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HEXANIC FRACTION OF TURMERIC POWDER ATTENUATES MURINE MODEL OF INDUCED-NOCICEPTION AND ITS POSSIBLE MECHANISMS OF ACTION

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Abstract

Background: The current study was conducted to further examine the antinociceptive activity of hexane fraction of turmeric powder (HFTP) and to elucidate the possible mechanisms of action underlying its antinociceptive activity in various experimental models of chemical- and thermal-induced nociception.
Materials and Methods: Acetic acid-induced abdominal constriction, hot-plate, formalin-, capsaicin- and glutamate-induced paw licking tests in mice were employed in the antinociceptive investigation of HFTP. In all experiments, HFTP was administered intraperitoneally at the doses of 0.1, 0.5, 1.0 and 5.0 mg/kg. In a separate group of experiments, the possible sedative and toxic effects of HFTP were tested in rota rod and preliminary acute toxicity tests, respectively.
Results: It was demonstrated that HFTP exerted significant dose-dependent antinociceptive responses in the acetic acid-induced abdominal constriction, hot-plate, formalin-, capsaicin- and glutamate-induced paw licking tests. It was also demonstrated that pretreatment with naloxone, produced no significant effect on the antinociception induced by HFTP. Moreover, administration of HFTP shows no significant interference in locomotor activity of the rota rod test, and in the preliminary acute toxicity test, neither abnormal behaviours nor mortality were observed.
Conclusion: Together, these results indicated that HFTP-induced antinociceptive activity at doses devoid of any detectable toxicity and sedative effects exerts pronounced peripheral and central antinociceptive effects, with no involvement of opioidergic system but possibly related to its ability to interact with TRPV1 receptors and the glutamatergic system.

Keywords: *Curcuma longa* L., Antinociceptive, TRPV1, Glutamatergic.

Introduction

The most common clinical treatment for a wide range of pain and inflammation conditions is the use of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. Despite its effectiveness, the use of NSAIDs and opioids has been known to cause numerous adverse effects (Wallace et al., 2008). Hence, there is a need for safer and more effective analgesic alternatives. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Natural products of plant origin that present minimal side effects are emerging as an interesting therapeutic resource for the development of new drugs in the management of inflammation and pain conditions (Mattison et al., 1988).

Curcuma longa Linn. (Family: Zingiberaceae), or locally known in Malaysia as 'kunyit', is one of the most important perennial herbs of the ginger species that are native and widely cultivated in most regions of South Asia (Goel et al., 2008). *C. longa* powder also known as turmeric powder, which is obtained by grinding its dried rhizomes, commonly used as a condiment for flavouring food, food preservative, source of certain colouring agents, and as an ingredient in cosmetic preparations (Ammon et al., 1991). Apart from its widespread commercial uses, turmeric powder holds great value as a health aid specifically in traditional folk medicine and aromatherapy (Joshi et al., 2009). Turmeric powder has long been used as a treatment by indigenous communities for gastrointestinal disturbances, skin diseases, anorexia, rheumatism and sinusitis (Ammon et al., 1992). Turmeric powder is also applied to cuts and wounds as an antiseptic as well as to stop bleeding (Leung, 1980). Pharmacologically, *C. longa* has been reported to exhibit significant antioxidant, anti-inflammatory, antinociceptive, anti-hepatotoxic, anti-bacterial, antimutagenic, anti-carcinogenic and insect repellent activities (Aratanechemuge et al., 2002; Jayaprakasha et al., 2002; Kiso et al., 1983; Liju et al., 2011; Norajit et al., 2007; Singh et al., 2002).

In spite of the extensive use of turmeric powder in folk medicine against various pain-related disorders, there is still lack of substantial pharmacological evidence of the antinociceptive effect of the turmeric powder. Therefore, the present study was conducted to evaluate the antinociceptive activity of hexanic fraction of turmeric powder (HFTP) and to elucidate its possible mechanisms of action.

