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An Evaluation of the Anti-inflammatory, Antipyretic and Antinociceptive Effects of Ficus exasperata (Vahl) Leaf Extract

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Abstract: The hydro alcoholic leaf extract of Ficus exasperata (Vahl) (family Moraceae) (FEE) was evaluated for its antinociceptive, anti-inflammatory and anti-pyretic properties in animal models. The leaf extract (10-300 mg kg^{-1}) showed a dose-dependent anti-inflammatory activity in carrageenan-induced foot oedema in chicks, with an IC_{50} of 46.05±12.3 mg kg^{-1} which was approximately 3.5 times less potent than diclofenac (IC_{50}:13.01±5.28 mg kg^{-1}) and about 130 times less potent than dexamethasone (0.36±0.45 mg kg^{-1}). In the formalin test, the extract showed dose dependent antinociceptive effects in both phases of the formalin test. The role of adenosinergic and opioidergic involvement in the antinociceptive effects was also investigated. While theophylline, a non-selective adenosine receptor antagonist, completely inhibited the antinociceptive effect of the extract, naloxone, an opioid antagonist had very little effect. The extract also showed weak activity in pyrexia induced by baker's yeast. These results suggest antinociceptive as well as anti-inflammatory activities a confirmation of its traditional use. Also, the results show the involvement of adenosinergic pathway in the antinociceptive effects of FEE.

Key words: Formalin, theophylline, naloxone, carrageenan, chicks, mice

INTRODUCTION

Inflammation and pain underlie almost every disease processes and as such the inflammatory response has received a great deal of interest in the field of medical research lately (Kapoor *et al*., 2005). Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including pain and inflammation (Balunas and Kinghorn, 2005; Braddock, 2007). In Ghanaian traditional medicine, various parts of several plants are used either alone or in combination therapy in the treatment of various painful inflammatory conditions.

Ficus exasperata Vahl, (family Moraceae), is a terrestrial afro-tropical shrub or tree that grows up to about 20 m tall and prefers evergreen and secondary forest habitats (Berg, 1989; Berg and Wiebes, 1992). In African traditional medicine, the leaf extract has been used to treat high blood pressure, rheumatism, arthritis, intestinal pains and colics, epilepsy, bleeding and wounds (Irvine, 1961). The roots are also used to manage asthma (Chhabra *et al*., 1990), dyspnoea (Chhabra *et al*., 1990) and venereal diseases. There are some reports on scientific validation of some of the traditional uses of the plant found in literature. Though, *F. exasperata* leaves are reputed to be useful in cancer, it was found to be non-toxic in some bioassays-the brine shrimp lethality test, inhibition of telomerase activity and induction of chromosomal aberrations *in vivo* in rat lymphocytes (Sowemimo *et al*., 2007).

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Similarly Macfoy and Cline (1990) demonstrated that the methanolic and hot and cold aqueous extracts of *F. exasperata* were in inactive against three Gram-negative and three Gram-positive bacteria species: *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Clostridium sporogenes*, *Escherichia coli* and *Staphylococcus aureus*. On the brighter side, the uses of the leaf in hypertension and in ulcers have been validated. A water extract of the leaf showed a dose related reduction in mean arterial blood pressure. The hypotensive effect of the extract was significantly reduced with a prior administration of either atropine or chlorpheniramine, suggesting the probable stimulation of muscarinic receptors in the heart or release of histamine into the circulatory system thereby causing the initial fall in blood pressure (Ayinde *et al*., 2007). Also, the extract had a significant anti-ulcerogenic property in aspirin-induced ulcerogenesis, delayed intestinal transit, increased the pH and decreased both the volume and acidity of gastric secretion. (Akah *et al*., 1998). These findings underscore the need to validate the traditional claims before the acceptance of the plant for general application in practice.

The present study evaluates the anti-inflammatory properties of the extract in carrageenan-induced foot oedema in chicks. Intraplantar injection of carrageenan into the footpad of the 7-day-old chick has been found to elicit a measurable, reliable and relatively short lasting state of oedema that is differentially attenuated by systemic administration of typical anti-inflammatory compounds (Roach and Sufka, 2003). Also, the antinociceptive activity of FEE was investigated in the formalin test in mice (Dubuisson and Dennis, 1977; Wheeler-Aceto *et al*., 1990). The formalin test is predictive of acute pain (Le Bars *et al*., 2001) and a valid model of clinical pain (Costa-Lotufo *et al*., 2004; Vasconcelos *et al*., 2003; Vissers *et al*., 2003, 2006) and hence suitable for the evaluation of analgesics. The yeast-induced hyperthermia in rats described by Tomazetti *et al*. (2005) was also employed to investigate the antipyretic activity of the extract.

