triterpenes, flavonoids, alkaloids, steroids, tannins and glycosides in various plant extracts may be responsible for their respective pharmacological properties (Singh *et al.*, 2002; Narendhirakannan *et al.*, 2007; Agarwal and Rangari, 2003; Mbagwu *et al.*, 2007; Liu *et al.*, 1996). In addition to this, flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (Rajnarayanan *et al.*, 2001). Further studies are required to establish the specific chemical constituents responsible for the pharmacological actions. However, the antinociceptive effect of the ethanolic root extract of *Capparis erythrocarpos* was demonstrated by its ability to dose-dependently reduced pain scores in both phases of formalin-induced nociception and since the principal targets of effective pain control are to ameliorate nociception and to reduce threshold of pain sensation this validates the traditional use of the plant in the treatment of conditions associated with pain.

In the toxicity study, using high concentrations of the extract (0.3-3 g kg<sup>-1</sup> p.o.) there were no significant differences found in most of the haematological indices and serum biochemical parameters suggesting that the extract has a high safety profile.

## CONCLUSION

The ethanolic extract of *Capparis erythrocarpos* roots exhibits central analgesic effect with possible involvement of adenosinergic mechanism. This finding provides some scientific support on the traditional use of roots of *Capparis erythrocarpos* for controlling inflammatory pain conditions.

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