Materials and methods

Preparation of the Hexanic Fraction of Turmeric Powder (HFTP)

The rhizomes of *C. longa* were purchased from the Serdang local market, Selangor, Malaysia and were identified by Dr. Shamsul

Khamis, a resident botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia, (UPM), Serdang, Selangor, Malaysia. A voucher specimen (SK 202/11) was deposited at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia. A small part of the rhizomes were cultivated in the Medicinal Plant Garden, Institute of Bioscience, Universiti Putra Malaysia for future reference. The rhizomes were cut into thin slices, air-dried for 5 days and then powdered to produce turmeric powder (1.5 kg). The dry powder was dissolved and shaken occasionally with distilled methanol for three days at room temperature. The extract was then filtered and the filtrate was dried using a rotary evaporator under reduced pressure to obtain about 57.6 g extract. The dried extract was then dissolved in distilled water and subjected to solvent-solvent extraction by using distilled hexane in a separating funnel. Upon evaporation of solvent on rotary evaporator, a hexanic fraction from methanolic extract about 10.7 g was obtained. The hexanic fraction was finally passed through silica gel column chromatography to remove the suspended particles and eluted with 100% hexane and then 2% ethyl acetate / hexane. As a result of column chromatography, a light brown hexanic sub-fraction of turmeric powder (10.3 g) was subjected to animal studies. The same extract was also subjected for GC-MS analysis.

Chemical Analysis

The chemical composition of HFTP was analysed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph (Model Agilent 7890A) directly coupled to a mass spectrometer system (Model Agilent 5975C inert MSD) with a triple-axis detector. A fused-silica capillary column AB-5MS (5% phenyl methylpolysiloxane; length 30 m x inner diameter 0.25 mm x film thickness 0.25 µm) was employed with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The column temperature was programmed as follows: at 60 °C for 1 min; then increased from 60 °C to 185 °C at the rate of 1.5 °C/min and held at 185 °C for 1 min; then again increased from 185 °C to 300 °C at the rate of 9 °C/min and held at 300 °C for 20 min. For GC-MS detection, electron ionization (EI) system was used with ionization energy of 70 eV. The MS scan parameters included a mass range of 40 to 600 amu, a scan interval of 0.5 s, a scan speed of 1000 amu/s, and a detector voltage of 1.5 kV. The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. The percentage composition of the extract was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and mass spectra with computer mass spectra libraries (NIST/EPA/NIH version 5.0).

Animals

Experiments were conducted using male ICR mice (20–-30 g) supplied by the animal house unit at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The animals were housed and maintained on a 12-h light/dark cycle. Standard laboratory food and tap water were made available *ad libitum* except during the experimental procedures. Animals were acclimatized and habituated to the laboratory environment for at least one week prior to the experiments and were used only once throughout the study. All experiments reported in this study adhered to the current guidelines for the care of laboratory animals and the ethical guidelines for experimental pain investigations in conscious animals (Zimmermann, 1983), approved by the Animal Care Unit Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (ACUC_UPM/FPSK/PADS/BR-UUH/00425). All efforts were made to minimize the number of animals and the intensity of noxious stimuli used to the level required to demonstrate consistent effects of the treatment. Each experimental group in the present study consisted of six animals.

Drugs and Chemicals

The following reagents and drugs were used: Tween 20, absolute ethanol (100%), formalin, acetic acid, morphine hydrochloride, acetylsalicylic acid (ASA), naloxone, diazepam, capsaicin, capsazepine and glutamate (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Morphine hydrochloride, acetylsalicylic acid, diazepam and naloxone were dissolved in physiological saline (0.9% NaCl), while capsaicin and glutamate were dissolved in phosphate buffered saline (PBS). HFTP was dissolved in vehicle (absolute ethanol: Tween 20: distilled water; 5:5:90; v/v) to the desired concentration. Respective controls received only the vehicle. All drugs and HFTP were prepared immediately before experiments were carried out and administered intraperitoneally (i.p.) at doses of 0.1, 0.5, 1 and 5 mg/kg, in a volume of 10 ml/kg unless otherwise stated. The vehicle had no effects *per se* on nociceptive responses. In all experiments, the experimenter was blinded to the treatment of the animals.

Acetic Acid-Induced Abdominal Writhing Test

The acetic acid-induced abdominal writhing test was performed as previously described (Mohamad et al., 2010). Briefly, 0.6% acetic acid (10 ml/kg) was injected intraperitoneally, and the abdominal writhing response, which consisted of a mild contraction and elongation of the abdominal wall, accompanied by bilateral stretching of the hind limbs, was counted over a period of 30 min beginning from 5 min immediately after acetic acid injection. HFTP (0.1, 0.5, 1 and 5 mg/kg, i.p.), vehicle (10 ml/kg, i.p.) or the reference drug, acetylsalicylic acid (ASA, 100 mg/kg, i.p.) were administered 30 min before the acetic acid injection **(n = 6)**. Antinociceptive activity was expressed as the percentage inhibition of the number of abdominal writhing between control and treatment groups.

