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## Anti-Nociceptive Effects of an Ethanolic Extract of the Whole Plant of *Synedrella nodiflora* (L.) Gaertn in Mice: Involvement of Adenosinergic Mechanisms

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**Abstract:** This study presents the effect of an ethanolic extract of the whole plant of *Synedrella nodiflora*, a plant used in Ghana for the treatment of epilepsy and pain, in formalin-induced pain and acetic acid-induced writhing assay and the possible mode(s) of action of its analgesic action. For comparison, morphine and diclofenac were used as standard opioid and NSAID respectively. The ethanolic extract (100-1000 mg kg<sup>-1</sup>; p.o.) and morphine (1-10 mg kg<sup>-1</sup>; i.p.) dose-dependently decreased both phases of the formalin-induced nociceptive behavior. The antinociceptive effect of *S. nodiflora* (300 mg kg<sup>-1</sup> p.o.) on the first and second phases of formalin induced pain was significantly blocked by caffeine but not by naloxone. In the acetic acid- induced writhing test, diclofenac and *S. nodiflora* significantly reduced the number of writhes dose dependently. Also, the effect of *S. nodiflora* (300 mg kg<sup>-1</sup> p.o.) was blocked by caffeine (3 mg kg<sup>-1</sup> i.p.) but the analgesic effect of diclofenac was enhanced significantly. The observed effects of caffeine on the central and peripheral analgesic effects of *S. nodiflora* in the formalin and acetic acid induced writhing suggest the possible involvement of adenosinergic mechanism(s).

**Key words:** *Synedrella*, morphine, diclofenac, naloxone, caffeine

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### INTRODUCTION

*Synedrella nodiflora* (L.) Gaertn. (Asteraceae) is an annual herb which grows to about 2 m high. It is a native tropical American weed but now dispersed pan-tropically and occurring throughout the West African sub-region. In Ghanaian traditional medicine, the aqueous extract of the whole plant is drunk for the treatment of epilepsy, whilst the leaves are used for the treatment of hiccup and threatened abortion (Mshana *et al.*, 2000). The plant is used extensively in Nigeria for cardiac troubles, wounds and for stopping bleeding (Idu and Onyibe, 2007). In Malaysia and Indonesia, the plant is for the treatment of headaches, earaches, stomachaches and in embrocation for rheumatism (Burkill, 1985). In agreement with its traditional uses, Abad *et al.* (1996) showed potent anti-inflammatory effects of ethanolic extract of the aerial parts. Analgesic properties of the whole plant have also been demonstrated in the hot plate and acetic acid induced writhing models of pain (Forestieri *et al.*, 1996). The present study investigates the antinociceptive effects of the hydroalcoholic extract of the whole plant of *S. nodiflora* in the formalin test, a predictive test of acute clinical pain, as well as the acetic acid induced writhing assay. The possible mechanism by which it exerts its analgesic effect in the formalin test and acetic acid- induced writhing test have also been investigated.

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## MATERIALS AND METHODS

### Plant Material

The whole plant of *Synedrella nodiflora* was collected from the Botanical Gardens, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in August, 2007. A voucher specimen has been kept at the Faculty of Pharmacy herbarium.

### Preparation of Extract

The plant was air-dried for seven days and powdered. Two kilograms of the powdered material was cold-macerated with 70% v/v of ethanol. The hydro-alcoholic extract was then evaporated to a green syrupy mass under reduced pressure, air-dried and kept in a dessicator. The yield was 7% w/w. This is subsequently referred to as the extract or SNE.

### Drugs

Diclofenac sodium was purchased from Troge, Hamburg, Germany, morphine hydrochloride from Phyto-Riker, Accra, Ghana, formalin, acetic acid and caffeine were also purchased from BDH, Poole, England whilst naloxone hydrochloride was also obtained from Sigma-Aldrich Inc., St. Louis, MO, USA.

### Animals

ICR mice (20-30 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of 6 in stainless steel cages (34×47×18 cm) 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). The protocols for the study were approved by the Departmental Ethics Committee.

### Phytochemical Analysis

The hydroalcoholic extract of *S. nodiflora* was screened for the presence of alkaloids, reducing sugars, glycosides, saponins and tannins as described by (Evans, 2001).