MATERIALS AND METHODS

Plant Material

Leaves of *Ficus exasperata* were collected from the campus of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in June, 2007. Leaves were authenticated in the Department of Pharmacognosy of the university where a voucher specimen (No.FP/08/023) has been kept.

Preparation of Extract

The leaves were shade-dried and pulverized in a hammer-mill. One kilogram of the powdered material was cold-macerated with 70% v/v of ethanol overnight. The hydroalcoholic extract was then evaporated to a greenish syrupy mass under reduced pressure in a rotary evaporator, air-dried and kept in a desiccator. Yield obtained was 10% w/w. This is referred to subsequently as the extract or FEE.

Phytochemistry

The presence of saponins, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars and tannins were tested by simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).

Drugs

Morphine hydrochloride and paracetamol were purchased from Phyto-Riker Pharmaceuticals, Accra, Ghana. Theophylline hydrochloride, naloxone hydrochloride and carrageenan sodium salt were from Sigma-Aldrich Inc., St. Louis, MO, USA. Diclofenac sodium was also purchased from Troge, Hamburg, Germany, whilst dexamethasone sodium phosphate was obtained from Pharm-Inter, Brussels.

Animals

Cockerels (Gallus gallus; strain Shaver 579, Akropong Farms, Kumasi, Ghana) were obtained 1-day post-hatch and were housed in stainless steel cages (34×57×40 cm^3) in groups of 12-13 chicks per cage. The chicks had free access to food (Chick Mash, Gafco, Tema, Ghana) and water. Room temperature was maintained at 29°C and an overhead incandescent illumination was maintained on a 12 h light-dark cycle. Chicks were tested at 7 days of age. Group sample sizes of six were utilized throughout the study.

Male Sprague-Dawley rats (150-200 g) and male ICR mice (20-25 g) 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^3) 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.

Carrageenan-Induced Oedema

Carrageenan-induced oedema in the footpad of chicks (Roach and Sufka, 2003) was used with some modifications (Woode *et al*., 2007) to evaluate the anti-inflammatory properties of the extract. Chicks were randomly divided into groups of six. Initial foot volumes were measured by water displacement as described by Fereidoni *et al*. (2000). Inflammation was induced by a subplantar injection of carrageenan (10 μL of a 1% solution in saline) into the right footpad of the chicks. Chicks were then randomly selected to perform one of the following study groups: control (vehicle-treated); FEE (10, 30, 100 and 300 mg kg^{-1}, p.o.); diclofenac (10, 30 and 100 mg kg^{-1}, i.p.) and dexamethasone (0.3, 1.0 and 3.0 mg kg^{-1}, i.p.). Drugs were given 30 min for i.p. route and 1 h for oral route before carrageenan injection. Foot volumes were measured at hourly intervals for 5 h. The oedema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection and at the various time points. The extract was prepared in 2% tragacanth mucilage. Diclofenac and dexamethasone, a non-steroidal and steroidal drug respectively, were used as positive controls.

Antipyretic Test

Rectal temperature (T_R) was recorded by inserting a lubricated digital thermometer (external diameter: 3 mm, 0.1°C precision) 2.8 cm into the rectum of rats. Animals presenting initial rectal temperature between 36 and 37°C were selected for the antipyretic tests. The effect of drugs on baker's yeast-induced hyperthermia were then determined as previously described by Tomazetti *et al*. (2005). After measuring basal T_R of the animals, they were injected with a pyrogenic dose of baker's yeast (0.135 g kg^{-1}). T_R changes were recorded every hour up to 4 h. Animal were divided into groups of five animals each. FEE (30, 100, 300 mg kg^{-1}; p.o.) and vehicle (control group) were administered and the T_R monitored over the following 8 h period. The antipyretic, paracetamol (10, 30, 100 mg kg^{-1}, p.o.) served as a positive control. Basal rectal temperature and changes in rectal temperature were expressed as Means±SEM.