Formalin-Induced Paw Licking Test

The procedures in the formalin-induced paw licking test were similar to those previously described (Mohamad et al., 2010). Animals **(n = 6)** were pretreated with HFTP (0.1, 0.5, 1 and 5 mg/kg, i.p.) 30 min before the injection of formalin. Control animals received only the vehicle (10 ml/kg, i.p.) while ASA (100 mg/kg, i.p.) and morphine (5 mg/kg, s.c.) were used as reference drugs. After 30 min, 20 µl of 2.5% formalin was injected into the right hind paw of mice, intraplantarly. The amount of time the animals spent licking the injected paw was recorded for 30 min after formalin injection whereby the first 5 min (first phase) and 15–30 min (second phase) were represented as neurogenic and inflammatory pain, respectively.

Hot-Plate Test

The hot-plate test was conducted to measure response latencies, according to a previously described method with slight modifications (Mohamad et al., 2010). The mice **(n = 6)** were treated with vehicle (10 ml/kg, i.p.), HFTP (0.1, 0.5, 1 and 5 mg/kg, i.p.) or morphine (5 mg/kg, s.c.) and placed individually on a hot-plate (Ugo Basile, Model 7280, Italy) maintained at 55 ± 0.2°C. All substances were administered 30 min before the beginning of the experiment. The time between placement of the animal on the heated surface of the hot-plate and the occurrence of licking of the hind paws, shaking the paw or jumping off the surface was recorded as response latency. All mice were observed before (0) and 30, 60, 120 and 180 min after substance administration. In order to avoid tissue injury to the animal paw, the cut-off time for response latency was taken as 20 s. Mice in this experiment were selected 24 h before the experiment, and only those showing response latency within the range of 5-8 s were used for the experiment.

Involvement of Opioid Receptors

The possible involvement of the opioidergic system in HFTP-induced antinociception was determined using the formalin-induced paw licking and hot-plate tests as previously described (Mohamad et al., 2010; Sulaiman et al., 2009). Separate groups of animals **(n = 6)** were pre-treated with a non-selective opioid receptor antagonist, naloxone (5 mg/kg, i.p.), which was administered 15 min prior to administration of HFTP (1 mg/kg, i.p.) or morphine (5 mg/kg, s.c.).

Capsaicin-Induced Paw Licking Test

The procedures used in the capsaicin-induced paw licking test were similar to of previously described (Ong et al., 2011). This test was carried out to determine whether HFTP antagonizes capsaicin-induced pain in the mouse paw. Animals **(n = 6)** were pre-treated (i.p.) either with vehicle (10 ml/kg), capsazepine (a TRPV1 antagonist; 0.17 mmol/kg) or HFTP (0.1, 0.5, 1 and 5 mg/kg) 30 min before a 20 µl capsaicin injection (1.6 µg/paw). Animals were then observed individually for 5 min after injection of capsaicin in the intraplantar surface of the right hind paw. The amount of time the animals spent licking and biting the injected paw was considered as nociception and recorded with a chronometer.

Glutamate-Induced Paw Licking Test

In order to evaluate the possible interaction between HFTP and the glutamatergic system, the glutamate-induced paw licking test was employed according to a previously described method (Perimal et al., 2011). Animals **(n = 6)** were treated with vehicle (10 ml/kg; i.p.) or HFTP (0.1, 0.5, 1 and 5 mg/kg; i.p.) 30 min before glutamate injection. A volume of 20 µl of glutamate (10 µmol/paw; in PBS solution) was injected subcutaneously into the plantar surface of the right hind paw of mice. Mice were observed individually for 15 min following glutamate injection. The amount of time animals spent on licking or biting the injected paw was recorded with a chronometer and considered as an indication of nociception

Rota-Rod Test

Evaluation of possible non-specific sedative effects of HFTP was conducted by the rota-rod test (Ong et al., 2011). Briefly, mice **(n = 6)** were placed on a rota-rod apparatus (Model 7600; Ugo Basile, Comerio VA, Italy), which consisted of a bar with a diameter of 3 cm, and subdivided into five compartments. The mice were subjected to pre-selection 24 h prior to experiments. Mice that remained successfully on the rota-rod at a fixed speed of 20 rpm for 120 s without falling were selected. On the day of the experiments, motor performance was evaluated 30 min after treatment with vehicle (10 ml/kg, i.p.), HFTP (5 mg/kg, i.p.) or diazepam (4 mg/kg, i.p.), and the amount of time of permanence(s) on the revolving bar during a 120 s period was recorded.

Acute Oral Toxicity Study

The method previously described was employed with minor modification to explore the toxic effects of HFTP (Lorke, 1983). A single oral dose of HFTP (5 g/kg) was administered to a group of six mice while the control group only received the vehicle (10 ml/kg; p.o.). Behavioural parameters such as convulsion, hyperactivity, amount of food and water intake, loss of weight, loss of righting reflex and increased or decreased respiration were observed for 1, 4 and 24 h after treatment administration. The mice were further observed for 7 days for any signs of toxicity and mortality.