### Formalin-Induced Nociception

The formalin test was carried out as previously described by Dubuisson and Dennis (1977) and Malmberg and Yaksh (1992) with a few modifications. Mice were randomly divided into groups of five for the following treatments: SNE (10, 30, 100 and 300 mg kg<sup>-1</sup>, p.o.), morphine (1, 3, 10 mg kg<sup>-1</sup>, i.p.) as positive control and saline-treated group as negative control. Each animal was assigned and acclimatized to one of twenty Perspex test chambers (15×15×15 cm) for 30 min before the treatments. Test drugs were given 30 min for i.p. route and 1 h for oral route before the induction of nociceptive behaviors in the animals by a subcutaneous injection of 10 µL of 5% formalin solution into the plantar tissues of the right hind paw. Animals were immediately returned individually into the testing chamber. A mirror was placed at an angle of 45° beneath the chambers to allow an unobstructed view of the hind paws. The behaviour of the animal was then captured (60 min) for analysis with a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed in front of the mirror.

In another experiment, the effect of naloxone (an opioid antagonist) and caffeine (a non-selective adenosine antagonist) on the actions of SNE and morphine was investigated. Naloxone (5 mg kg<sup>-1</sup>) or caffeine (3 mg kg<sup>-1</sup>) was administered intra-peritoneally 30 min before the extract (100 mg kg<sup>-1</sup>) or morphine (3 mg kg<sup>-1</sup>).

Pain responses were scored for 60 min, starting immediately after formalin injection. A nociceptive score was determined for each 5 min time block by measuring the time spent biting/licking of the injected paw (Hayashida *et al.*, 2003). Behavioural 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 time block was calculated by multiplying the frequency and time spent in biting/licking. Data were expressed as the Mean±SEM scores between 0-10 and 10-60 min after formalin injection.

### Writhing Assay

The acetic acid-induced abdominal writhing test was performed as described by Koster *et al.* (1959), with some modifications. Total number of writhings following intraperitoneal (i.p.) administration of 0.6% acetic acid (10 mL kg<sup>-1</sup>) was recorded for 30 min, starting 5 min after the injection. Animals were pretreated with SNE (100-1000 mg kg<sup>-1</sup>, p.o.) and diclofenac sodium (10-100 mg kg<sup>-1</sup>, i.p.) 30 and 15 min, respectively before acetic acid administration. Control animals were treated with normal saline. The animals were placed in a test chamber and the procedure recorded as described for the formalin test. The total time and frequency of writhes was scored for a period of 30 min following the injection of the acetic acid.

In another experiment, caffeine (3 mg kg<sup>-1</sup>, i.p.) was administered 15 min before the extract (300 mg kg<sup>-1</sup>) and diclofenac (30 mg kg<sup>-1</sup>) treatments. Acetic acid was then injected 30 min and 1 h later after the diclofenac and extract, respectively.

Nociceptive scores were determined for each 5 min time block as described above.

### Data Analysis

Time-course curves were subjected to two-way Analysis of Variance (ANOVA), where treatment is the between-subject factor and time post-formalin or post-acetic acid as the within-subjects (repeated measures) factor. Mean differences were calculated with Bonferroni's post hoc test. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC) with GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego, CA, USA). To determine the percentage effect for each treatment, the following equation was used.

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

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

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

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 ( $ED_{50}$ s) of the curves were compared statistically using F-test (Motulsky and Christopoulos, 2003; Miller, 2003). GraphPad Prism for Windows version 4.03 was used for all statistical analyses and  $ED_{50}$  determinations.  $p < 0.05$  was considered statistically significant.

## RESULTS

### Phytochemical Analysis

The phytochemical screening revealed that the hydroalcoholic extract of *S. nodiflora* contains alkaloids, saponins, tannins and reducing sugars.

### Formalin-Induced Nociception Test

Formalin induced a characteristic nociceptive response exhibited as biting or licking of the injected paw. The response to pain was biphasic as previously reported by Dubuisson and Dennis (1977) and Wheeler-Aceto *et al.* (1990), 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 from 10-60 min after formalin injection with maximum effect at approximately 20-30 min after formalin injection (second phase) (Wang *et al.*, 1999; Hayashida *et al.*, 2003).