Formalin-Induced Nociception

The formalin test was carried out as previously described (Dubuisson and Dennis, 1977; Malmberg and Yaksh, 1992) with a few modifications. Mice were randomly divided into groups of five

for the following treatments: FEE (10, 30, 100 and 300 mg kg^{-1}, p.o.), morphine (1, 3, 10 mg kg^{-1}, i.p.) as positive control and saline-treated group. Each animal was assigned and acclimatized to one of twenty Perspex test chambers (15×15×15 cm^3) for thirty minutes before the start of the experiment. 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 a 45° angle beneath the chambers to allow an unobstructed view of the hind paws. The behaviour 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.

A second set of experiments were carried out to determine the effect of naloxone (an opioid antagonist) and theophylline (a non-selective adenosine antagonist) on the actions of FEE and morphine. Naloxone (1 mg kg^{-1}) or theophylline (10 mg kg^{-1}) was administered intra-peritoneally 30 min before the extract (100 mg kg^{-1}) or morphine (3 mg kg^{-1}).

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 amount of 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). 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.

Data Analysis

Raw scores for right foot volumes were individually normalized as percentage of change from their values at time 0 and then averaged for each treatment group. The time-course curves were subjected to two-way (treatment × time) repeated measures Analysis of Variance (ANOVA) with Bonferroni's post hoc test. Total oedema, total change in R_T and total 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.

$$\text{inhibition}\,(\%) = \left(\frac{AUC_{control} - AUC_{treatment}}{AUC_{control}}\right) \times 100$$

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

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

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

RESULTS AND DISCUSSION

Phytochemistry

General tests showed the presence of tannins, pseudotannins, saponins and alkaloids.

Carrageenan-Induced Oedema

Injection of carrageenan into the foot of chicks caused an increase in foot volume (oedema) which was maximal by 2 h and persisted, slowly declining, for the duration of the experiment (Fig.1a-f). FEE (10, 30, 100 and 300 mg kg^{-1}) administered orally, dose-dependently and significantly [$F_{4, 100}$=4.07; p = 0.0143; two-way ANOVA (treatment group × time)] reduced the increase in foot volume induced

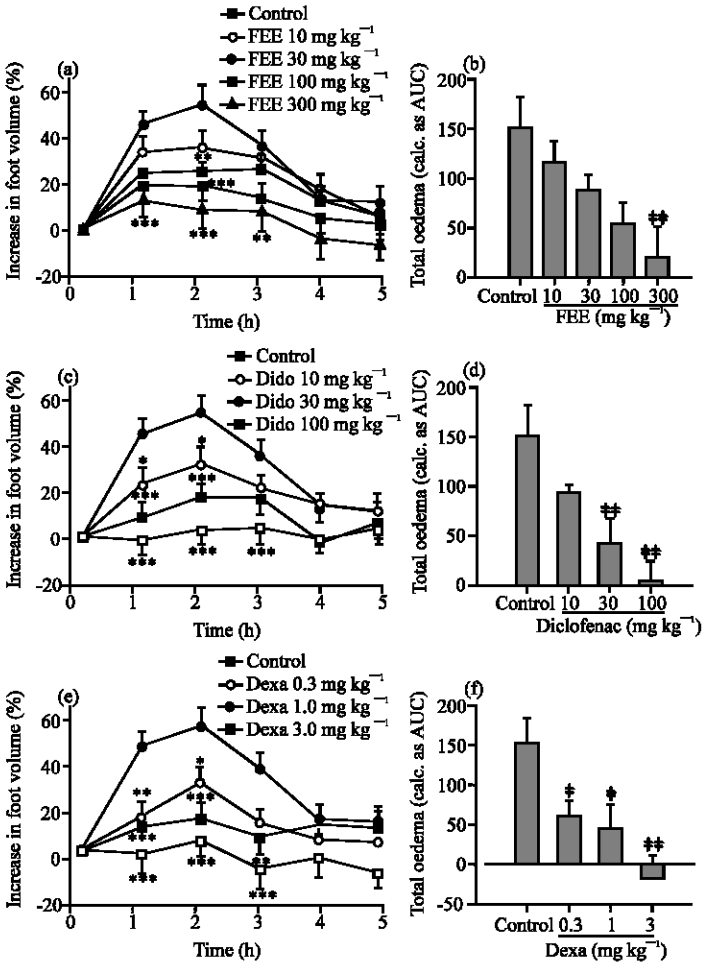

Fig. 1: Dose-response effects of FEE (30-300 mg kg^{-1}, p.o.) (a and b); diclofenac (10-100 mg kg^{-1}, i.p.) and (e, f) dexamethasone, Dexa (0.3-3 mg kg^{-1}, i.p.) on carrageenan-induced foot oedema in chicks. Left panels show the time course of effects and the right panels show the total oedema calculated as AUCs over the 5 h period. Data are Mean±SEM (n = 6). ***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<0.01; ‡p<0.05 compared to vehicle-treated group (one-way ANOVA followed by Newman-Keul's post hoc test)