Statistical Analysis

The data obtained were expressed as means ± S.E.M. for six mice in each group. The statistical analysis was estimated by one-way ANOVA followed by Dunnett's multiple comparison tests for all experiments, except hot-plate test, which was analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test. Differences between means were considered as statistically significant at $P < 0.05$. When appropriate, the ED_{50} values (dose of extract producing a 50% reduction in nociceptive response as compared to the control value), and 95% confidence interval limits (CI) values in the test models were determined by linear regression using GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, CA, USA).

Results

Chemical Analysis

The results of GC-MS analysis on the HFTP sample used in the current studies indicated the presence of 17 compounds contributing

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83.41% of the extract were identified as shown in Table 1. The main components of the extract was ar-Tumerone (44.21%) followed by curlone (19.01%), linoleic acid (4.30%), sesquiphellandrene (4.16%) and ar-Curcumene (3.03%).

Acetic Acid-Induced Abdominal Writhing Test

The results illustrated in Figure 1 shows that i.p. administration of HFTP (0.1, 0.5, 1 and 5 mg/kg) exerted significant dose-dependent inhibition of the number of abdominal writhings with 17.38, 41.84, 88.48 and 99.65% of inhibition as compared to control, respectively. The calculated mean ED_{50} value (and its respective 95% confidence limits) for HFTP in this model was 0.62 mg/kg (0.58 - 0.67 mg/kg). In contrast, ASA (100 mg/kg, i.p.) produced an inhibitory effect with 38.12% of inhibition as compared with the control group.

Table 1: The main constituents of hexanic fraction of turmeric powder (HFTP).

Compound	Retention time(min)	Peak area (%)
ar-Curcumene	43.385	3.03
alpha-Zingiberene	44.320	0.27
beta-Bisabolene	45.344	0.62
Sesquiphellandrene	46.559	4.16
Caryophyllene oxide	50.262	0.24
α-Turmerone	50.434	1.18
alpha-Bisabolol	53.112	0.44
ar-Turmerone	57.432	44.21
alpha-2-epi-Funebrene	58.851	0.65
Curlone	59.811	19.01
Terpinolene	66.714	0.61
Artemisia ketone	66.809	0.88
Palmitic acid	77.771	0.57
3-Decen-5-one	78.083	1.16
Linoleic acid	87.658	5.13

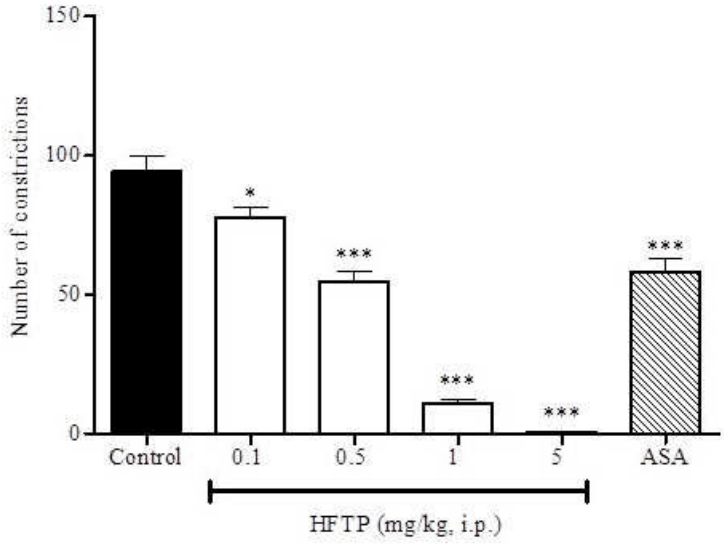

Figure 1: Effect of HFTP in the acetic acid-induced abdominal constriction test in mice. Each column represents the mean ± S.E.M. of six mice. **P < 0.05, ***P < 0.001* compared with control (C) group (one-way ANOVA followed by Dunnett's *post hoc* test). ASA: acetylsalicylic acid.