Figure 1 shows the effect of pre-treatment with SNE, morphine and diclofenac on formalin-induced pain in mice. Generally, responses to pain (defined by pain scores) were lower in the drug-treated groups than the vehicle-treated group as shown by the time course curves (Fig. 1a-c). Oral administration of SNE (100-1000 mg  $kg^{-1}$ ; p.o.) 30 min before formalin injection significantly and dose-dependently inhibited both first and second phases of formalin-induced paw licking and biting ( $F_{3,20} = 26.31$ ;  $p < 0.0001$  and  $F_{3,200} = 17.21$ ;  $p < 0.0001$ , respectively, two-way ANOVA (treatment group $\times$ time) (Fig. 1a). Analysis of the AUCs showed that SNE, at the doses used, attenuated formalin-induced pain/behaviours by 72.51-89.65 and 68.11-89.90% in the early and late phases respectively (Fig. 1b). Similarly morphine (1-10 mg  $kg^{-1}$ ; i.p.), an opioid agonist significantly attenuated the formalin-induced biting/licking in both the first and second phases [ $F_{3,20} = 26.31$ ;  $p < 0.0001$  and  $F_{3,200} = 17.21$ ;  $p < 0.0001$ , respectively, two-way ANOVA (treatment group $\times$ time)] in a dose-dependent manner (Fig. 1a). One-way ANOVA followed by Bonferroni's post hoc test also revealed a significant dose-dependent decrease in total responses in the first and second phases in the presence of morphine ( $F_{3,20} = 8.60$ ;  $p = 0.0007$  and  $F_{3,200} = 8.06$ ;  $p = 0.001$ , respectively) (Fig. 1d). Also, intraperitoneal administration of diclofenac (10-100 mg  $kg^{-1}$ ; i.p.) dose-dependently decreased the nociceptive behaviours induced by formalin in the first and second phases [ $F_{3,20} = 3.22$ ;  $p = 0.0446$  and  $F_{3,200} = 22.414$ ;  $p = 0.0001$ , respectively, two-way ANOVA (treatment group $\times$ time)] (Fig. 1e). Percentage inhibitions as shown by the AUCs were 5.01-43.43% in the first phase and 79.28-93.42% in the second phase (Fig. 1f). Comparison of  $ED_{50}$ s obtained by non-linear regression (Table 1), revealed that both the extract and morphine were equipotent in both phases ( $F_{1,32} = 0.175$ ,  $p = 0.68$  and  $F_{1,32} = 0.279$ ,  $p = 1.212$ , respectively). By contrast, diclofenac was about seven fold more potent in the second phase compared to the first phase ( $F_{1,32} = 7.982$ ,  $p = 0.0081$ ).

Figure 2 shows the effect of non-selective adenosine inhibitor, caffeine and naloxone, an opioid antagonist on the anti-nociceptive effects of SNE and morphine on formalin-induced nociceptive behaviours. Caffeine (3 mg  $kg^{-1}$ ) completely reversed the effects SNE (300 mg  $kg^{-1}$ ) in both phases (Fig. 2a, b; middle panels) but did not have any effects on inhibitory effects of morphine (Fig. 2a, b; outer panels). Naloxone (1 mg  $kg^{-1}$ ) injected 30 min before formalin did not have any significant effects on anti-nociceptive actions of the extract (Fig. 2a, b; middle panels). By contrast, naloxone completely reversed the inhibitory effects of morphine in both phases of the formalin test (Fig. 2a, b; outer panels).