by injection of carrageenan (Fig. 1a). Furthermore, when total oedema over the period of the experiment is represented in arbitrary units as AUC of the time-course curves (Fig. 1b), FEE produced a significant [$F_{4,20}$= 4.39; p = 0.0104] and dose-dependent decrease in total oedema. Similarly, the NSAID, diclofenac reversed the increase of foot volume on carrageenan injection dose-dependently [$F_{3,80}$= 6.80; p = 0.0036; two-way ANOVA (treatment group × time); Fig. 1c] and thus also the total increase in foot volume [$F_{4,20}$= 8.1; p = 0.0017; Fig. 1d]. The effects of dexamethasone (Fig. 1e, f) were comparatively much greater than the extract and diclofenac. Dexamethasone very significantly [$F_{3,80}$= 5.73; p = 0.0073; two-way ANOVA (treatment group × time); Fig. 1e] decreased the carrageenan-induced oedema. At the highest dose administered (3 mg kg^{-1}), dexamethasone completely prevented the increase in foot volume.

Dose-response curves for the inhibition of foot oedema are shown in Fig. 2. FEE (IC_{50}: 46.05±12.63 mg kg^{-1}) was approximately three and a half times less potent than diclofenac (IC_{50}:13.01±5.28 mg kg^{-1}; $F_{1,33}$ = 6.24; p = 0.0177) and about 130 times less potent than dexamethasone (0.36±0.45 mg kg^{-1}; $F_{1,33}$ = 82.47, p<0.0001).

Antipyretic Test

Intraperitoneal injection of yeast caused a steady incremental change in rectal temperature of rats which peaked at about 5 h (Fig. 3). Whereas paracetamol produced a sustained inhibition of pyrexia, the extract (30-300 mg kg^{-1}, p.o.) produced only a slight inhibition of pyrexia (2 h) occurring between 5th and 7th h ($F_{4,120}$= 37.57, p<0.0001, Fig. 3a) after which a gradual rise in temperature was observed. The AUC shows the extract has some antipyretic activity only at doses of 100 and 300 mg kg^{-1} (Fig. 3b). On the other hand, administration of the antipyretic paracetamol (10-100 mg kg^{-1}; p.o) at 4 h, significantly attenuated the change in rectal temperature ($F_{4,120}$= 84.31, p<0.0001) in a dose-dependent manner as depicted in the time course curves and AUCs (Fig. 3c, d).

Formalin Test

Formalin induced a clear nociceptive response exhibited as biting or licking of the injected paw. The nociceptive response was biphasic as previously described (Le Bars *et al*., 2001; Saddi and Abbott, 2000; 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 (Hayashida *et al*., 2003; Wang *et al*., 1999).

Figure 4 shows the effect of pre-treatment with FEE and morphine on formalin-induced pain in mice. Generally, responses to pain (defined by pain scores) were lower in the drug-treated groups than

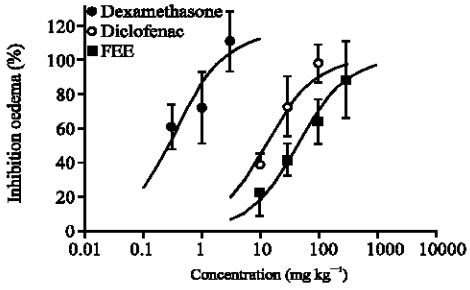

Fig. 2: Dose response curves for FEE (30-300 mg kg^{-1} p.o.), diclofenac (10-100 mg kg^{-1} i.p.) and dexamethasone (0.3-3.0 mg kg^{-1} i.p.) on carrageenan-induced foot oedema in chicks