Formalin-Induced Paw Licking Test

The results illustrated in Figure 2 shows that HFTP (0.1, 0.5, 1 and 5 mg/kg; i.p.) had significant analgesic effects on both the first (0-5 min) and second phase (15-30 min) of formalin-induced nociception. At all doses administered, HFTP elicited greater inhibition of pain in the second phase than the first phase. The calculated mean ED_{50} value (and its respective 95% confidence limits) for HFTP in the first phase and second phase were 0.70 mg/kg (0.60 - 0.83 mg/kg) and 0.86 mg/kg (0.69 - 1.08 mg/kg), respectively. ASA (100 mg/kg, i.p.) caused significant inhibition (42.98%) on the second phase, but not the first phase of the formalin-induced pain model. In contrast, morphine (5 mg/kg, s.c.) significantly reduced the nociceptive response in both phases.

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Hot-Plate Test

As shown in Table 2, HFTP (0.1, 0.5, 1 and 5 mg/kg; i.p.) significantly increased the latency time to a nociceptive response in the hot-plate test. A marked increase in the latency period was observed at 60 until 180 min for the doses of 1 and 5 mg/kg. The calculated mean ED_{50} value (and its respective 95% confidence limits) for HFTP at 120 min was 0.62 mg/kg (0.33 - 1.16 mg/kg).

Involvement of the Opioid Receptors

In formalin and hot-plate test, pre-treatment with the non-selective opioid receptor antagonist, naloxone (5 mg/kg, i.p.) 15 min beforehand did not reverse the anti-nociception caused by HFTP (1 mg/kg, i.p.). In contrast, naloxone significantly reversed the antinociception caused by morphine (5 mg/kg, s.c.) (Figure 2 and Table 2).

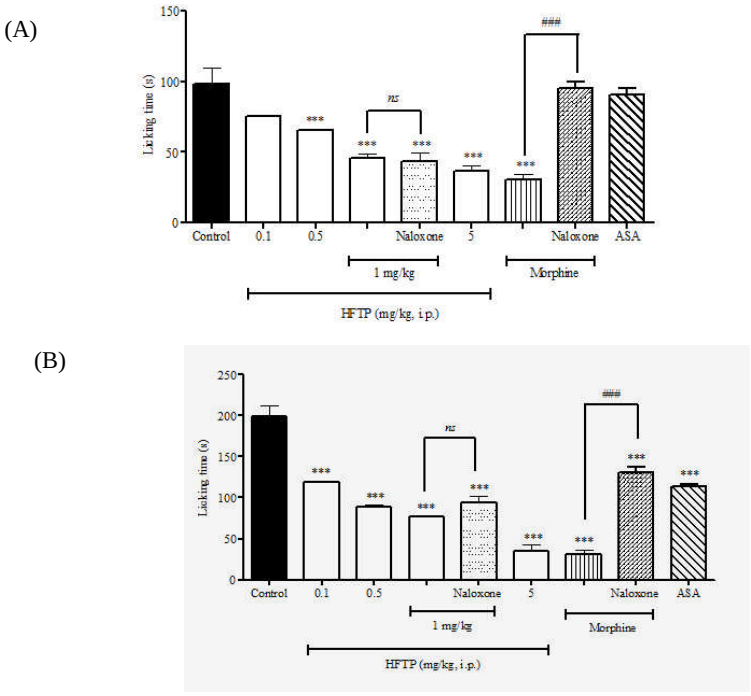

Figure 2: Effect of HFTP in the formalin-induced paw licking test (early phase, panel A, and late phase, panel B) in mice. Each column represents the mean ± S.E.M. of six mice. $^{***}P < 0.001$ compared with control (C) group, while $^{\#\#\#}P < 0.001$ compared to the group receiving appropriate extract/drug (one-way ANOVA followed by Dunnett's *post hoc* test). *ns* - denote no significance level compared to HFTP-treated group. ASA: acetylsalicylic acid.

Capsaicin-Induced Paw Licking Test

Figure 3 shows that HFTP (0.1, 0.5, 1 and 5 mg/kg, i.p.) produced significant attenuation of capsaicin-induced neurogenic nociception with 25.11, 43.89, 58.44 and 83.33% inhibition, respectively. The calculated mean ED_{50} value (and its respective 95% confidence limits) for HFTP in this test was 0.82 mg/kg (0.70 – 0.96 mg/kg). In addition, capsazepine (Cap), a selective capsaicin receptor antagonist, also demonstrated a significant suppression (88.57%) against capsaicin-induced nociception.

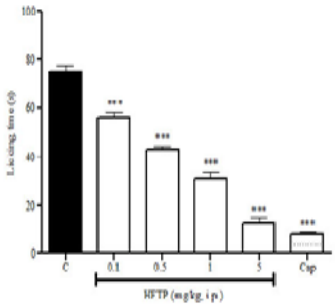

Figure 3: Effect of HFTP in the capsaicin-induced paw licking test in mice. Each column represents the mean ± S.E.M. of six mice. $^{***}P < 0.001$ compare with control (C) group (one-way ANOVA followed by Dunnett's *post hoc* test). Cap: Capsazepine.