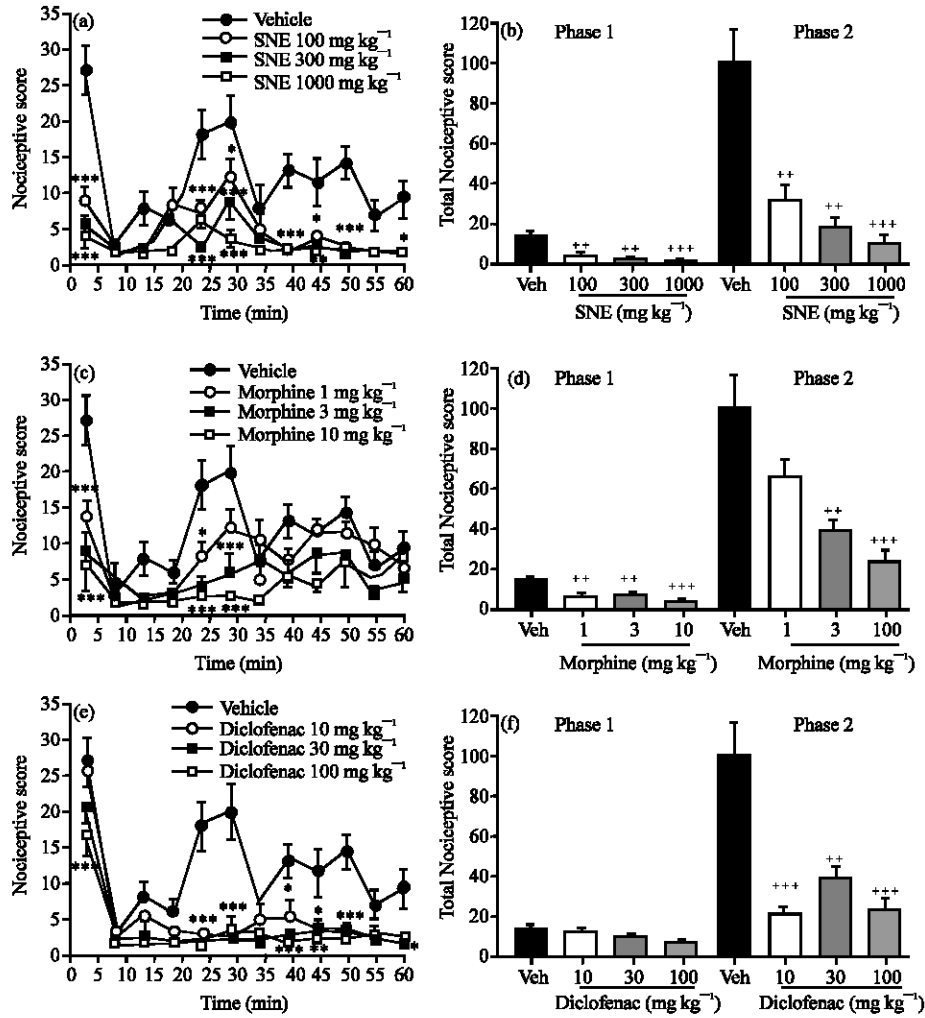


Fig. 1: Dose-response effects of SNE (100-1000 mg kg<sup>-1</sup>, p.o.) (a and b); morphine (1-10 mg kg<sup>-1</sup>, i.p.) (c and d) and diclofenac (10-100 mg kg<sup>-1</sup>, i.p.) (e and f) on formalin-induced nociceptive behaviors in mice. Left panels show the time course of effects over the 60 min period and the right panels show the total nociceptive score calculated from AUCs over the first (0-10 min) and second (10-60 min) phases. Nociceptive/pain scores are shown in 5 min time blocks up to 60 min post-formalin injection. Data are Means±SEM (n = 6). \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001 compared to vehicle-treated group (two-way ANOVA followed by Bonferroni's post hoc test). †p≤0.05, ††p≤0.01, †††p≤0.001 compared to vehicle-treated group (one-way ANOVA followed by Neuman-Keul's post hoc test)

Table 1: ED<sub>50</sub> values for *S. nodiflora* extract, diclofenac and morphine in the formalin test

Drug	ED <sub>50</sub> (mg kg <sup>-1</sup> )	
	Phase 1	Phase 2
SNE	25.98±14.59	30.24±18.08
Morphine	1.30±0.53**	2.14±0.61***
Diclofenac	27.12±12.45	3.97±9.90*††

\*\*p<0.01 and \*\*\*p<0.001 compared to SNE values and ††p<0.01 compared to phase 1 diclofenac value