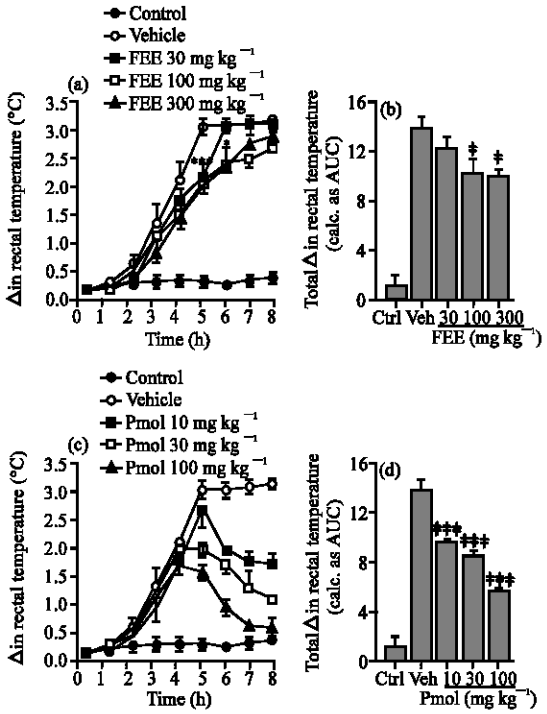

Fig. 3: Dose-response effects of FEE (30-300 mg kg^{-1}, p.o.) (a and b) and paracetamol, Pmol (10-100 mg kg^{-1}, p.o.) (c and d) on baker's yeast-induced changes of rectal temperatures in rats. Left panels show the time course of effects and the right panels show the total change in temperature calculated as AUCs over the over the 8 h period. Control (Ctrl) represents naive animals (no treatment with yeast). Data are Mean±SEM (n = 5). ***$p<0.001$; *$p<0.05$ 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, (one-way ANOVA followed by Newman-Keul's post hoc test)

Table 1: ED_{50} values for the effect of the FEE and morphine in the formalin test

	ED_{50} (mg kg^{-1})	
Drug	Phase 1	Phase 2
FEE	187.00±49.75	34.08±6.50**
Morphine	0.84±0.27	2.72±1.05***

Values are Mean±SEM. (n = 5). ***$p<0.001$, **$p<0.01$ compared to respective phase 1 values (F-test)

the vehicle-treated group as shown by the time course curves (Fig. 4a, c). Oral administration of FEE (30-300 mg kg^{-1}) 30 min before the injection of formalin significantly and dose-dependently inhibited both first and second phases of formalin-induced paw licking and biting [$F_{3,21}$= 10.15; p = 0.0002 and $F_{3,210}$= 11.72; p = 0.0001 respectively, two-way ANOVA (treatment group × time)] (Fig. 4a). Analysis of the AUCs showed that FEE attenuated formalin-induced pain/behaviours by 14.87-66.06% and 52.03-76.86% in the early and late phases respectively (Fig. 4b). Morphine, the positive analgesic control, was also effective in the formalin test (Fig. 4c, d). Intraperitoneal administration of morphine (1-10 mg kg^{-1}, i.p.) dose-dependently decreased the nocifensive behaviors induced by formalin in the first and second phases [$F_{3,21}$= 18.86; p = 0.0001 and $F_{3,210}$= 3.4; p = 0.0366 respectively, two-way ANOVA (treatment group × time)] (Fig 4c). Percentage inhibitions

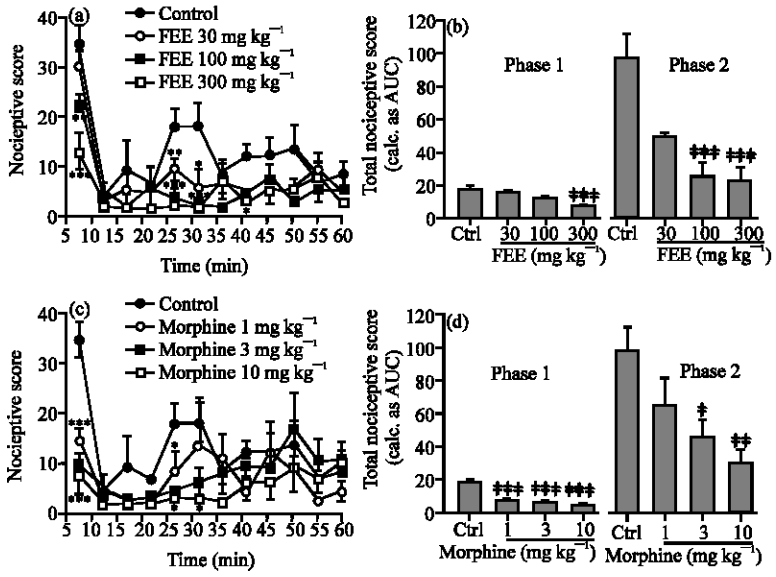

Fig. 4: Dose-response effects of FEE (30-300 mg kg^{-1}, p.o.) (a and b) and morphine (1-10 mg kg^{-1}, i.p.) (c and d) on formalin-induced nocifensive behaviors in mice. Left panels show the time course of effects over the 60 min period and the right panels show the total nociceptive score calculated 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 Mean±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)