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Table 2: Effect of HFTP on the hot-plate test in mice.

Treatments	Dose	Latency time (s)				
	(mg/kg)	0 min	30 min	60 min	120 min	180 min
Control (i.p.)		6.17 ± 0.17	6.83 ± 0.31	6.67 ± 0.33	6.83 ± 0.31	6.83 ± 0.31
HFTP (i.p.)	0.1	6.50 ± 0.34	7.83 ± 0.54	7.83 ± 0.31	9.33 ± 0.49	10.00 ± 0.26*
	0.5	7.00 ± 0.26	7.00 ± 0.86	8.67 ± 0.88	10.00 ± 0.73*	10.17 ± 0.70**
	1	7.00 ± 0.37	8.50 ± 0.62	10.67 ± 0.99***	12.33 ± 0.71***	10.00 ± 1.00*
	5	7.33 ± 0.21	8.67 ± 0.33	9.50 ± 0.43*	12.50 ± 1.15***	11.00 ± 1.24***
HFTP (i.p.) + naloxone (i.p.)	1 + 5	7.17 ± 0.31	8.33 ± 0.67	10.00 ± 0.82	10.33 ± 0.67	12.00 ± 1.13
Morphine (s.c.)	5	7.50 ± 0.34	18.33 ± 0.67***	17.00 ± 0.82***	16.33 ± 0.99***	15.17 ± 0.40***
Morphine (s.c.) + naloxone (i.p.)	5 + 5	6.33 ± 0.21	6.67 ± 0.33#	6.67 ± 0.33#	7.00 ± 0.37#	6.83 ± 0.17#

Results are expressed as mean ± S.E.M of six mice (n=6) in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control group; #$P < 0.05$ compared to the group receiving appropriate drug/extract at the same dose without naloxone (two-way ANOVA followed by the Bonferroni *post hoc* test)

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Glutamate-Induced Paw Licking Test

The results presented in Figure 4 shows that HFTP (0.1, 0.5, 1 and 5 mg/kg, i.p) caused significant and dose-related inhibition of glutamate-induced nociception with percentage inhibitions of 22.91, 30.98, 45.40 and 60.40%, respectively. The calculated mean ED_{50} value (and its respective 95% confidence limits) for HFTP in this model was 0.85 mg/kg (0.73 – 0.99 mg/kg).

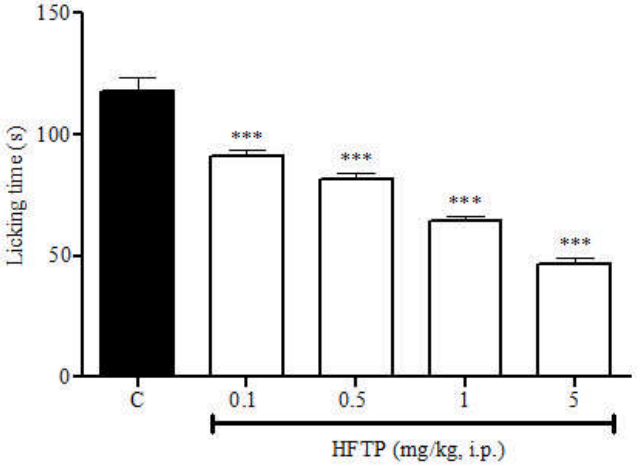

Figure 4: Effect of HFTP in the glutamate-induced paw licking test in mice. Each column represents the mean ± S.E.M. of six mice. $^{***}P < 0.001$ compared with control (C) group (one-way ANOVA followed by Dunnett's *post hoc* test).

Rota-Rod Test

Administration of HFTP (5 mg/kg; i.p.) did not cause any significant alterations in the motor performance of the mice as compared to the control group (vehicle; 10 ml/kg; i.p.). In contrast, diazepam (4 mg/kg; i.p.) significantly reduced the time of permanence on the rota-rod. The mean ± S.E.M. in the rota-rod test for control and HFTP were 120.0 ± 0.00 s while diazepam was 38.83 ± 4.38 s.

Acute Oral Toxicity Study

Single oral administration of HFTP (5 g/kg) did not cause mortality and any noticeable signs of toxicity such as convulsion, loss of weight, hyperactivity and other abnormal behaviours after 7 days of observation.