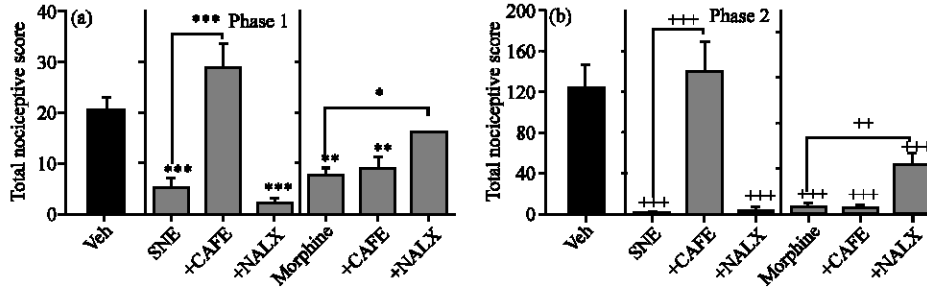


Fig. 2: Effects of caffeine (3 mg kg<sup>-1</sup>, i.p) or naloxone (3 mg kg<sup>-1</sup>, i.p.) pre-treatment on the anti-nociceptive effects of SNE (300 mg kg<sup>-1</sup>, p.o.) and morphine (3 mg kg<sup>-1</sup>, i.p.) in the (a) first phase (upper panel) and (b) second phase (lower panel) of the formalin test. Each bar represents Mean±SEM (n = 5). \*\*\*p≤0.001; \*\*p≤0.01 compared to vehicle-treated group (Veh) and +++p≤0.001; ++p≤0.001 compared to respective drug-treated (one-way ANOVA followed by Newman-Keul's post hoc test)

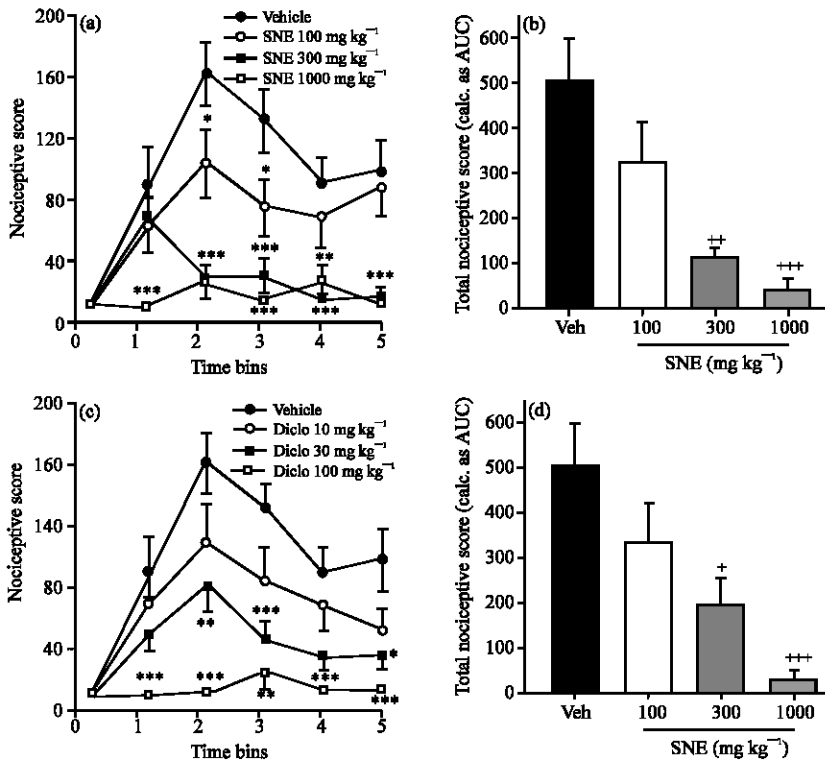


Fig. 3: Dose-response effects of SNE (100-1000 mg kg<sup>-1</sup>, p.o.) (a and b) and diclofenac (10-100 mg kg<sup>-1</sup>, i.p.) (c and d) on acetic acid-induced writhing in mice. Left panels show the time course of effects and the right panels show the total nociceptive scores calculated as AUCs over the over the 30 min period. Nociceptive/pain scores are shown in 5 min time blocks up to 30 min post-acetic acid injection. Data are Means±SEM (n = 5). \*\*\*p≤0.001; \*\*p≤0.01; \*p≤0.05 compared to vehicle-treated group (two-way ANOVA followed by Bonferroni's post hoc test). +++p≤0.001; ++p≤0.01; +p≤0.05 compared to vehicle-treated group, Veh (one-way ANOVA followed by Newman-Keul's post hoc test)