as shown by the AUCs were 63.32-83.66% in the first phase and 32.60-66.32% in the second phase (Fig. 4d). Comparison of ED_{50}s obtained by non-linear regression (Table 1), revealed that the extract was more potent in the second phase ($F_{1,34}$ = 25.75, p<0.0001) than the first. In contrast, morphine was three fold more potent in the first phase compared to the second phase ($F_{1,34}$= 6.722, p = 0.0139). Figure 5 shows the effect of naloxone pre-treatment on the inhibitory effects of FEE and morphine on formalin-induced nocifensive behaviours. Naloxone (1 mg kg^{-1}) injected 30 min before formalin did not have any significant effects on the anti-nociceptive actions of the extract in both phases (Fig. 5a, b). Though, naloxone slightly blocked the effect of the extract, this was not statistically significant. By contrast, naloxone completely reversed the inhibitory effects of morphine in both phases (Fig. 5c, d).

Effects of treatment of non-selective adenosine inhibitor, theophylline is shown in Fig. 6. Theophylline (10 mg kg^{-1}) completely reversed the effects FEE (Fig. 6a, b) but did not have any effects on inhibitory effects of morphine (Fig. 6c, d).

This present study evaluated the anti-inflammatory, antinociceptive and anti-pyretic properties of the leaf extract of Ficus exasperata in experimental animals.

The result shows FEE has anti-inflammatory activity in the carrageenan-induced foot oedema in chicks. Carrageenan-induced local oedema is a standard experimental model of acute inflammation which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Roach and Sufka, 2003). In the present study, chicks were used instead of the commonly used rodents. Carrageenan-induced oedema has been validated in the chicks by (Roach and

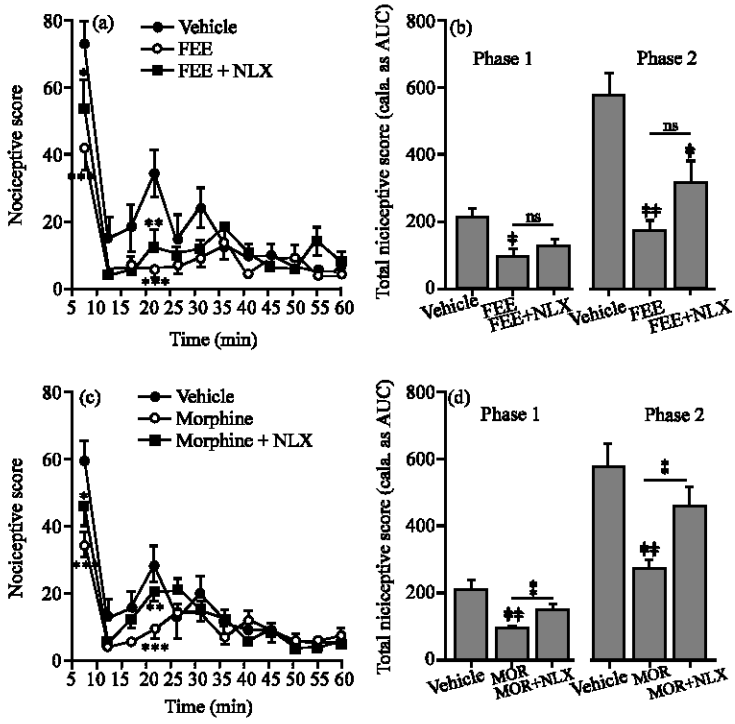

Fig. 5: Time course curve effects of (a) FEE (100 mg kg^{-1}, p.o.) + naloxone, NLX (1 mg kg^{-1}, i.p), (c) Morphine, MOR (3 mg kg^{-1}, i.p.) + NLX (1 mg kg^{-1}, i.p) and their total nociceptive score in (b) phase 1 and (d) phase 2 of formalin test in mice. Nociceptive scores are shown in 5 min time blocks up to 60 min post formalin injection for the time course curves. Values are Mean±SEM. (n = 5). ns: Not significant, **p<0.01; *p<0.05 compared to vehicle-treated group (two-way ANOVA followed by Bonferroni's post hoc test). ‡‡p<0.01; ‡p<0.05 compared to vehicle-treated group (one-way ANOVA followed by Newman-Keul's post hoc test)