Discussion

The potential antinociceptive activity of hexanic fraction from turmeric powder was examined using various chemical- and thermal-induced models of nociception in mice. Because of different profiles of sensitivity of each nociception model, a combination of several animal pain test models was employed in the present study to better illustrate the antinociceptive property of HFTP. In addition, we further evaluated some of the possible mechanisms of action underlining the antinociceptive effects of this extract.

The acetic acid-induced abdominal writhing test has long been used as a screening tool for the assessment of both peripherally and centrally acting drugs (De Souza et al., 2009). This test model has been associated with an increased level of cyclooxygenase (COX) and lipooxygenase (LOX) products in peritoneal fluids that in turn stimulate the release of various inflammatory mediators, such as bradykinin, serotonin and substance P. These endogenous mediators further excite afferent sensory C fibres to send pain signals into dorsal horn neurons of the central nervous system (Ikeda et al., 2001). In addition, it is well established that the nociceptive response caused by acetic acid is also dependent on the release of some cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-1 and interleukin-8 via modulation of macrophages and mast cells localised in the peritoneal cavity (Lucena et al., 2007). It was demonstrated in the present study that HFTP elicited significant dose- dependent inhibition of acetic acid-induced visceral nociceptive responses in mice, which is consistent with the previous observations (Liju et al., 2011). The ASA-treated group also manifested similar inhibition properties. Therefore, the present results of the acetic acid-induced abdominal constriction test strongly suggests that the mechanism of HFTP may be linked partly to the inhibition of COX and/or LOX and other inflammatory mediators in peripheral tissues, thereby, interfering with the mechanism of signal transduction in primary afferent nociceptors. Despite being considered to be a very sensitive test used to evaluate analgesia of various drugs, this test however is not specific as it cannot distinguish between peripheral and central antinociceptive as well as muscle relaxant activity of drugs, which may lead to possible misinterpretation of the results (Le Bars et al., 2001). Thus, formalin-induced paw licking and hot-plate test models were selected to further corroborate the effects of HFTP.

The formalin-induced paw licking test is the acute tissue injury-induced cutaneous pain that is characterised by the occurrence of two distinct phases: the first phase (neurogenic phase) corresponds to neurogenic pain due to direct activation of formalin on sensory nerve fibres, while the second phase (inflammatory phase) is related to the release of various inflammatory mediators associated with the increased level of prostaglandins, induction of COX and release of nitric oxide (NO) in peripheral tissue (Tjolsen et al., 1992). The biphasic nature of the pain response in this test, which reflects different pathological processes, can be used to elucidate the possible mechanisms involved in analgesia. It is well documented that centrally acting drugs, such as opioids, inhibit both phases of pain, while peripheral-acting drugs such as ASA, that inhibit COX activity, only inhibit the second phase (Shibata et al., 1989). The present study showed that time spent in paw licking was significantly

reduced in both neurogenic and inflammatory phases, with stronger inhibition of the nociceptive response observed in the inflammatory phase than in the neurogenic phase. The inhibitory effect observed in the inflammatory phase suggests that HFTP may be acting via inhibition of any inflammatory mediator liberated in the mouse paw. This effect may be due to inhibition of the formation and/or liberation of mediators on local tissue or due to blockage of receptors to the different mediators. These results corroborate the inhibitory effect of HFTP on the acetic acid-induced writhing response. It was also demonstrated in the present study that morphine, a well-known centrally acting drug, inhibited both phases equally, while the peripherally acting drug, ASA inhibited only the inflammatory phase. Hence, based on the results obtained, the possible mechanism for this antinociceptive activity of HFTP could be attributed to both peripheral and central involvement.

It is interesting to note that the central antinociceptive effect of HFTP in the present study is strongly supported by the findings obtained from the hot-plate test, which is a specific test to elucidate the involvement of a central mechanism (Sulaiman et al., 2009). Our results demonstrated that HFTP (1 and 5 mg/kg; i.p.) exhibits significant prolongation in the response latency time to a heat stimulus. As anticipated, morphine significantly increased the response latency time to the nociceptive stimulus in the hot-plate test, and its effect was significantly antagonised by a non-selective opioid receptor antagonist, naloxone. However, unlike morphine, naloxone failed to antagonise the inhibitory effect of HFTP (Table 2). A similar effect was also observed in both phases of the formalin-induced paw-licking test (Figure 2) where pre-treatment with naloxone, failed to antagonise the inhibitory effect of HFTP. Together, these results strongly suggest that both the peripheral and central mechanisms of HFTP-induced anti-nociception seem to be independent of the activation of the opioidergic system.