### Acetic Acid-Induced Writhing Assay

In acetic acid induced writhing assay, oral administration of SNE (100-1000 mg kg<sup>-1</sup>) 30 min before the injection of acetic acid significantly and dose-dependently inhibited writhes induced by the acid [ $F_{3,80} = 9.69$ ;  $p = 0.0007$ , two-way ANOVA (treatment group×time)] (Fig. 3a). Analysis of the AUCs showed that SNE attenuated acetic acid-induced writhing by 7.61-64.12% (Fig. 3b). Similarly the non-steroidal drug, diclofenac (10-100 mg kg<sup>-1</sup>; i.p.) significantly reduced acetic acid-induced writhing in mice [ $F_{3,80} = 7.11$ ;  $p = 0.003$ , two-way ANOVA (treatment group×time)] (Fig. 3c). Calculated AUCs showed that diclofenac at the doses used, reduced writhing by 5.02-66.00% compared to vehicle-treated mice (Fig. 3d). Comparison of ED<sub>50</sub>s obtained by non-linear regression (Fig. 4), revealed that the extract (ED<sub>50</sub>: 141.9±37.16 mg kg<sup>-1</sup>) was ≈8× less potent than diclofenac (18.17±6.75 mg kg<sup>-1</sup>) in the writhing assay ( $F_{1,26} = 6.78$ ;  $p = 0.015$ ).

Effects of treatment of non-selective adenosine inhibitor, caffeine is shown in Fig. 5. One-way ANOVA confirmed significant effect of caffeine (1 mg kg<sup>-1</sup>) treatment on the effect on acetic acid-induced writhing ( $F_{2,14} = 8.13$ ,  $p < 0.0001$ ). post hoc between-group comparisons showed that naloxone (0.3 mg kg<sup>-1</sup> and higher) antagonized the effects of SNE in this assay (Fig. 5). By contrast, caffeine pre-treatment did not have any effect on the antinociceptive effect in the writhing assay (Fig. 5) but rather seemed to ‘potentiate’ the effects of diclofenac.

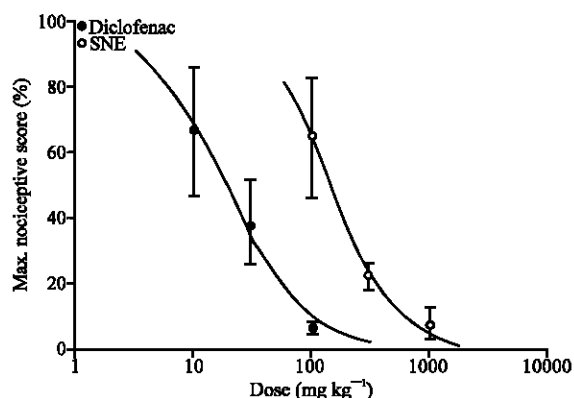


Fig. 4: Dose-response effects of SNE and diclofenac in the acetic acid induced writhing assay in mice

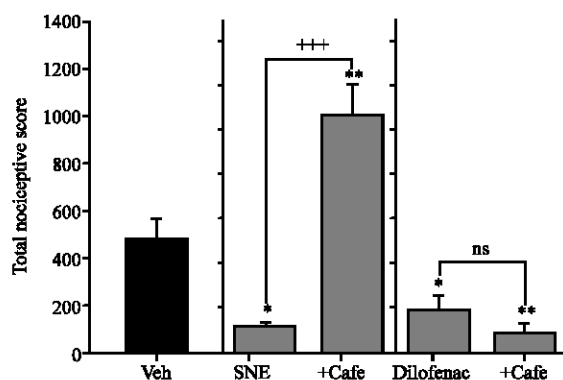


Fig. 5: Effects of caffeine (3 mg kg<sup>-1</sup>, i.p) pre-treatment on the anti-nociceptive effects of SNE (300 mg kg<sup>-1</sup>, p.o.) and diclofenac (3 mg kg<sup>-1</sup>, i.p.) in the acetic acid-induced writhing test. Each bar represents Mean±SEM (n = 5). \*\* $p < 0.001$ ; \* $p < 0.01$  compared to vehicle-treated group (Veh) and \*\*\* $p < 0.001$  compared to SNE effects (one-way ANOVA followed by Newman-Keul’s post hoc test)