Sufka, 2003) and is much more economical than rodent models. Also, chicks are easier to handle. Studies have shown that intraplantar injection of carrageenan in the 7-day-old chick elicits a measurable, reliable and relatively short-lasting state of oedema, that is differentially attenuated by the systemic administration of typical anti-inflammatory compounds (Roach and Sufka, 2003) and compares favourably with the more commonly used rodent models (rat and mice) in the screening of drugs with anti-inflammatory activities. Carrageenan-induced oedema is a biphasic event (Vinegar *et al*., 1969). The initial phase of inflammatory response (0-1 h), which is not inhibited by NSAIDs such as indomethacin or aspirin, has been attributed to the release of histamine, 5-hydroxytryptamine and bradykinins (Crunkhorn and Meacock, 1971). This is followed by a complement-dependent late phase (1-6 h) sustained mainly by prostaglandin release and has been attributed to the induction of COX-2 in tissue (Di Rosa *et al*., 1971). The second phase is sensitive to most clinically effective anti-inflammatory drugs (Di Rosa *et al*., 1971; Vinegar *et al*., 1969). The extract FEE, inhibited the oedema from the first hour, acting in both the early and later phases of inflammation. This indicates the extract could inhibit various chemical mediators involved in both the early and later phases (histamine, serotonin, bradykinin, prostaglandins). The extent of inhibition of foot oedema by FEE was less than the standard anti-inflammatory drugs diclofenac and

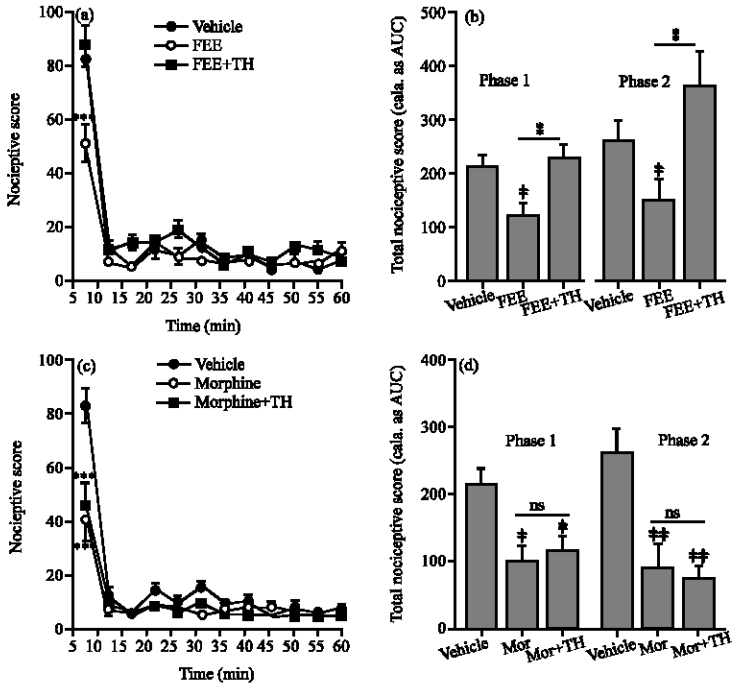

Fig. 6: Time course curve effects of (a) FEE (100 mg kg^{-1}, p.o.) + theopylline, TH (10 mg kg^{-1}, i.p), (c) Morphine, MOR (3 mg kg^{-1}, i.p.) + TH (10 mg kg^{-1}, i.p) and their total nociceptive score in (b) phase 1 and (d) phase 2 of formalin test in mice. Nociceptive scores are shown in 5 min time blocks up to 60 min post formalin injection for the time course curves. Values are Mean±SEM. (n = 5). ns: Not significant, ** $p<0.01$; *$p<0.05$ compared to vehicle-treated group (two-way ANOVA followed by Bonferroni's post hoc test). ‡‡$p<0.01$; ‡$p<0.05$ compared to vehicle-treated group (one-way ANOVA followed by Newman-Keul's post hoc test)

dexamethasone. It is however not surprising since the crude extract contains principles other than the anti-inflammatory principle(s). For example, it has been shown in some instances that complex interaction may occur between constituents of plants, which then produces both synergistic and antagonistic responses between the various components (Savelev *et al*., 2003).