Of great interest to the present study were the findings demonstrating that HFTP produced a significant antinociceptive effect in capsaicin and glutamate tests. The capsaicin-induced paw licking test is an experimental model used for the investigation of substances that act on pain of a neurogenic origin (Sakurada et al., 2003). In the present study, it was demonstrated that capsaicin-evoked nociceptive pain behaviours were significantly attenuated in mice treated with HFTP in a dose-dependent manner. Capsaicin, a pungent ingredient of red peppers, is well known to stimulate a ligand-gated cation channel, the vanilloid receptor (transient receptor potential cation channel V1 or TRPV1) located on poly-modal C-fibres of nociceptive sensory neurons (Schmidt, 2002). Stimulation of these receptors initiates a complex cascade of events, including neuronal excitation, release of excitatory amino acids, nitric oxide and pro-inflammatory mediators (Sakurada et al., 2003). In a similar experimental condition, it was also observed that capsazepine, a selective capsaicin receptor antagonist, significantly reduced the nociception induced by capsaicin. Thus, our results demonstrate that HFTP exerts its antinociceptive effects via inhibition of the TRPV1 receptor.

Numerous studies have demonstrated that glutamate is an important mediator of pain not only in the central nervous system, but also in the periphery (Fonnum, 1984; Fundytus, 2001). Another interesting finding of the present study is the demonstration that administration of HFTP produced a significant and dose-dependent inhibition of the nociceptive response caused by i.pl. injection of glutamate. Glutamate exerts its excitatory effects either by activation of several G-protein-coupled metabotropic glutamate receptors or by induction of ion fluxes by different classes of inotropic receptors such as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite receptors. In addition, the nociceptive response induced by glutamate is also mediated by the release of nitric oxide or nitric oxide-derived substances, which eventually enhances the synthesis of inflammatory mediators such as cytokines and prostanoids (Beirith et al., 2002). Thus, the results presented in this study suggest that, at least in part, HFTP exerts its antinociceptive action through interaction with the glutamatergic system or possibly through the inhibition of NO production.

The present chemical analysis of HFTP indicated the presence of a number of constituents that appear to be likely candidates for the observed antinociceptive activity. These major constituents have been reported to possess various important pharmacological properties including anti-oxidant (Jayaprakasha et al., 2002), anti-inflammatory (Liju et al., 2011) and anti-venom (Ferreira et al., 1992). Moreover, constituents such as caryophyllene oxide, palmitic acid and linoleic acid, although present in small percentage in HFTP, have been reported to possess antinociceptive activity (Inceoglu et al., 2006; Rocha-Gonzalez et al., 2010; Romero et al., 2012). Therefore, the antinociceptive activity observed in the present study may be attributed to the overall synergetic effects of the constituents or the compounds having action similar to non-steroidal anti-inflammatory or opioid drugs, albeit in a complimentary manner, but the exact mechanism of action remains to be elucidated.

A major concern in experiments designed to evaluate the analgesic action of new agents is whether pharmacological treatment causes other behavioural alterations, such as altering motor coordination, which could be misinterpreted as analgesia. It is worth indicating that at the dose administered HFTP produced neither an occurrence of mortality in the preliminary acute toxicity test nor any significant effect on motor coordination in the rota-rod test. These results indicate that HFTP might have a reasonable safety margin with regards to toxicity, and the anti-nociception induced by HFTP is unlikely to be secondary to its depressant and/or non-specific central effects. Besides that, the acute oral toxicity study was also conducted in the present study. Oral administration of 5000 mg/kg of HFTP, similar to intraperitoneal treatment of experimental dose of HFTP, did not show any signs of toxicity up to 7 days post-administration. This observation justified the safety and efficacy of turmeric powder, which is traditionally consumed as route of treatment for the Indian and Malay communities.

Conclusions

Collectively, the present study demonstrated that HFTP, at doses devoid of any detectable toxicity and sedative effects, exerts pronounced peripheral and central antinociceptive effects when assessed in chemical and thermal experimental models of nociception in mice. Although the precise mechanism underlying the antinociceptive action of HFTP has yet to be determined, it seems that the peripheral activity is probably mediated through the inhibition of COX and /or LOX and other inflammatory mediators. On the other hand, both peripheral and central antinociceptive effects of HFTP seem to be independent of the activation of the opioid system. Moreover, the results of the present study also strongly suggest that the antinociceptive effect exerted by HFTP could involve however, at least in part, its ability to interact with TRPV1 receptors and the glutamatergic system. The antinociceptive activity of HFTP and its possible mechanisms of action reported herewith support, at least in part, the use of turmeric powder in traditional medicine for the treatment of some painful and inflammatory conditions and also establish the presence of biologically active principles whose activities may need further investigation.

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