## DISCUSSION

The present study demonstrates the antinociceptive effect of the hydroalcoholic extract of *S. nodiflora* in the formalin and acetic acid induced writhing assays and shows the possible involvement of adenosinergic mechanisms in the antinociceptive effect. The formalin test first described by Dubuisson and Dennis (1977) has been shown to be very predictive of acute pain (Le-Bars *et al.*, 2001) and thus a valid model of clinical pain (Costa-Lotufu *et al.*, 2004; Vasconcelos *et al.*, 2003; Vissers *et al.*, 2003). The formalin test, is a well characterized and accepted method in pre-clinical screening of analgesics (Abbott, 1988; Vissers *et al.*, 2003). Intradermal injections of formalin into the rat paw resulted in a biphasic nociceptive response evidenced by flinching, licking or biting of the injected paw as reported by Dubuisson and Dennis (1977) and Wheeler-Aceto *et al.* (1990). An analgesic drug would tend to decrease the incidence of flinching, licking or biting of the injected paw (Courteix *et al.*, 1998). It is suggested that the first phase of the formalin response results essentially from the direct stimulation of nociceptors (Szolcsanyi *et al.*, 2004; Chau, 1989; Le-Bars *et al.*, 2001) leading to the activation of C-fibers (Martindale *et al.*, 2001) whereas the second phase involves inflammatory components with the release of different pain mediating substances that possibly activate small afferents sensory neurons (Le-Bars *et al.*, 2001; Yashpal and Coderre, 1998; Malmberg and Yaksh, 1992). Hence, the analgesic properties exhibited by the extract and morphine in both the first and second phases are characteristic of analgesics with central effects and peripheral anti-inflammatory properties (Mino *et al.*, 2004; Le-Bars *et al.*, 2001). In fact, an earlier study showed that the plant possesses potent anti-inflammatory properties (Abad *et al.*, 1996). Results obtained for diclofenac were in sharp contrast to reports by several researchers who suggest that diclofenac and other NSAIDs have no effect on the first phase of the formalin test (Mino *et al.*, 2004; Yashpal and Coderre, 1998; Malmberg and Yaksh, 1992; Rosland *et al.*, 1990). This inconsistency may be due to lower doses (1-20 mg) used by other researchers in comparison to the higher doses used in this experiment. Furthermore, there is considerable evidence to suggest that in addition to its inhibitory actions on prostaglandin synthesis, diclofenac may also block sensitization to inflammatory processes (Tonussi and Ferreira, 1994). The latter action of diclofenac has been attributed to the activation of a NO-cyclic GMP-K<sup>+</sup> channel pathway in the periphery. It is worth noting that morphine, an opioid, also activates the same pathway to produce its anti-nociceptive effect (Duarte *et al.*, 1992; Aguirre-Banuelos and Granados-Soto, 1999; Ortiz *et al.*, 2001, 2003). Therefore, it is likely that antinociceptive effect of diclofenac on the first phase in the formalin test could be due to the activation of the NO-cyclic GMP pathway, leading to direct blockade of inflammatory sensitization. There is also evidence indicating that not all NSAIDs may act via., the NO-cGMP-potassium channel pathway to produce anti-nociception in the periphery (Ortiz *et al.*, 2003; Torres-Lopez *et al.*, 2002). Also, Ortiz *et al.* (2008) have recently shown that the overall antinociceptive effect induced by systemic diclofenac is the outcome of central and peripheral mechanisms. Since, *S. nodiflora* inhibits the second phase of formalin algesia it is likely to possess some peripheral anti-inflammatory properties. This assertion supports the findings, that the plant extract possesses potent anti-inflammatory effect (Abad *et al.*, 1996).

The aqueous extract of *Synedrella nodiflora* is traditionally used in the management of epilepsy (Mshana *et al.*, 2000). We have pharmacologically confirmed the anticonvulsant effect of the hydroalcoholic extract using acute seizure models in our laboratory (unpublished results). It has been discovered that anticonvulsants such as gabapentin and lamotrigine among others have exhibited analgesic effects in the formalin test (Sawynok, 2001; Chesler *et al.*, 2003; Vissers *et al.*, 2003). In contemporary practice, it is common for clinicians to use anticonvulsant such as phenytoin, carbamazepine, oxcarbazepine and gabapentin in the management of neuropathic pain (Jensen, 2002; Carrazana and Mikoshiba, 2003). Thus it is not surprising that the whole plant extract of *S. nodiflora* is used traditionally in treating epilepsy and pain (Mshana *et al.*, 2000; Burkill, 1985).