The formalin test is one of the most predictive of acute pain (Le Bars *et al*., 2001) and a valid model of clinical pain (Costa-Lotufo *et al*., 2004; Vasconcelos *et al*., 2003; Vissers *et al*., 2003). It is a widely accepted method for the rapid and easy screening of pharmacological targets in drug evaluation (Saddi and Abbott, 2000; Vissers *et al*., 2003).

Intraplantar injections 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 (Le Bars *et al*., 2001; Szolcsanyi *et al*., 2004) that corresponds to acute neurogenic pain which is sensitive to central analgesics. The late phase involves inflammatory components with the release of different pain mediating substances sensitive to NSAIDs (Le Bars *et al*., 2001; Malmberg and Yaksh, 1992; Yashpal and Coderre, 1998) corticosteroids (Vasconcelos *et al*., 2003) as well as analgesics with central effects.

The analgesic effect of morphine and FEE in both phases is characteristic of centrally acting analgesics. This means FEE has central analgesic effect as well as anti-inflammatory analgesic activity. The traditional use of FEE in the control of convulsion/epilepsy further supports a possible central

analgesic effect since some anti-convulsants/anti-epileptics used in the management of chronic pain have shown analgesic activity in the formalin test (Laughlin *et al*., 2002; Vissers *et al*., 2003). The analgesic effect of morphine is mediated via the activation of opioid receptors which is also linked to the adenosine system (Mantegazza *et al*., 1984; Ribeiro *et al*., 2002; Sawynok and Liu, 2003). Naloxone blocked the antinociceptive effect of morphine as expected in this study. Theophylline, however, did not block morphine antinociception as reported by Mantegazza *et al*. (1984), Ribeiro *et al*. (2002) and Sawynok and Liu (2003). This may be accounted for by various reasons including the type of species used (Malec and Michalska, 1990), the doses of theophylline used (Sawynok, 1998) as well as pharmacokinetic factors (Misra *et al*., 1985). Although, the inhibition of the anti-nociceptive effect of FEE by naloxone was statistically insignificant to implicate the involvement of opioid receptors, the complete antagonism of its analgesic effect by theophylline suggests the involvement of the adenosine system. Adenosine interacts with at least four P1 receptor subtypes coupled with G protein, namely A_1, A_{2a}, A_{2b} and A_3 (Fredholm *et al*., 2001). These receptors can influence nociception at peripheral, spinal and supraspinal sites and affect nociceptive, inflammatory and neuropathic pain states (Dickenson *et al*., 2000; Sawynok, 1998; Sawynok and Liu, 2003). Current evidence shows that A_1 receptor activation produces antinociception while activation of A_{2a} and A_3 mediates pronociceptive actions in rodents (Sawynok, 1998; Sawynok *et al*., 1998). The effect of FEE on the adenosine system may involve the activation or antagonism of any of adenosine receptors. However, further studies are needed to confirm this assertion.

Although, the extract showed analgesic and anti-inflammatory activity, its weak anti-pyretic effect occurring at 100 and 300 mg kg^{-1} is not sufficient to infer anti-pyretic activity. Further studies with higher doses may be necessary confirm its antipyretic activity. Fever is a result of a finely tuned, complex event that involves both the peripheral immune system and the brain, through which a series of inflammatory and metabolic processes are regulated (Inoue *et al*., 2008; Roth *et al*., 2006) and it now commonly accepted that prostaglandin E_2 (PGE_2) is the final fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus (Li *et al*., 2008), thus it may be plausible to conclude that the extract inhibits the synthesis of prostaglandins, albeit to a very little extent.

Though the present study did not investigate the exact chemical responsible for the actions described, the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins (Ayinde *et al*., 2007; Ijeh and Ukwemi, 2007) in the leaf may account for pharmacological effects in this study. Alkaloids were present in the sample used in this as confirmed by the work Ijeh and Ukwemi (2007).

In conclusion, *Ficus exasperata* leaf extract used in this study, possess analgesic, anti-inflammatory activity and weak antipyretic activity. This validates its traditional uses in the management of pain and inflammatory conditions.

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