Earlier phytochemical screening of the aqueous and methanolic extracts of *S. nodiflora* by Martin-Rathi and Gopalakrishnan (2006) revealed the presence of steroids, reducing sugar, alkaloids, phenolic compounds, tannins and aromatic acid. These substances were also detected during the phytochemical screening of the hydroalcoholic extract of *S. nodiflora* with the alkaloids being present. The analgesic, anti-inflammatory and antioxidant effects of alkaloids and phenolic compounds have been reported in literature (Calixto *et al.*, 2000; Henriques *et al.*, 1996). Thus the potent analgesic effect exhibited by *S. nodiflora* may be related to the presence of these constituents.

In an attempt to investigate into the mode of the analgesic activity of *S. nodiflora*, the effect of the extract was antagonized by naloxone and caffeine. Naloxone, an opioid antagonist, did not block the analgesic action of *S. nodiflora* but significantly blocked the analgesic effect of morphine in both phases of the formalin test. On the other hand, caffeine, a non-specific adenosine receptor antagonist, (Sawynok and Reid, 1996; Fredholm *et al.*, 1994) significantly blocked the analgesic of *S. nodiflora* but had little effect on morphine-induced analgesia. Similarly, caffeine inhibited the *S. nodiflora*-induced analgesia in the writhing test as well. Caffeine is a non-selective adenosine A<sub>1</sub> and A<sub>2</sub> receptor antagonist with comparable affinity at both receptors, but lacks activity at the adenosine A<sub>3</sub> receptor (Daly, 1993; Fredholm *et al.*, 1994). Current animal-based studies have repetitively demonstrated that caffeine enhances the antinociceptive activity of acetaminophen and non-steroidal anti-inflammatory drugs when combined with them and studies have also revealed consistent intrinsic antinociceptive properties in the formalin test (Poon and Sawynok, 1995). However, when administered with morphine, caffeine exhibits both inhibition (De-Lander and Hopkins, 1986; Sweeney *et al.*, 1987; Sawynok and Yaksh, 1993) and enhancement of the antinociceptive action of morphine (Ahlijanian and Takemori, 1985; Person *et al.*, 1985; Misra *et al.*, 1985; Malec and Michalska, 1988). Morphine has been shown to cause the release of adenosine in both *in vivo* and *in vitro* spinal preparations and the adenosine released has been shown to contribute to its anti-nociceptive effect (Sweeney *et al.*, 1987, 1989). Hence, the ability of caffeine to inhibit the antinociceptive effect of morphine reflects antagonism of adenosine. Quite similarly, the ability of caffeine to inhibit the antinociceptive effect of SNE in the formalin and acetic acid induced writhing test indicates that adenosine probably plays a role in the antinociceptive effect of SNE. There is ample evidence to prove that adenosine and its analogs alter pain transmission by actions on both nociceptive afferent and transmission neurons and these actions are principally mediated by adenosine A<sub>1</sub> receptor (Sawynok and Liu, 2003; Sawynok, 1998; Sawynok *et al.*, 1999). Thus the effect of *S. nodiflora* on nociception may probably be due to the release of adenosine or direct activation of adenosine A<sub>1</sub> receptor. Further experiments involving the use of specific agonists and antagonists of A<sub>1</sub> receptors are necessary to confirm this assertion. Furthermore, findings have shown that adenosine A<sub>1</sub> receptor agonists exert anticonvulsant effects within a number of models of epilepsy (Dunwiddie and Worth, 1982; Dragunow, 1990; Khan *et al.*, 2001) and also some anticonvulsants like diazepam and sodium valproate have been found to exhibit their anticonvulsant properties in part through the activation of adenosine receptors (Gupta and Malhotra, 1997; Candenias *et al.*, 1991). Hence, it is not inconceivable to deduce that antinociceptive effect of SNE may probably be linked to adenosinergic mechanisms, which could possibly play a role also in its anticonvulsant effect.

## CONCLUSION

Collectively, the results from this study indicate that the hydroalcoholic extract of the whole plant of *S. nodiflora* exhibits central analgesic effect possibly mediated through adenosinergic mechanism and a peripheral anti-inflammatory activity.

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